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Effect of cropping system and age of plant at harvest on tuber rot and performance of elite cassava varieties in derived savannah

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ABSTRACT

Devastated tuber rot disease among farmers prompted the evaluation of the elite improved varieties in the intercrop and the practice of delaying harvesting when there is glut in the market necessitated this study. Trial was carried out at the Federal University of Agriculture, Abeokuta between 2011 and 2014 to evaluate yield performance of 21 elite cassava varieties planted as sole crop verse intercropped and harvested at different age. The 2 x 21 x 3 factorial experiment was laid out in randomized complete block design and replicated three times. The tuber yield obtained from sole plot in 2011/2012 cropping season was significantly higher than intercrop whereas those of 2012/2014 cropping season were similar. Land Equivalent Ratio was above one in both cropping seasons indicating that the performance of the improved varieties in intercrop was efficient. The pooled mean tuber yield showed that TMS 30572, 92/0326, 95/0211, 01/1371, 00/0338, 01/0046, 00/0098, 01/1097, 01/0085, 98/0581 and 98/510 were among the top eight varieties. Harvesting could be delayed up to 15 months after planting to reduce tuber rot.

Key words: cassava; intercrop; tuber rot; delay harvest; Nigeria

IZVLEČEK

UČINKI NAČINA GOJENJA IN STAROSTI RASTLIN OB SPRAVILU NA POJAVLJANJE GNILOBE GOMOLJEV PRI ELITNIH SORTAH MANIOKE V ANTROPOGENI SAVANI NIGERIJE

Zaradi prakticiranja odloga spravila pridelka manioke, kadar se pojavlja njen višek na trgu in pojavljanja uničujoče gnilobe gomoljev, se je pojavila potreba po ovrednotenju elitnih sort te tropske gomoljevke, gojene v medsadnji. Poskus je bil izveden na Federal University of Agriculture, Abeokuta med 2011 in 2014 z namenom ovrednotenja pridelka 21 elitnih sort manioke, posajene v monokulturi ali v kombinaciji z drugimi kulturami in pospravljene v različnih časovnih obdobjih. Faktorski 2 × 21 × 3 poskus je bil izveden po sistemu naključnih blokov s tremi ponovitvami. Pridelek gomoljev na površinah z monokulturo je bil v rastni sezoni 2011/2012 značilno večji kot na površinah z medsadnjo, v rastni sezoni 2012/2014 pa sta bila pridelka podobna. Ekvivalent zemljišča je bil nad ena v obeh rastnih sezonah in kaže prednost izboljšanih sort, gojenih v medsadnji. Analiza povprečnih vrednosti pridelka gomoljev je pokazala, da so bile sorte TMS 30572, 92/0326, 95/0211, 01/1371, 00/0338, 01/0046, 00/0098, 01/1097, 01/0085, 98/0581 in 98/510 med osmimi najdonosnejšimi. Za zmanjšanje gnilobe je izkop gomoljev lahko zamaknjen do 15 mesecev po sadnji.

Ključne besede: manioka; medkultura; gniloba gomoljev; poznejši izkop; Nigeria

1 INTRODUCTION

Cassava (*Manihot* spp.) belongs to the family of Euphorbiaceae. Cassava is one of the most important food crops in Africa, South America and Asia. It derives its importance from the fact that its starchy, thickened, tuberous roots are a valuable source of cheap calories, especially in the developing countries where calorie deficiency and malnutrition are widely spread. Its usage as a source of ethanol for fuel, energy in animal feed,

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and starch for industries is increasing. Cassava contributes the largest share of daily per capita food consumption (1.6 kg) in Nigeria (FAOSTAT, 2003) and ranked number one among the top 20 commodities produced in Nigeria (www.fao.org/faostat/en/#rankings/commodities_by_countries) for more than estimated 800 million people around the world (Akparobi et al., 1998; Lebot, 2009).

Nigeria, Thailand, Indonesia and Brazil were ranked as first, second, third and fourth respectively, among the top 20 countries producing cassava in the world. The current estimated cassava production in 2013 for Nigeria, Thailand, Indonesia and Brazil were 47.4, 30.2, 23.0 and 21.5million tonnes, respectively (www.fao.org/faostat/en/#rankings/countries_by_commodities). Total area harvested in 2009 was 3.13 million ha, with an average yield of 11.7 t ha^{-1} (FAO, 2010). It is produced predominantly (99 %) by small farmers with 1-5 ha of land intercropped with yams, maize, or legumes in the rainforest and savannah agro-ecologies of Southern, Central, and lately Northern Nigeria. The world production of cassava root was estimated to be 184 million tonnes in 2002.

IFSERAR, (2009) conducted a diagnostic survey in South West Nigeria and reported that the local varieties grown among the farmers were not only low yielders but their ability to tolerate, or resist new strains of diseases and pests occasioned, perhaps, by climate change. Mwangi et al. (2004) similarly reported that the root rots are an important constraint to cassava production in humid forest and forest transition of Central and West Africa and can impact negatively on food security to several millions people inhabiting the regions. Rotting is known to increase significantly if mature plants are left in the soil for extended period of time (Oyeka, 2004). Yield loss was estimated at 20 to 100 % in Democratic Republic of Congo (Mwangi et al., 2004). These challenges necessitated the evaluation of 21 promising varieties collected from International Institute of Tropical Agriculture (IITA) in maize/cassava intercrop. IITA have released several high yielding varieties but there performances in the intercrop as well as their tolerant or resistant level to cassava root rot disease have not been documented. There is therefore the need to ascertain the performance of these elite cassava varieties under the predominant intercropping systems among the resource constraint farmers in the region. Besides, the highly perishable nature of cassava tubers has compelled the farmers to harvest only when there is availability of market or family need. This delay harvesting enables farmers to leave the mature plant in the soil as a form of storage. This storage period enable the farmers to keep the fresh tuber in good quality for an extended period. Growth and dry matter accumulated will continue since cassava is believed to mature 7-24 months. Most cassava varieties attain optimum weight at 18 months after planting when starch accumulation is highest (Ekanayake et al., 1997). Hammer et al. (1987), who evaluated sequential harvests to age 24 months, reported that root rot occurred in the second year. Sagrilo et al. (2006) quoted Sagrilo et al. (2002) that cassava harvested at 21 months could improve storage root yield compared to 12 months. Ebah-Djedji et al.(2012) who harvested cassava sequentially at 11, 13, 15 and 17 months after planting in Cote d' Ivoire recommended that tuberous root of improved cassava varieties should be harvested at 13 MAP to obtain optimum dry matter content.

These inconsistencies in the appropriate time of harvesting to obtain optimum dry matter content and quality is further aggravated by the prevailing tuber rot. Consequently, there is the need to ascertain the appropriate time to harvest these elite cassava varieties. This will ensure maximum dry matter accumulation without losing much of the tuber to root rot, particularly when harvesting is delayed because of poor market arrangement. The objectives of this study therefore were to**:** evaluate the performance of the improved varieties, 2) investigate the effect of intercropping on the elite cassava varieties and 3) determine the effect of delay harvest on the cassava tuber rot.

2 MATERIALS AND METHODS

The study was carried out at the Institute of Food Security, Environmental Resource and Agricultural Research (IFSERAR) farm, Federal University of Agriculture, Abeokuta in 2011/2012 and repeated in 2012-2014 cropping seasons. The experiment was laid out in a Randomized Complete Block Design in split plot and replicated three times in the 2011/2012. Cropping systems and variety factors were assigned to the main plot and sub plot, respectively. However, in 2012-2014 cropping season, harvesting date (12, 15 and 18 months) was varied as the third factor i.e. sub sub-plot (split split-plot) to gain additional information. The intercrop proportion mixture and population adopted was additive series. Table 1 shows the twenty one elite cassava varieties collected from International Institute of Tropical Agriculture (IITA), Ibadan. Benlate treated stem cuttings of 25 cm were planted into plot size of 9 m x 7 m (1.2 ha experimental field) at spacing of 1 m x 1 m in July 2011 and harvested in July, 2012. The cuttings obtained from the harvest were replanted in

July 2012 and harvested sequentially in July (12 months after planting MAP), October 2013 (15 MAP) and January 2014 (18 MAP). Three seeds per hole of treated maize 'SUWAN 1' variety was alternately planted in-between cassava stands (in the intercrop plots only) to evaluate the performance of cassava under intercropping (i.e. additive series). Maize was harvested at green stage. Weeding was carried out at 3, 9 12 WAP. Other weedings were done once in a month. Fertilizer 400 kg/ha N: P: K: Mg (12:12:17:2) was applied in the 2011/2012 cropping season whereas 2012/2014 trial did not receive fertilizer because of circumstance beyond our control.

2.1 Data collection on cassava

2.1.1 Plant height (cm):

5 randomly selected cassava plants within the plot were measured with aid of graduated meter rule from the ground level to the highest leaf.

2.1.2 Stem girth (mm):

Vernier caliper was used to determine the stem girth (at 10 cm above the ground) of 5 randomly selected cassava stems within the plot.

2.1.3 Tuber girth (mm):

Vernier caliper was used to determine the tuber girth of 5 randomly selected freshly harvested tubers from ten up rooted cassava stands samples

2.1.4 Number of tubers per plant:

Determined by average number of freshly harvested tuber from the ten samples uprooted.

2.1.5 Rot incidence (%):

This was done by dividing the rotted tubers by total tuber multiplied by 100.

2.1.6 Tuber yield (t/ha):

The mass of uprooted tuber from the ten sampled cassava stand was converted to t/ha. (i.e. mass of sampled/sampled area*10000/1000}

2.2 Data analysis:

Data collected were subjected to analysis of variance using GenStat Edition 12. Significant means were separated by using DMRT at 5 % probability.

Table 1: Selected cassava varieties used for the experiment

Variety	Tuber color
TMS98/0581	White
TMS 01/1797	White
TMS 95/0211	White
TME 1	White
TMEB 693	White
TMS 01/0046	White
TMS 01/0093	White
TMS 00/0338	White
TMS 01/1097	White
TMS 01/1086	White
TME B 419	White
TMS 30572	White
TMS 01/1371	Yellow
TMS 01/0085	White
TMS 98/0510	White
TMS 01/0131	White
TMS 98/0505	White
TMS 92/0326	White
TMS 01/0098	White
TMS 01/1368	Yellow
TMS 97/JW2	Yellow

3 RESULTS AND DISCUSSION

3.1 Influence of intercropping on the plant height of elites cassava varieties at 12 MAP

Plant height and stem girth are essential component to determine plant growth particularly when intercrop is involved. The plant height of the 21 varieties obtained at 12 months after planting varied significantly (P>0.05) from each other in the 2011/2012 cropping season (Table 2). TME B 419 had the tallest plants but comparable to TMEB 693, TMS 01/1097, TMS 01/1797, TME 1, TMS 01/1086, TMS 01/1371,TMS 97/JW2 and TMS 92/0326. Whereas, TMS 98/505 had the shortest plant which was similar to those of TMS 01/0098, TMS 01/0131, TMS 01/0046, TMS 01/0093 and TMS 00/0338. In 2012/2014 cropping season, TMS 97/JW2 and TMS01/0093 had the tallest plants at 12 MAP but were similar to those of TMS 95/0211, TME 1, TMS 00/00338, TME B 419, TMS 30572, TMS 01/1371, TMS 01/0085, TMS 98/0510, TMS 92/0326, TMS 01/0098 and TMS 01/1386 (Table 3). However, TMS 01/1797, TMEB 693, TMS 01/0046, TMS 01/1097, TMS 01/1086, TMS 01/0131 and TMS 98/0505 had the shortest plants in 2012/2014.The plant heights in the two seasons were at variance except those of TMS 97/JW2, TME B 419 and TMS 01/1371 which were consistently top on the list, whereas TMS 01/0131, TMS 01/0046 and TMS 98/0505 constantly had the shortest plants. This consistency in plant height implies that the varieties were stable in the different environment, whereas the others were influenced more by the environment.

3.2 Influence of intercropping on the tuber number of elites cassava varieties at 12 MAP

The number of fresh tubers observed in 2011/2012 on cassava varieties TME 1, TMS 98/0505, TMS 97/JW2, TMS 98/0581, TMS 01/1097, TMS 01/1386, TMS 30572, TMS 01/1086, TMS 01/0085 and TMS 00/0338 were similar but significantly higher than those of TMS 01/1371 and TMS 01/0131 varieties in 2011/2012 cropping season (Table 2). The varieties TMS 30572, TME 1, TMS 01/0093, TMS 00/0338, TMS 1097, TMS 01/0046, TMEB 693, TMS 98/0510 and TMS 92/0326 in 2012/2014 were among the top varieties with high number of fresh tuber while TME B 419 had the least (Table 3). TME 1, TMS 01/1097, TMS 30572 and TMS 00/0338 were constantly ranked amongst the top varieties with high number of tubers in 12 MAP of the two seasons. The variance in tuber number could be genetically inherent and was considered as vital yield attribute that contributes immensely to the increase in tuber yield. It has been documented that the increase in yield were attributed to increase in number of tuber/stand and single root mass (Kogram et al., 2002).

3.3 Influence of intercropping on the tuber girth of elites cassava varieties at 12 MAP

The cropping system and variety did not influence tuber girth in 2011/2012 (Table 2), however, in 2012/2014 the varieties varied significantly among each other in 2012/2014 at 12 MAP (Table 3). ‘TMS 98/0510’ had the highest tuber girth while ‘TMS 97/JW2’ had the least. The stem girths of the varieties were influenced by cropping systems in both seasons of the trial at 12 MAP (Tables 2 and 4). However, the varieties TMS 00/0338 and TMS 98/0505 consistently recorded the highest and the lowest, respectively in 12 MAP of both cropping season.

3.4 Influence of intercropping on the root rot of elites cassava varieties at 12 MAP

The cropping systems did not influence tuber rot infection in both seasons but there were significant differences among the varieties in 12 MAP of 2011/2012 (Table 2), they were however similar in 2012/2014 (Table 4). The rot incidence observed in 2011/2012 was high and ranges between 9.8 and 22.5 % while that of 2012/2014 was low and range between 0.00 and 0.94 % at 12 MAP. The lost incurred during 2011/2012 cropping season is in consonant with the finding of Mwangi et al. (2004) who documented 20 to 100 % tuber lost.

3.5 Influence of intercropping on the LER and tuber fresh mass of elites cassava varieties at 12 MAP

The Land Equivalent Ratio (LER) was similar in both cropping season but above one suggesting that intercrop plots was more productive. The fresh tuber mass of the varieties varied in the two cropping seasons at 12 MAP (Tables 2 and 4). Sole cassava plots had significantly higher tuber mass than intercrop in 2011/2012 (Table 2) but similar 2012/2014 cropping seasons (Table 4). In 2011/2012 cropping season, TMS 98/0505, TMS 97/JW2, TME 1, TMS 30572, TMS 95/0211, TMS 92/0326, TMS 01/0085, TMS 01/0098, and TMS 98/0581 varieties were the nine topmost in terms of fresh tuber mass at 12 MAP. Whereas the following varieties: TMS 01/1086, TMS 01/1368, TMS 98/0510, TMS 01/1097, TMS 01/1371, TMS 01/1797 and TMS 01/0046 closely followed. However, ‘TMEB 693’ had the lowest tuber yield. The fresh tuber yields range between 27.5 and 57.4 t ha^{-1} in 2011/2012 while those of 2012/2014 was 12 to 32.3 t ha^{-1}. The yield range obtained in 2011/2012 was substantially higher than that of 2012/2014 at 12 MAP as expected. However, the results obtained in 2012/2014 was comparable to range of 9.9 to 30.1, 8.49 to 28.38, and 10.0 to 26.9 t ha^{-1} as reported by IITA (1987), Maroya et al. (2010) and Ssemakula and Dixon (2007), respectively.

The eleven topmost varieties in 2012/2014 cropping season at 12 MAP, were TMS 00/0338, TMS 30572, TMS 92/00326, TMS 01/1097, TMS 98/0581, TMS 01/0046, TME 1, TMS 98/0510, TMS 01/0085, TMS 01/1371,and TMS 95/0211 in that order. These were closely followed by TMS 01/1086, TMS 01/0093, TMS 01/1797, TMS 01/0098, TMS 01/1368, TME B 419 and TMS 01/0131 varieties. While TMS 97/JW2 variety had the lowest tuber yield. The variation in tuber yield of the varieties agreed with the finding of Howeler (2007); Mulualem and Ayenew (2012); Odedina et al. (2012) who reported that yields of cassava roots vary with cultivars, plant growth conditions (soil, climate, rainfall) and agronomic practices. It is pertinent to note that the variation in tuber yield of the 21 varieties was only observed in 12 MAP (Tables 2 and 4) while the harvest made at 15 and 18 MAP had similar tuber yield. This is, perhaps, an indication that maturity had not been attained and thus dry matter accumulation were at variance at 12 MAP. Although, number of tuber, stem girth and tuber girth varies among the varieties at 15 and 18 MAP (Table 3) but all the varieties had similar tuber yield (Table 4). This is an indicative of the fact that all the varieties tested in this trial attained maturity period after 12 MAP. Based on the definition of maturity period of cassava by Benesi et al. (2008) is the point where maximum or near maximum yield is obtained.

The topmost 3 consistent varieties in the two cropping seasons were TMS 30572, TMS 92/0326 and TMS 98/0581. Although 'TMS 01/1371' and 'TMS 01/1386' were not listed among top yielder, but had beta carotene as an advantage and statistically comparative yield with the top varieties in the two cropping seasons.

Table 2: Influence of intercropping on the tuber rot, agronomic parameters and tuber yield performance of elites cassava varieties in 2011/2012 cropping season

Treatment	Plant height (m)	Fresh tuber no.plant^{-1}	Tuber girth (mm)	Stem girth (mm)	Rot incidence (%)	LER	Fresh tuber mass (t ha^{-1})
Cropping System (CS)							
Sole	2.74	7.2	63.66	28.93	14.3	-	44.2a
Intercrop	2.72	6.9	62.22	28.52	14.3	-	39.4b
LSD	NS	NS	NS	NS	NS	-	2.07
Variety (V)							
TMS 98/0581	2.78bcde	7.3a-e	69.13	32.75ab	22.5a	1.96	41.7abc
TMS 01/1797	2.98abc	7.0b-f	59.97	27.94abc	18.3a-d	1.73	38.5bcd
TMS 95/0211	2.61cdef	6.8b-f	67.04	28.75abc	11.7ef	1.40	46.5abc
TME 1	2.93abcd	8.7a	64.40	28.26abc	10.2f	1.96	47.6abc
TMEB 693	3.13ab	7.0b-f	57.22	27.12abc	13.4d-f	1.56	27.5d
TMS 01/0046	2.49efg	6.7c-f	66.14	32.22abc	16.3b-f	1.86	38.1bcd
TMS 01/0093	2.49efg	6.2d-f	60.44	25.76bc	18.2a-d	1.60	35.3cd
TMS 00/0338	2.31fg	7.5a-e	58.90	34.24a	12.0ef	1.56	34.5cd
TMS 01/1097	3.00abc	6.0ef	61.23	29.72abc	11.8ef	1.73	40.1bcd
TMS 01/1086	2.69adef	8.0abc	60.02	26.88abc	13.5c-f	1.96	41.2bc
TME B 419	3.23a	6.7c-f	61.85	27.96abc	12.9d-f	1.73	37.0cd
TMS 30572	2.78bcde	8.0abc	65.23	28.68abc	13.6c-f	1.73	47.2abc
TMS 01/1371	2.90abcd	5.7f	58.45	29.19abc	20.1ab	1.56	39.4bcd
TMS 01/0085	2.75cde	7.3a-e	63.83	27.81abc	16.6a-e	1.50	45.8abc
TMS 98/0510	2.66cdef	6.2d-f	73.30	31.51abc	19.5abc	2.03	40.6bcd
TMS 01/0131	2.54defg	5.7f	60.41	27.23abc	16.6a-e	1.56	36.6cd
TMS 98/0505	2.21g	8.2ab	66.08	24.52c	12.9d-f	1.70	57.4a
TMS 92/0326	2.85abcde	7.0b-f	68.70	28.65abc	13.9c-f	1.50	45.9abc
TMS 01/0098	2.40fg	6.7c-f	61.75	28.95abc	15.0b-f	1.90	45.1abc
TMS 01/1368	2.82bcde	7.7abc	61.01	31.16abc	11.5ef	1.80	40.3bcd
TMS 97/JW2	2.85a-e	8.2ab	56.58	25.97abc	9.8f	1.90	51.3ab
SE (V)	0.201	0.75	12.53	4.147	3.07	NS	6.69
CS X V	NS	NS	NS	NS	NS	NS	NS

NS = not significant

Table 3: Influence of intercropping on agronomic performance of elite cassava varieties at different age of plant in 2012/2014 cropping season

Treatment	Plant height (m) 12 MAP	15 MAP	18 MAP	Fresh tuber No. 12 MAP	15 MAP	18 MAP	Tuber girth (mm) 12 MAP	15 MAP	18 MAP
Cropping systems (CS)									
Sole	2.25	2.71	2.87	6.1	6.6	5.71	57.22	67.1	64.1
Intercrop	2.31	2.76	2.94	6.3	6.6	5.67	58.66	66.9	65.0
LSD	NS	NS	NS	NS	NS	NS	NS	NS	NS
Variety (V)									
TMS 98/0581	2.66ab	3.15abc	3.04abc	6.2b-f	6.7a-d	5.6a-e	64.1ab	66.1a-e	69.5abc
TMS 01/1797	1.70d	2.12d	2.21bc	5.5c-f	4.8cd	4.5cde	55.0bc	64.9a-e	57.4cde
TMS 95/0211	2.27a-d	2.41bcd	2.73abc	6.2b-f	6.2a-d	6.0a-d	62.0abc	66.2c-e	68.1a-d
TME 1	2.27a-d	2.93a-d	3.13abc	7.2a-d	7.2abc	7.6ab	59.4abc	67.7a-e	56.9de
TMEB 693	1.97cd	2.35bcd	2.53a	8.0a-c	8.5a	7.7ab	52.2bc	57.4e	57.7b-e
TMS 01/0046	1.94cd	2.22cd	2.20bc	7.0a-e	6.8a-d	6.3abc	61.1abc	66.6a-e	69.3a-d
TMS 01/0093	2.80a	3.78a	3.66a	8.2ab	8.8a	6.7abc	55.4bc	58.5e	66.2a-e
TMS 00/0338	2.49abc	2.74bcd	2.85abc	7.3a-d	6.5a-d	5.7a-e	53.9bc	63.0b-e	62.6a-e
TMS 01/1097	2.09bcd	2.43bcd	2.90abc	6.3a-f	6.3a-d	6.0a-d	56.2abc	74.0ab	63.8a-e
TMS 01/1086	2.11bcd	2.79bcd	2.94abc	5.0def	6.3a-d	4.7cde	55.0bc	61.1c-e	61.3a-e
TME B 419	2.41abc	2.54bcd	2.78abc	4.2f	5.1bcd	3.2e	56.9abc	74.1ab	72.6ab
TMS 30572	2.26a-d	2.62bcd	3.07abc	8.7a	8.3ab	8.3a	60.2abc	71.2a-d	63.7a-e
TMS 01/1371	2.43abc	3.10abc	3.16ab	5.8b-f	7.5abc	6.5abc	53.5bc	68.5a-e	68.4a-d
TMS 01/0085	2.23a-d	2.77bcd	3.00abc	5.8b-f	7.7abc	5.5b-e	58.8abc	72.5abc	63.3a-e
TMS 98/0510	2.25a-d	2.79bcd	2.98abc	7.0a-e	6.7a-d	5.7a-e	68.3a	74.9a	74.3a
TMS 01/0131	1.94cd	2.34bcd	2.23bc	5.2def	5.0b-d	4.3cde	55.4bc	67.6a-e	60.7b-e
TMS 98/0505	2.05bcd	2.32bcd	2.12c	4.7ef	3.7d	3.3de	61.1abc	71.4a-d	65.2a-e
TMS 92/0326	2.41abc	3.02a-d	3.40a	7.3a-d	7.5a-c	6.0a-d	63.7abc	72.1a-d	71.6abc
TMS 01/0098	2.48abc	3.26ab	3.43a	5.8b-f	6.8a-d	5.3b-e	56.8abc	62.0c-e	53.9e
TMS 01/1368	2.35abc	2.53bcd	3.09abc	4.7ef	5.7a-d	5.3b-e	56.0abc	67.7a-e	68.8a-d
TMS 97/JW2	2.77a	3.12ab	3.52a	4.7ef	6.5a-d	5.3b-e	51.6c	60.4de	60.9a-e
SE	0.65	0.96	1.02	2.5	3.4	2.8	12.5	11.9	13.8
CS x V	NS	NS	NS	NS	NS	NS	NS	NS	S

NS = not significant, S = significant

Table 4: Influence of intercropping on root rot and tuber yield of elite cassava varieties at different age of plant in 2012/2014 cropping season

Treatment	Stem girth (mm) 12 MAP	15 MAP	18 MAP	Root rot incidence (%) 12 MAP	15 MAP	18 MAP	LER	Fresh tuber mass (t ha^{-1}) 12 MAP	15 MAP	18 MAP
Cropping systems (CS)										
Sole	23.9	23.1	23.3	0.29	0.48	11.61	-	23.7	31.0	27.0
Intercrop	23.52	23.7	23.0	0.31	0.73	11.06	-	24.6	31.5	26.6
LSD	NS	NS	NS	NS	NS	NS	-	NS	NS	NS
Variety (V)										
TMS 98/0581	27.8ab	24.7abc	24.4a	0.39	1.60	35.14	1.46	29.3ab	30.0	23.6
TMS 01/1797	22.9c-g	21.2bcd	23.1ab	0.14	0.44	4.93	1.23	22.1a-d	29.2	26.7
TMS 95/0211	23.8b-f	24.9abc	24.1a	0.5	2.39	10.99	1.10	24.5abc	38.9	35.8
TME 1	23.3c-g	25.0abc	23.0ab	0.12	0.94	28.02	1.46	26.7abc	26.5	22.4
TMEB 693	22.1d-g	22.2a-d	20.7ab	0.0	0.50	14.40	1.06	19.7cde	25.4	27.2
TMS 01/0046	25.2a-e	20.7cd	21.0ab	0.94	1.40	11.20	1.36	27.4abc	37.3	25.5
TMS 01/0093	20.8f-g	22.2a-d	22.2ab	0.24	0.56	12.10	1.10	22.6a-d	30.7	26.2
TMS 00/0338	29.2a	22.7a-d	27.1a	0.73	0.73	7.57	1.06	32.3a	29.1	29.5
TMS 01/1097	24.7b-f	25.1abc	23.3ab	0.24	1.43	5.36	1.23	29.6ab	31.7	25.8
TMS 01/1086	21.9efg	21.6a-6	21.6ab	0.00	0.78	17.48	1.46	23.1a-d	29.2	24.4
TME B 419	23.0c-g	24.7abc	24.4a	0.27	0.80	4.74	1.23	21.3a-d	27.0	20.8
TMS 30572	23.7b-f	22.5a-d	25.3a	0.14	2.09	21.97	1.23	32.0a	39.0	35.4
TMS 01/1371	24.2b-f	26.3a	24.4a	0.00	1.78	20.02	1.06	25.0abc	37.5	32.7
TMS 01/0085	22.8c-g	23.1a-d	23.3ab	0.56	0.65	4.61	1.10	25.6abc	33.6	30.5
TMS 98/0510	26.5abc	23.5a-d	26.2a	0.27	1.24	18.34	1.53	26.6abc	30.4	24.7
TMS 01/0131	22.2d-g	23.7a-d	21.8ab	0.27	1.49	4.25	1.06	20.1a-d	24.2	20.2
TMS 98/0505	19.5g	22.0a-d	17.7b	0.14	1.01	11.33	1.20	15.2cd	23.1	17.7
TMS 92/0326	23.7b-f	26.2ab	23.7a	0.00	1.43	16.31	1.10	30.1ab	38.5	31.8
TMS 01/0098	24.0b-f	24.4a-d	23.0ab	0.71	1.01	2.85	1.40	22.0a-d	33.3	32.3
TMS 01/1368	26.2a-d	25.0abc	23.8a	0.27	0.56	2.92	1.30	21.6a-d	39.4	21.2
TMS 97/JW2	21.0fg	19.5d	21.4ab	0.94	0.27	8.80	1.50	12.0d	21.5	28.3
SE	4.2	5.0	5.8	NS	NS	NS	NS	12.3	NS	NS
CS X V	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS = not significant, S = significant

3.6 Influence of age at harvest and intercropping on the plant height, tuber girth and tuber rot of elites cassava varieties at 12, 15 and 18 MAP

The pooled mean plant height, tuber girth and tuber rot obtained from 2012/2014 showed significant difference among the ages of plant at harvest (Table 5). The similarity in height and tuber girth of plants harvested at 15 and 18 MAP buttressed the fact that maturity had been attained compared to 12 MAP. The higher incidence root rot recorded in 18 MAP accounted for the apparent decline in fresh tuber mass. This result contradicts the finding of Mulualem and Ayenew (2012) who recommended 18 months as the appropriate age to harvest cassava to get the desired yield.

The pool mean of varieties TMS 01/0093, 97/JW2 and 01/0098 had similar but the tallest plants. However, varieties 97/JW2 and 01/0098 were not significantly taller than those of TMS 92/0326, TMS 98/0581 and TMS 01/1371. The following varieties TMS 01/0131, TMS 98/0505, TMS 01/1797, TMEB 693 and TMS 01/0046 had the shortest plants. The pooled mean fresh tuber number of varieties TMS 30572, TMEB 693, TMS 01/0093 and TME 1 were similar and highest among the others. But the tuber number of varieties TMS 01/0093 and TME 1 were not significantly higher than at TMS 92/0326. Variety TMS 98/0505 had the minimum number of fresh tubers but not significantly lower than TMS 01/1797, TME B 419 and TMS 01/0131.The tuber girth of varieties TMEB 693, TMS 01/0093, TMS 00/0338, TMS 01/1086, TMS 97/JW2, TMS 01/1797 and TMS 01/0098 were similar but significantly lower than those of TMS 98/0510, TMS 92/0326, TME B 419 and TMS 98/0581. The stem girth of varieties TMS 98/0510, TMS 00/0338 and TMS 98/0581 were significantly higher compared to others whereas TMS 97/JW2 and TMS 98/0505 had the lowest.

3.7 Influence of age at harvest and intercropping on the tuber yield of elites cassava varieties at 12, 15 and 18 MAP

Although the tuber yield recorded for the three ages were similar but dropped at 18 MAP evidently due to rot damage. Ebah-Djedji et al. (2012) reported decline in cassava tuber at 17 months old, however, the decline was not linked to root rot. Hammer et al. (1987) reported that root rot occurred in the second year but was not specific on the number of months.

The tuber fresh mass of varieties were significantly different from one another. The following varieties TMS 30572, TMS 92/0326, TMS 95/0211, TMS 01/1371, TMS 00/0338, TMS 01/0046, TMS 00/0098 and TMS 01/1097 were among the topmost eight varieties whereas TMEB693, TMEB 419, 01/0131, TMS 97/JW2 and TMS 98/0505 were the least.

Generally, the consistence in plant heights values at 12 MAP of varieties TMS 97/JW2, TME B 419 and TMS 01/1371 (which ranked among the top) and those of TMS 01/0131, TMS 01/0046 and TMS 98/0505 (at the bottom of the list) in both cropping seasons are indication of their stability despite differences in crop management. Besides, the ability of TMS 30572, TMS 92/0326 and TMS 98/0581 to constantly rank among the first six varieties at 12 MAP in the two seasons makes them candidates to be recommended to farmers (Table 6). The wide gap in tuber yield between the two cropping seasons could be attributed to the fertilizer application. Although, farmers hardly use fertilizer for cassava production because of the notion that cassava can thrive on marginal soils that cannot sustain other crops. This trial connotes that the addition of fertilizer can substantially enhance tuber yield. Odedina et al. (2012) who worked on integrated nutrient management reported similar gap between control and other sources of nutrient. Ironically, appreciable quantity of root rot was observed in the first cropping season compared to the second, it was not quite clear if the addition of fertilizer was responsible for the tuber rot. Consequently, there is the need to validate whether or not fertilizer application to cassava influences root rot.

4 CONCLUSIONS

The study has shown that the cassava varieties were not affected by intercropping but Land Equivalent Ratio was above one in both cropping seasons indicating that the performance of the improved varieties in intercrop was efficient. Plant height and tuber girth were higher in 15 and 18 MAP than 12. On the bases of their consistent performance at 12 MAP, in the two cropping seasons, TMS 30572, TMS 92/0326 and TMS 98/0581 are candidate varieties to be recommended to farmer with or without resource constraints. The pooled mean tuber yield showed that TMS 30572, TMS 92/0326, TMS 95/0211, TMS 01/1371, TMS 00/0338, TMS 01/0046, TMS 00/0098, TMS 01/1097, TMS 01/0085, TMS 98/0581 and TMS 98/510 are top eight varieties. The incidence of tuber rot was highest at 18 MAP hence; harvesting could be delayed up to 15 MAP to reduce tuber rot. The three yellow flesh tuber varieties identified had comparable performance with their white counterpart.

Table 5: Performance of elite cassava varieties as influenced by age of plant at harvest and intercropping in 2012/2014 cropping season

Treatment	Plant height (m)	Fresh tuber no.plant^{-1}	Tuber girth (mm)	Stem girth (mm)	Root rot incidence (%)	Fresh tuber mass (t ha^{-1})
Age at harvest (H)						
12 MAP	2.28b	6.2	57.9b	23.73	1.95b	24.12
15 MAP	2.73a	6.6	67.0a	23.39	0.90c	31.23
18 MAP	2.90a	5.7	64.6a	23.17	3.43a	26.78
LSD	0.30	NS	5.23	NS	0.67	NS
Cropping systems (CS)						
Sole	2.61a	6.1a	62.8a	23.44a	2.09a	27.21a
Intercrop	2.67a	6.2a	63.5a	23.42a	2.09a	27.55a
LSD	NS	NS	NS	NS	NS	NS
H x CS	NS	NS	NS	NS	NS	NS
Variety (V)						
TMS 98/0581	2.95bcd	6.1defg	66.6bc	25.6ab	2.89a	27.76bcdefg
TMS 01/1797	2.01j	4.9hijk	59.1fgh	22.4fgh	1.52a	26.01defgh
TMS 95/0211	2.47fghi	6.1defg	65.5bcd	24.2bcdef	2.33a	32.98abc
TME 1	2.78cdef	7.3abc	61.4defg	23.8bcdef	2.49a	25.21defgh
TMEB 693	2.28ghij	8.1a	55.8h	21.7gh	1.76a	24.10efghi
TMS 01/0046	2.12ij	6.7cd	65.7bcd	22.6efg	2.71a	30.09abcde
TMS 01/0093	3.41a	7.9ab	60.0efgh	21.7gh	1.99a	26.53cdefgh
TMS 00/0338	2.69def	6.5cde	59.8efgh	26.4a	2.25a	30.31abcde
TMS 01/1097	2.48fgh	6.2cdefg	64.3bcde	24.4bcde	1.61a	29.04abcdef
TMS 01/1086	2.61defg	5.3fghi	59.2fgh	21.7gh	1.89a	25.57defgh
TME B 419	2.58efg	4.1jk	67.9abc	24.0bcdef	1.77a	23.04fghi
TMS 30572	2.65def	8.4a	65.1bcd	23.8bcdef	2.27a	35.48a
TMS 01/1371	2.90b-e	6.6cde	63.5cdef	25.0abcd	1.98a	31.72abcd
TMS 01/0085	2.67def	6.3cdefg	64.9bcd	23.1defg	2.09a	28.88bcdef
TMS 98/0510	2.67def	6.4cdefg	72.5a	25.4ab	2.37a	27.21bcdefg
TMS 01/0131	2.17hij	4.8ijk	61.2defg	22.6efg	1.64a	21.52ghi
TMS 98/0505	2.16hij	3.9k	65.9bcd	19.7i	1.93a	18.64i
TMS 92/0326	2.94bcd	6.9bcd	69.1ab	24.5abcde	2.02a	33.68ab
TMS 01/0098	3.06abc	6.0defgh	57.5gh	23.8bcdef	2.00a	29.19abcdef
TMS 01/1368	2.65def	5.2ghij	64.2cde	25.0abcd	1.66a	27.40bcdefg
TMS 97/JW2	3.15ab	5.5efghi	57.6gh	20.6hi	2.74a	20.58hi
SE (V)	0.36	1.18	4.9	2.0	NS	6.56
H X V	NS	NS	NS	NS	NS	NS
CS x V	NS	NS	NS	NS	S	NS
H x CS	NS	NS	NS	NS	NS	NS
H X CS x V	NS	NS	NS	NS	NS	NS

NS = not significant, S = significant

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Multivariate analysis to assess abscisic acid content association with different physiological and plant growth related traits of *Petunia*

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ABSTRACT

Petunia is an important and beautiful ornamental flowering plant, grown throughout the world for its beauty and attraction. Different *Petunia* hybrids have been developed by petunia growing countries of the world. The prescribed study was conducted to investigate the association of abscisic acid with seed yield and its contributing traits of petunia line. Data for different physiological, morphological and petunia seed yield traits was recorded, analyzed and interpreted for final inferences. From results it was showed that the petunia lines IAGS-P8, IAGS-P9 and IAGS-P11 performed well for most of the studied traits. It was shown from multivariate analysis techniques that stomata conductance, chlorophyll b contents, seed area, chlorophyll a contents, flower fresh mass, flowers per plant, seed mass and abscisic acid contributed higher to seed yield per plant in petunia. The abscisic acid contents showed positive and significant association and contribution towards seed yield of petunia genotypes. It was suggested that selection on the basis of abscisic acid may be useful to develop good seed yield per plant and large number of flowers per plant in petunia under stressful environmental conditions.

Key words: petunia; multivariate analysis; heritability; genetic advantage; abscisic acid content; seed yield

IZVLEČEK

UPORABA MULTIVARIATNE ANALIZE ZA OCENITEV POVEZAVE MED VSEBNOSTJO ABSCIZINSKE KISLINE IN RAZLIČNIMI Z RASTJO POVEZANIMI FIZIOLOŠKIMI ZNAKI PRI PETUNIJI

Petunija je pomembna in lepa okrasna rastlina, ki se goji širom po svetu zaradi lepote in privlačnosti. V številnih državah, kjer jo gojijo, so bili vzgojeni različni križanci. Pričujoča raziskava je bila opravljena z namenom preučiti povezavo med vsebnostjo abscizinske kisline in lastnostmi, povezanimi s pridelkom semena preučevanih linij petunij. Izmerjene so bile različne fiziološke in morfološke lastnosti, ki vplivajo na pridelek semen, analizirana in pojasnjena je bila njihova povezava. Izsledki so pokazali, da so se linije petunij IAGS-P8, IAGS-P9 in IAGS-P11 izkazale kot primerne za večino analiziranih lastnosti. Multivariatna analiza je pokazala, da so parametri kot so stomatarna prevodnost, vsebnost klorofila b in a, površina semen, sveža masa cvetov, število cvetov na rastlino, masa semen in vsebnost abscizinske kisline prispevali največ k večjemu pridelku semena na rastlino. Vsebnost abscizinske kisline je imela značilen pozitiven vpliv na pridelek semena vseh genotipov petunij. Zaradi tega se priporoča, da je izbor genotipov petunije na osnovi večje vsebnosti abscizinske kisline primeren za vzgojo rastlin z velikim pridelkom semena na rastlino in velikim številom cvetov v stresnih okoljskih razmerah.

Ključne besede: petunija; multivariatna anliza; dednost; genetska prednost; vsebnost abscizinske kisline; pridelek semena

1 INTRODUCTION

The genus *Petunia* is an important ornamental plant of high economic imperativeness on the whole agriculture. It gives dominant qualities to serve as model plant for contemplating plant improvement. The history of *Petunia* development as a crop is accent with implications for an advanced array of added ornamental crops (Cantor et al., 2015; Gerats and Vandenbussche, 2005). This generally developed genus of flowering plants fits in with Solanaceae gang. It is an important ornamental plant in landscape because of colour diversity. A large number of hybrids and varieties have been developed with diverse color and patterns (Ganga,

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2011). This plant is native to Brazil, Argentina or Uruguay and included over 35 species (Dole and Wilkins, 1999). Petunias are considered an annual ornamental plant but may be perennial in warmer climates (Berenschot et al., 2008). Petunia genus includes about 20 species of South American origin, mostly perennials but developed as annuals (Mallona et al., 2010), 14 annual species (Toma, 2009), but *Petunia hybrida* Hort. (*P. axillaris* Lam. × *P. violacea* Lindl.) is a species which presents the biggest decorative value (Berenschot et al., 2008; Vandenbussche et al., 2016). Presently the researches have also confirmed that the genus *Petunia* is consisted of 14 closely related species (Stuurman et al., 2004). The modern petunias have been developed through hybrid breeding like the violet petunia (*P. violacea*, and *P. integrifolia* (Hook.) Schinz & Thell.) and ambrosial agrarian or white petunia (*Petunia axillaris*). A large number of researchers are also working on finding the real ancestors because their ancestors are rarely cultivated today. Petunia is a small sprawling plant with large number of flowers, grown throughout the world for its beauty and interactive colors (Anderson, 2006; Băla, 2007). Petunias blossom abundantly even in hot summers and new varieties even in seasons with top clamminess (Băla, 2007; Florin et al., 2012). The petunia is long day plant due to which it is used for landscape proposes (Anderson, 2006; Currey and Lopez, 2013). The present study was conducted to develop inbred lines of petunia through selfing for growing seasons. The data of various morphological, physiological and seed yield traits was recorded to access the performance of inbred lines under development. The identification of promising inbred lines for the development of petunia hybrids.

2 MATERIAL AND METHODS

Prescribed research work was conducted in the research area of Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan. Twelve petunia lines, IAGS-P1, IAGS-P2, IAGS-P3, IAGS-P4, IAGS-P5, IAGS-P6, IAGS-P7, IAGS-P8, IAGS-P9, IAGS-P10, IAGS-P11 and IAGS-P12 were selected and grown in the field during 2015. Selfing of all the lines was carried out for 4 successive growing seasons (2011-14) to develop inbred lines. The selfed seed was collected to develop next generation, grown in 2015 and for obtaining data for various traits, such as leaf temperature (LT), photosynthetic rate (A), stomata conductance (gs), water use efficiency (WUE), sub-stomata CO_2 concentration (Ci) and transpiration rate (E) (by using IRGA-LI-6262 (Infrared Gas Analyzer, LI-COR Biosciences designs, USA), chlorophyll a content (Chl. a), chlorophyll b content (Chl. b) in fresh matter (measured through the dimethyl sulfoxide extraction method (Hiscox and Israelstam, 1979), plant height (PH), leaves per plant (LPP), flowers per plant (FPP), leaf area (LA), stem diameter (SD), leaf length (LL), fresh leaf mass (FLM), seeds per fruit (SPF), leaf width (LW), flower mass (FM), seed mass (SM), fresh stem mass (FSM), seed area (SA, measusered by using Digital Micrometer Screw Guage, Model: 1658DGT/25), 100-seed mass (HSM), abscisic acid (ABA) contnets (using HPLC method (Seo and Koshiba, 2002)), and seed yield per plant (SYP). The data were statistically analyzed by using analysis of variance technique (Steel et al., 1997).

3 RESULTS AND DISCUSSION

The results from Table 1 persuaded that significant differences among all the studied traits were found. The highest heritability (h^2bs) was found for photosynthetic rate, sub-stomata CO_2 concentration, stomata conductance, water use efficiency, plant height, leaves per plant, flowers per plant, seeds per fruit, leaf area, seed yield per plant and abscisic acid contents. The genetic advantage was found higher for all studied traits except sub-stomata CO_2 concentration, flowers per plant, seeds per fruit, seed mass while moderate for abscisic acid content and leaves per plant. The higher broad sense heritability referred the dominance type of gene action and suggested that the selection for such traits may be helpful to develop petunia hybrids with much of vigor and ability to tolerate harsh environmental conditions as higher value of h^2bs was recorded for abscisic acid content. Higher concentration of abscisic acid contnets in plant body gives an extra advantage to grow in drought conditions with higher and well performance. Various researchers while working on different crop plants have described about higher heritability for these traits as reported in our study (Aaliya et al., 2016; Ali et al., 2015; Ali et al., 2013; Ali et al., 2014a; Mahmood and Haider, 2016). Genetic advance indicated the presence of additive type of gene action hence the traits with higher genetic advance suggested that the selection of lines may also be helpful to develop synthetic varieties. The similar findings for different crops have been reported by various researchers (Ali and Ahsan, 2015; Ali et al., 2014b; Ali et al., 2014c; Khorasani et al., 2011; Mahmood and Haider, 2016). The results

(Supplementary Material Table S1) indicated that the lines IAGS-P8, IAGS-P9 and IAGS-P11 were better performing than all of the other lines also seen form figure 1a (principal component analysis) that the lines IAGS-P2, IAGS-P8, IAGS-P9 and IAGS-P11 fall in quadrant I which indicates the highest and best performance for respective traits. The lines which showed the best performance may be used for the development of good quality cultivars, with large number of flowers, stress tolerant and multicolor petunia hybrids and varieties (Ali et al., 2013; Florin et al., 2012; Mahmood and Haider, 2016).

Table 1: Genetic components for morpho-physiology and yield traits of petunia

Traits	M.S	G.M	GV	GCV %	PV	PCV %	EV	ECV %	h^2bs%	GA%
Photosynthetic rate (μg CO_2 s^{-1})	123.533*	14.104	39.803	167.991	43.927	176.480	4.124	54.074	90.612	74.728
Leaf tTemperature (^oC)	137.453*	21.194	38.440	134.674	60.574	169.058	22.134	102.194	63.459	40.898
Chlorphyll a (mg g^{-1} fr. mass.)	14.245*	3.199	4.238	115.095	5.770	134.298	1.532	69.203	73.447	96.786
Chlorphyll b (mg g^{-1} fr. mass)	17.345*	1.578	3.037	138.730	11.271	267.256	8.234	228.429	26.945	100.608
Stomata conductance (mmol m^{-2} s^{-1})	1.323*	0.031	0.417	366.764	0.489	397.167	0.072	152.400	85.276	337.958
Transpiration rate (mm day^{-1})	1.025*	0.884	0.258	54.013	0.509	75.873	0.251	53.286	50.678	71.773
sub-stomata CO_2 concentration (μmol mol^{-1} CO_2)	234.534*	148.889	77.000	71.914	80.533	73.546	3.533	15.404	95.613	10.114
Water use efficiency (%)	36.345*	6.792	11.700	131.248	12.945	138.055	1.245	42.814	90.382	84.026
Leaves per plant	233.342*	86.750	77.452	94.489	78.438	95.089	0.986	10.661	98.743	17.692
Plant height (cm)	219.245*	55.818	70.571	112.441	78.104	118.290	7.533	36.736	90.355	25.107
Stem diameter (cm)	1.026*	0.511	0.308	77.594	0.411	89.647	0.103	44.896	74.919	164.889
Flowers per plant	287.345*	141.000	92.791	81.123	101.764	84.955	8.973	25.227	91.183	11.449
Leaf length (cm)	36.124*	6.324	11.333	133.868	13.458	145.880	2.125	57.967	84.210	85.731
Leaf width (cm)	4.897*	1.358	1.017	86.539	2.863	145.198	1.846	116.591	35.522	77.676
Leaf area (cm^2)	41.255*	6.353	12.934	142.685	15.387	155.628	2.453	62.138	84.058	91.087
Fresh leaf mass (g)	3.522*	0.654	0.629	98.096	2.263	186.031	1.634	158.066	27.806	112.255
Fresh stem mass (g)	214.255*	49.224	67.574	117.166	79.107	126.771	11.533	48.404	85.421	27.088
Flower mass (g)	2.148*	0.601	0.391	0.806	1.366	1.945	0.975	127.396	28.608	97.648
Seeds per fruit	996.357*	866.167	326.331	61.380	343.694	62.992	17.363	14.158	94.948	3.567
100-seed mass (mg)	64.235*	12.049	16.357	116.515	31.520	161.741	15.163	112.180	51.895	42.437
Seed area (mm)	2.087*	0.357	0.434	110.317	1.218	184.745	0.784	148.192	35.657	193.490
Seed yield per plant	1.024*	0.117	0.313	163.648	0.397	184.283	0.084	84.732	78.859	745.625
Seed mass (mg)	97.573*	50.140	24.671	70.146	48.231	98.078	23.560	68.548	51.152	12.434
Abscisic Acid contents (mg/100g fresh leaf mass)	524.156*	115.124	162.969	118.979	198.219	131.217	35.250	55.335	82.217	17.646

* = significant at 5 % probability level, mean sum of squares (M.S), grand mean (G.M), genotypic variance (GV), genotypic coefficient of variance (GCV %), phenotypic variance (PV), phenotypic coefficient of variance (PCV %), environmental variance (EV), environmental coefficient of variance (ECV %), broad sense heritability (h^2bs %), genetic advance (GA)

The correlation analysis provides best opportunity to the researchers for selecting genotypes of crop plant to improve crop plant growth and production (Ali et al., 2016; Ali et al., 2014c). The results from table 2 indicated that significant correlation was found for photosynthetic rate with chlorophyll a contents, plant height, sub-stomata CO_2 concentration, leaf width, abscisic acid and seeds per fruit. Abscisic acid contents was found to be significantly correlated with most of the studied traits including photosynthetic rate, chlorophyll a contents, chlorophyll b contents, plant height, sub-stomata CO_2 concentration, transpiration rate, water use efficiency, leaf temperature, leaf area, leaf width, fresh shoot mass, seeds per fruit, 100-seed mass, seed mass and seed yield per plant. Seed yield per plant was significantly correlated with photosynthetic rate, transpiration rate, leaf temperature, leaves per plant, fresh leaf mass, stem diameter, seed area, seed mass, seeds per fruit, flowers per plant and abscisic acid contents. The positive and significant correlation revealed that the selection of lines to develop hybrids and synthetic varieties may be helpful to improve the growth and development of petunia. The significant correlation of abscisic acid content with morphological traits, seed yield and physiological traits indicated that the selection of petunia lines on the basis of good abscisic acid production may be fruitful to improve drought tolerance in petunia (Aaliya et al., 2016; Abbas et al., 2016; Filipović et al., 2014).

The growth of petunia was adversely affected by changing the environmental optimum temperature of 25 °C with minimum circadian light intensity to be 13 Wm^{-2} (Kaczperski et al., 1991). It has been observed that temperature and light caused major effects on growth and development of petunia. Therefore, new petunia varities and hybrids should be developed which can tolerate varying environmental conditions to continue optimal plant growth and development. However, the holdup of plant growth, development, the access of CO_2 by stomata in an optimized environmental condition has shown not any extensive adverse effect on petunia plants (Blanchard and Runkle, 2009).

Stepwise regression analysis was performed to predict the trait(s) that were highly contributing towards the petunia seed yield per plant. Stepwise regression analysis provides an opportunity to select crop plant genotypes with higher contribution traits to improve crop yield and production (Aaliya et al., 2016; Abbas et al., 2016). The results from Table 3 showed that stomata conductance, chlorophyll a contents, flowers per plant, leaf area, flower fresh mass, seed area, seeds per fruit, seed mass and abscisic acid contenta contributed more to seed yield per plant but it could be biased as preceding literature has also been reported the error effects of stepwise regression (El-Badawy and Mehasen, 2011) while handling a large number of independent variables. The Intercept = 145.754, R^2 = 0.863, Adjust R^2 = 0.336 and Standard Error = 0.812 was found with expected regression equation as fellow:

$Y = 145.754 + (7.144X_1) + (-1.254\ X_2) + (3.898\ X_3) + (-6.651X_4) + (121.14X_5) + (-4.582X_6) + (0.018X_7) + (-0.042X_8) + (1.145X_9) + (0.163X_{10}) + (-0.063X_{11}) + (2.463X_{12}) + (-0.125X_{13}) + (-17.215X_{14}) + (41.006X_{15}) + (11.267X_{16}) + (-11.175X_{17}) + (2.825X_{18}) + (8.982X_{19}) + (0.256X_{20}) + (6.902X_{21}) + (21.267X_{22}) + (25.926X_{23})$

The use of PCA (principal component analysis) to overcome the error effect of large number of independent variables in breeding experiments and find overall attributed variation in dependent structure (Ali et al., 2015; Filipović et al., 2014; Goodarzi et al., 2015; Marjanović-Jeromela et al., 2011). It has also been reported that the eigenvalues (in PCA) showed primary significance for numerical diagnostics to evaluate variation endorsed by a large number variables on the dependent structure and their data matrix in a graphical display (Greenacre, 2010). Therefore, we have also performed principle component analysis (PCA) to inspect the traits which were contributing higher towards petunia seed yield per plant. Our data generated four PCA as shown in Table 4 with diverse variation among all of the studied traits. It was found that the PC1, PC2, PC3 and PC4 contributed variation of 35.60 %, 24.60 %, 17.90 % and 11.3 % while their cumulative proportion was 25.2 %, 43.20 %, 57.20 % and 73.10 % respectively. PC1 and PC2 contributed higher variation for respective studied traits (Fig. 1a) the eigenvalues of these four PCs was higher than 1 (Fig. 1b). The Figure 1a also showed that the petunia lines IAGS-P2, IAGS-P8, IAGS-P9 and IAGS-P11 showed better performance for most of the studied traits.

Table 2: Correlation among various morpho-physiological and yield traits of petunia

Traits	A	LT	Chl. a	Chl. b	gs	E	Ci	WUE	LPP	PH	SD	FPP	LL	LW	LA	FLM	FSM	FM	SPF	HSW	SA	SM	ABA
LT	0.2024																						
Chl. a	0.8228*	0.0852																					
Chl. b	0.0197	-0.2476	0.0282																				
gs	-0.2867	-0.2754	-0.2040	0.4668*																			
E	0.0079	0.4140*	-0.0727	0.8246*	0.4179*																		
Ci	0.4950*	-0.2424	0.4296*	0.0152	0.4287*	0.0697																	
WUE	0.1222	-0.2718	0.4619*	0.7206*	0.4996*	0.8442*	0.2587																
LPP	-0.2284	0.2949	-0.2064	-0.2864	-0.2048	0.6049*	-0.2224	0.6517*															
PH	0.4889*	0.0884	0.1840	0.5719*	0.2407	0.4948*	0.1474	-0.0464	-0.2226														
SD	-0.0772	0.2017	0.2204	-0.2140	-0.2642	0.4087*	0.2221	-0.2726	0.0482	0.1820													
FPP	0.1809	-0.4272*	0.4499*	0.4462*	0.6668*	-0.2286	-0.2616	0.4426*	-0.2204	0.2420	0.2846												
LL	-0.0172	0.1487	-0.0152	0.4146*	0.2267	0.5144*	-0.0225	0.4184*	0.2298	0.6272*	0.7846*	-0.4044*											
LW	0.4454*	-0.2097	0.2184	0.2649	0.4011*	0.2166	0.1872	0.4426*	0.4649*	0.4250*	0.2819	0.4487*	-0.0998										
LA	-0.0988	0.1709	0.0484	0.4282*	0.4186*	0.6729*	0.2268	0.4761*	-0.0258	0.4480*	0.6828*	-0.4292*	0.9210*	0.2848									
FLM	-0.2756	-0.0711	0.2048	0.4242*	0.2044	0.0602	0.2902	0.2690	0.4792*	-0.0848	-0.2441	0.4787*	0.2478	-0.2162	0.2826								
FSM	0.2586	0.0874	-0.0951	0.8227*	0.7284*	0.7286*	-0.2976	0.6617*	0.2229	0.4228*	0.4016*	0.7602*	0.1291	0.4848*	0.6144*	0.4962*							
FM	0.4268*	0.4892*	0.2216	0.5122*	-0.2800	0.8444*	0.0062	-0.0209	0.2824	-0.0691	-0.2471	-0.0049	0.4442*	0.2229	-0.4496*	-0.2210	0.4468*						
SPF	0.4417*	-0.2624	-0.2290	0.0262	0.6072*	0.2427	-0.4290*	0.0702	-0.1222	-0.0821	-0.2815	0.6472*	-0.0104	-0.2489	-0.2488	-0.2559	0.4417*	0.0109					
HSM	0.2220	0.0852	0.0289	0.2801	0.2698	0.2241	-0.2069	0.2428	0.2402	0.4799*	-0.2092	-0.4772*	0.4224*	0.4472*	0.4400*	-0.0049	0.1089	0.2227	-0.2910				
SA	0.2224	-0.0276	0.4487*	-0.4649*	-0.4144*	-0.4196*	-0.2204	-0.2422	0.0090	0.4724*	0.1642	0.2487	0.4104*	-0.2822	-0.4246*	0.4184*	0.1262	0.4474*	-0.0584	-0.4251			
SM	-0.2780	0.4446*	0.2201	0.2084	0.2294	-0.0742	-0.0224	0.2144	-0.2874	0.2422	0.4928*	-0.2649	0.4114*	-0.4291	-0.1608	0.0275	-0.0047	-0.2452	-0.2056	-0.4778*	-0.2148		
ABA	0.4874*	0.5678*	0.4291*	0.6019*	0.6201*	0.5211*	0.2949	0.5404*	0.7011*	0.0150	-0.2548	0.5052*	0.2578	0.4029*	0.4122*	-0.2109	0.4672*	-0.0241	0.4402*	0.4122*	-0.0252	0.5046*	
SYP	0.4686*	0.4744*	-0.2714	-0.2221	0.4062*	0.4179*	-0.2422	0.4978*	0.4494*	-0.0664	0.4180*	0.4444*	-0.2852	-0.2274	-0.2542	0.4222*	0.2217	0.0212	0.4284*	0.0642	0.4851*	0.4295*	0.5642*

*= Significant at 5 % probability level, A = photosynthetic rate, LT = leaf temperature, Chl. a = chlorophyll a content, Chl. b = chlorophyll b content, E = transpiration rate, gs = stomata conductance, Ci = sub-stomata CO_2 concentration, WUE = water use efficiency, LPP = leaves per plant, PH = plant height, SD = stem diameter, FPP = flowers per plant, LL = leaf length, LW = leaf width, LA = leaf area, FLM = fresh leaf mass, FSM = fresh stem mass, FM = flower mass, SPF = seeds per fruit, HSM = 100-seed mass, SA = seed area, SM = seed mass, ABA = abscisic acid content, SYP = seed yield per plant

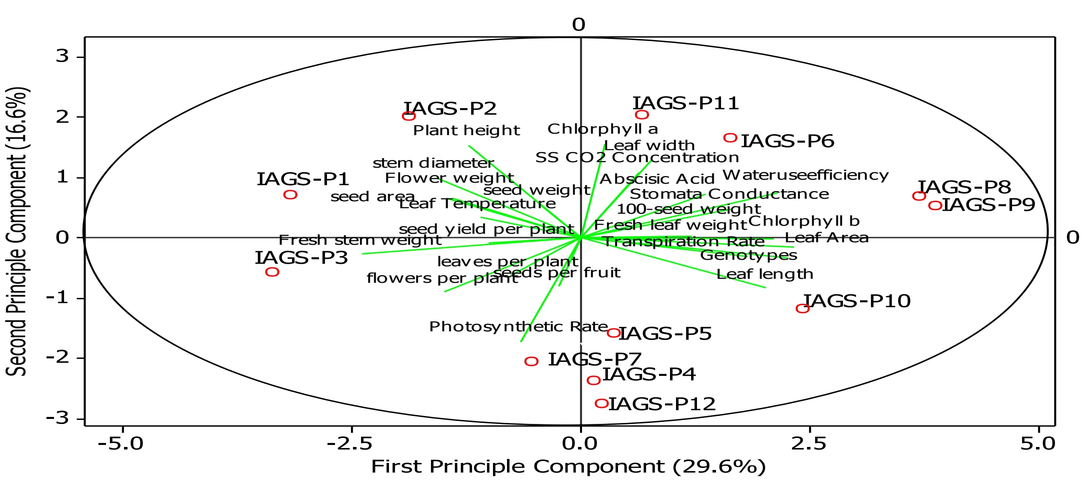
Table 3: Stepwise regression analysis for various traits of petunia for seed yield

	Traits	*Coefficients B*	*Standard Error*	*t Stat*	*Cumulative R^2*	*Partial R^2 %*
X_1	Photosynthetic rate	7.144	0.553	2.013	0.1673	16.73
X_2	Leaf temperature	-1.254	0.127	-2.320	0.2151	21.51
X_3	Chlorphyll a	3.898	2.531	1.114	0.2573	25.73
X_4	Chlorphyll b	-6.651	1.632	1.052	0.2661	26.61
X_5	Stomata conductance	121.14	45.125	2.525	-0.235	23.50
X_6	Transpiration rate	-4.582	3.153	-1.172	0.266	26.60
X_7	Sub-stomata CO_2 concentration	0.018	0.053	-1.512	-0.263	26.30
X_8	Water use efficiency	-0.042	0.351	-0.153	0.5631	56.31
X_9	Leaves per plant	1.145	0.121	1.522	0.2634	26/34
X_{10}	Plant height	0.163	0.086	-1.315	0.2534	25.34
X_{11}	Stem diameter	-0.063	0.015	0.063	0.4743	47.43
X_{12}	Flowers per plant	2.463	8.535	0.279	0.5386	53.86
X_{13}	Leaf length	0.125	0.015	2.233	-0.2157	21.57
X_{14}	Leaf width	-17.215	16.815	-1.037	0.2353	23.53
X_{15}	Leaf area	41.006	24.150	-0.137	-0.2327	23.27
X_{16}	Fresh leaf mass	11.267	8.759	0.521	0.0333	3.33
X_{17}	Fresh stem mass	-11.175	5.115	-1.255	-0.0847	8.47
X_{18}	Flower mass	2.825	0.131	0.522	0.3562	35.62
X_{19}	Seeds per fruit	8.982	10.525	-1.248	-0.2237	22.37
X_{20}	100-seed mass	0.256	0.052	-0.113	0.3644	36.44
X_{21}	Seed area	6.902	4.315	1.522	-0.1245	12.45
X_{22}	Seed mass	21.267	15.517	1.535	0.3252	32.52
X_{23}	Abscisic acid contents	25.926	11.258	0.463	0.5437	54.37

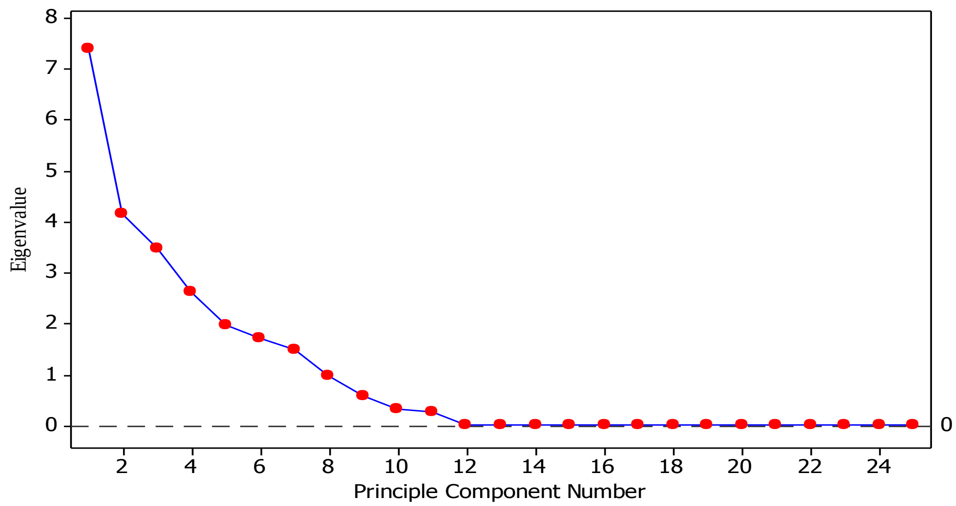
Intercept = 145.754, R^2 = 0.863, Adjust R^2 = 0.336, Standard Error = 0.812

Table 4: Principal component analysis

Eigen value	6.147	3.9536	4.-0717	3.0313
Proportion	0.356	0.246	0.179	0.113
Cumulative	0.252	0.432	0.572	0.731
Traits	**PC2**	**PC2**	**PC3**	**PC4**
Photosynthetic rate	0.689	0.425	0.026	0.055
Leaf temperature	-0.25	0.083	-0.336	-0.093
Chlorphyll a	0.035	0.376	0.07	0.036
Chlorphyll b	0.286	-0.005	-0.025	-0.227
Stomata conductance	0.222	0.228	-0.297	-0.047
Transpiration rate	0.323	-0.037	0.252	-0.262
Sub-stomata CO_2 concentration	0.087	0.163	0.029	0.002
Water use efficiency	0.291	0.183	0.223	-0.202
leaves per plant	-0.204	-0.125	-0.34	0.257
Plant height	-0.166	0.372	0.237	-0.033
Stem diameter	-0.113	0.143	-0.044	-0.224
flowers per plant	-0.413	-0.216	0.325	-0.208
Leaf length	0.114	-0.202	-0.02	0.258
Leaf width	0.214	0.323	0.076	0.248
Leaf area	0.301	-0.017	0.028	0.242
Fresh leaf mass	0.231	-0.007	-0.245	-0.042
Fresh stem mass	-0.314	-0.064	0.216	0.002
Flower mass	-0.291	0.216	0.018	0.337
Seeds per fruit	-0.031	-0.216	0.414	-0.234
200-seed mass	0.262	0.109	-0.074	0.372
Seed area	-0.291	0.212	0.012	-0.206
Seed yield per plant	-0.231	-0.012	-0.126	-0.056
Seed mass	-0.061	0.135	-0.105	-0.499
Abscisic acid contents	0.284	0.218	0.136	0.075



a: Principle components



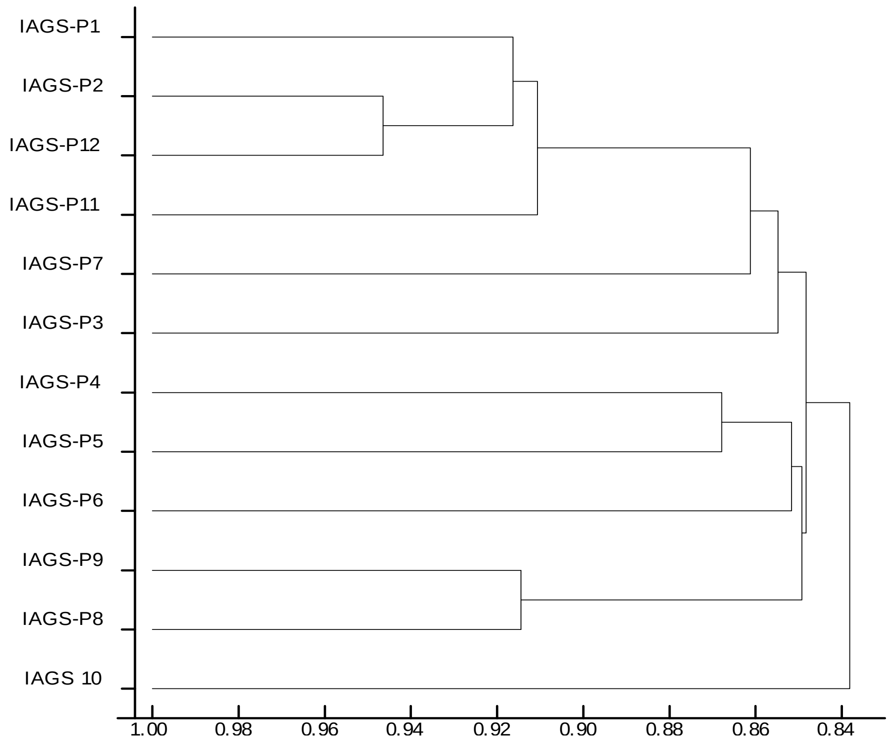
b: Scree plot

Figure 1: a. Principle component analysis of yield and its attributing traits, **b**. Scree plot and respective eigen values

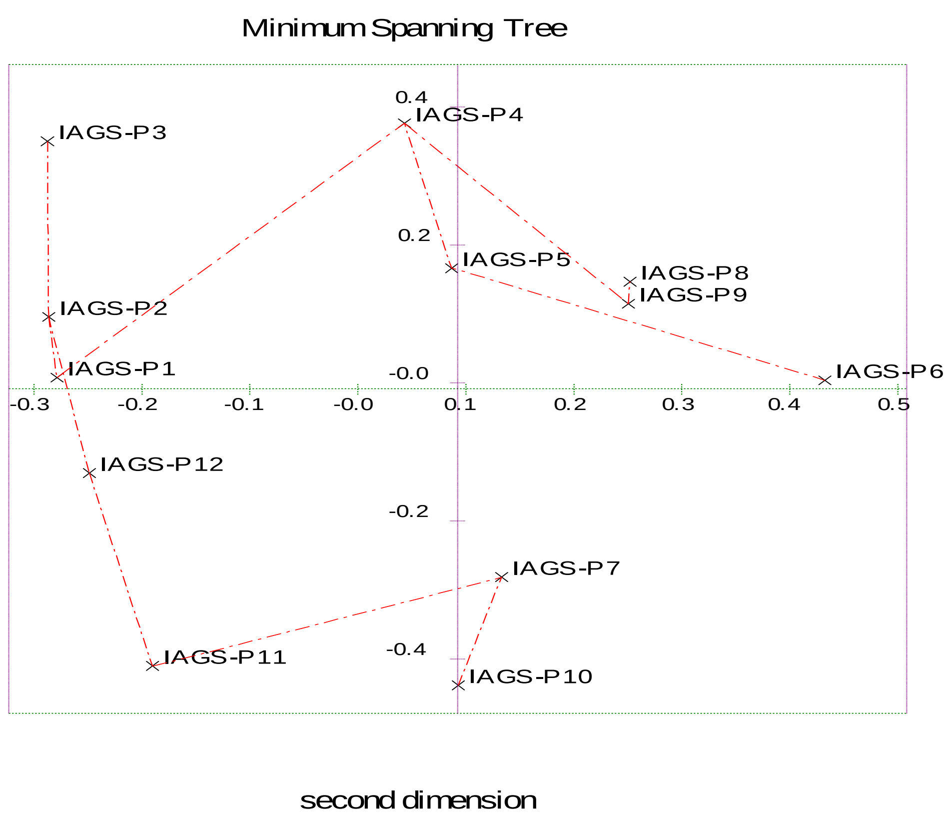
Principal factor analysis was performed by using principle component analysis values, to check that traits which were directly contributing and highly associated with petunia seed yield per plant. The factor 1 was found to be highly contributing factor trait which contributes 48.20 % in total variation were chlorophyll a, chlorophyll b, transpiration rate, stomata conductance, leaves per plant, water use efficiency, leaf area, leaf length, seed yield per plant stem diameter and abscisic acid content (Table 5). Abscisic acid content and seed yield per plant were found the most contributing traits of petunia. Various researchers have suggested that the selection of crop plant genotypes on the basis of the factor analysis (traits from factor 1) may be supportive to develop higher yield hybrids and synthetic varieties of crop plants. While the traits which fall in factor 2 (from factor loading table 5) indicated that the selection of crop plant genotypes on the basis of such traits will not be helpful as the segregation will take place in the next growing generations (Ali et al., 2016; Filipović et al., 2014; Mahmood and Haider, 2016). The better performance of petunia lines for chlorophyll a, transpiration rate, chlorophyll b, leaves per plant, stomata conductance, leaf length, water use efficiency, leaf area, stem diameter, seed yield per plant, seed mass and abscisic acid content revealed that the accumulation or assimilation of organic matter/compounds will be higher in the plant body. It has been also found the accumulation or assimilation of organic biomass in plant body is very essential for the proper enhanced growth and development of petunia plant (Hladni et al., 2011; Huang, 2007; Huang and Yeh, 2009; Mahmood and Haider, 2016). The accumulation of organic compounds generally takes place in the leaves, stem and flowering parts of a plant body. The results from our study were well supported by results which demonstrated the role of factor analysis for effective selection criteria in maize breeding program (Filipović et al., 2014). In order to understand about the genetic association among petunia lines, cluster analysis was performed (Khorasani et al., 2011; Mostafavi et al., 2011). The results from clustering showed that the petunia lines IAGS-P2 and IAGS-P12 followed by IAGS-P8 and IAGS-P9 were highly associated with each other as compared with other petunia lines (Fig. 2a) the association was verified through the development of minimum spanning tree (Fig. 2b) that showed smaller distance between petunia lines IAGS-P8 and IAGS-P9 while IAGS-P2 and IAGS-P12 were having IAGS-P1 in between them through the use of eigen values. So, from results it may be revealed that the petunia lines IAGS-P8 and IAGS-P9 were highly associated with each other and may be used as two separate male or female lines to develop petunia hybrids as also verified by mean performance and principal component analysis Figure 1a results of these lines. Also the petunia line IAGS-P11 showed better performance for almost all under studied traits, so it may also be used as male to develop good quality petunia hybrids (Mahmood and Haider, 2016). It was also suggested that in future breeding program of IAGS-P8, IAGS-P9 and IAGS-P11, these traits may be important for primary selection of synthetic petunia varieties and hybrids to increase seed yield per plant of petunia under various environmental regimes of Pakistan and other growing countries. Moreover, the hybrid seed production technology proved to be more efficient as it reduced the cost, time and increase efficacy for better selection in petunia improvement programs. Still, further studies are required which should cover different years and locations.

Table 5: Factor loadings for different traits of petunia

Factor1	Factor loadings	% Communality
Chlorophyll a	0.674	
Chlorophyll b	0.735	
Stomata conductance	0.643	
Transpiration rate	0.743	
Water use efficiency	0.879	
Leaves per plant	0.568	
Stem diameter	0.789	
Leaf length	0.678	
Leaf area	0.568	
Seed yield per plant	0.567	
Abscisic acid content	0.876	
		48.2
Factor2		
Sub-stomata CO2 concentration	-0.563	
Plant height	-0.577	
Fresh leaf mass	-0.636	
		22.1
Factor3		
Leaves per plant	0.323	
Leaf length	0.325	
Leaf area	0.327	
Fresh leaf mass	0.241	
Seed yield per plant	0.263	
		11.1
Factor4		
Photosynthetic rate	0.221	
Leaves per plant	0.135	
Plant height	0.119	
Stem diameter	0.219	
Leaf area	0.287	
Flower mass	0.153	
100-seed mass	0.206	
		8.62
Cumulative variance		90.02



a: Dendrogram



b: Minimum spanning tree

Figure 2: a. Dendrogram analysis based on hierarchal clustering. Association of petunia lines on genetic basis of all studied traits, **b**. Minimum spanning tree using eigene values for petunia lines on the basis of all studied traits

4 CONCLUSION

The present study concluded that abscisic acid contents showed positive and significant association and contribution towards seed yield of petunia genotypes. It was suggested that selection on the basis of abscisic acid content may be useful to develop good seed yield per plant and large number of flowers per plant in petunia under stressful environmental conditions.

5 CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Molecular genetic analysis of some North African barley germplasms

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ABSTRACT

Isozyme and RAPD markers were used to characterize 29 barley accessions, which were collected from North Africa. In addition, resistance gene sequences were employed to develop molecular markers using RT-PCR approach. High level of polymorphism was found with both RAPD and isozyme markers, where RAPD showed that 60 % of amplified bands were polymorphic. Peroxidase showed three polymorphic loci (7 allelic bands). Isozymes cluster analysis successfully separated the barley accessions into three geographically distinct groups. RAPD investigation demonstrated that Egyptian accessions were grouped into two obvious groups. Moreover, the Tunisian accessions showed no distinct clustering, while high dissimilarities were revealed by the Algerian accessions. In the RT-PCR, from six primer pairs selected, primer pair AF092524P1P2 successfully amplified two specific amplicons of approximately (340 & 220 bp) and (360 & 270 bp), respectively in two Egyptian barley genotypes (El-Awamah and Awlad-Ali). One primer pair DN988165P1P2 gave only one specific amplicon in both barley genotypes of 250 and 270 bp, respectively. The markers developed could be used in improving barley crop by assisting in breeding selection of resistance genotypes.

Key words: RT-PCR; resistance genes; barley; genetic diversity; RAPD

IZVLEČEK

MOLEKULARNA GENETSKA ANALIZA NEKATERIH SEVERNOAFRIŠKIH GENSKIH VIROV JEČMENA

Z izoencimskimi in RAPD markerji je bilo ovrednoteno 29 akcesij ječmena, nabranih v Severni Afriki. Za razvoj molekularnih markerjev na osnovi RT-PCR so bila uporabljena nukleotidna zaporedja genov za odpornost. Ugotovljen je bil velik polimorfizem RAPD in izoencimskih markerjev, kjer je bilo 60 % namnoženih RAPD markerjev polimorfnih. Peroksidaza je pokazala tri polimorfne lokuse (7 alelelov). Z analizo izoencimskih skupin so bile akcesije ječmena uspešno razdeljene v tri razločne geografske skupine. Raziskava RAPD je pokazala, da se egiptovske akcesije razločno povežejo v dve skupini. Tunizijske akcesije niso pokazale razločnega povezovanja, pri alžirskih pa so bile ugotovljene velike razlike. V RT-PCR analizi, je od šestih izbranih začetnih oligonukeltodiov par AF092524P1P2 uspešno namnožil dva specifična amplikona s približno 340 in 220 in 360 in 270 baznih parov pri dveh egiptovskih genotipih ječmena (El-Awamah in Awlad-Ali). Par začetnih oligonukleotidov DN988165P1P2 je pomnožil le en specifični fragment pri obeh genotipih ječmena z 250 in 270 baznimi pari. Razviti markerji se bodo lahko uporabili pri izboru genotipov za izboljšanje ječmena v žlahtnjenju na odpornost.

Ključne besede: RT-PCR; geni za odpornost; ječmen; genska raznolikost; RAPD markerji

1 INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most pivotal cereal crops in the world. It is cultivated in the temperate zones. The haploid genome of barley is about 5.1 Gbp (Mayer et al., 2012). Due to compatibility and inter-fertility of the cultivated and wild species (share a common genome, n =7), wild species of barley and primitive landraces provide precious sources of genetic variability in a number of beneficial traits (Nevo, 1992; Ceccarelli et al., 1995). Consequently, search for genetic variation that might be useful for plant breeding programs is very essential as well as collection and conservation of wild relatives of the cultivated species and the endemic varieties (Brown et al., 1990).

PCR-based molecular markers (e.g. RAPD, SSR, STS, and ISSR) have been used in barley to uncover genetic variation, genotype identification and mapping of genes (Sánchez et al., 1996; Fernández et al., 2002; Matus and Hayes, 2002; Tanyolac, 2003). Particularly, RAPD markers are found to be valuable in case of self-

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pollinating species, which show a relatively low level of intraspecific polymorphism, as in hexaploid wheat (Devos and Gale, 1992; Joshi and Nguyen, 1993) and in cultivated barley (Barua et al., 1993; Chalmers et al., 1993).

Recently, cDNA sequences are being used to develop markers, which are very useful since they are gene-based markers (Parchman et al., 2010). Moreover, the progress in sequencing and documentation in public database has facilitated sequence data mining and development of DNA markers without any difficulty (Bhattachryya et al., 2014).

Resistance gene analogs (RGAs) were frequently found to be in close genetic distance to known resistance loci, hence suggesting their possible role in disease resistance responses in plants (Fourmann et al., 2001). The gene sequences, which encode proteins containing a nucleotide-binding site (NBS) and C-terminal leucine-rich repeats (LRRs) constitute the largest class of R genes in flowering plants (Dangl and Jones, 2001).

Wild barley has been known to carry valuable sources of useful genes for barley breeding. For example, genes that are associated with resistance to diseases (Fetch et al., 2003), tolerance to abiotic stress (Ellis et al., 2000), other key agronomic traits (Vanhala and Stam, 2006), and quality traits (Shen et al., 2011).

The development of new barley lines, tolerant to abiotic and biotic stresses is an essential part of the continued improvement of the crop. Moreover, it can assist in the amelioration of other relative crops. Wild barley would be a valuable source of novel genetic variation for environmental stress tolerance. However, it depends on identifying of suitable genetic variation and the development of marker-assisted selection, which allows effective cultivar development (Ellis et al., 2000).

The aims of this study are to uncover the genetic variability in a barley germplasm collected from North Africa, compare peroxidase isozymes and RAPD diversity in the studied materials, and amplify disease resistance sequences from some Egyptian landraces, which could be used as molecular markers in assisted marker selection of the resistance lines of barley.

2 MATERIALS AND METHODS

2.1 Plant materials

Twenty–nine cultivars, genotypes and landraces of barley were obtained from National Institute of Agricultural Research Tunisia (INRAT), National Institute of Agricultural Research Algeria (INRAA), National Research Centre, Egypt (NRC), and Agricultural Genetic Engineering Research Institute, Egypt (AGERI). For resistance genes amplification, two Egyptian barley genotypes El Awamah & Awlad Ali were selected. The barley cultivar names, places and country of origin are listed in Table 1.

2.2 Isozyme analysis

To electrophoretically examine peroxidase (PER, E.C.1.11.1.7) isozymes, crude extraction of the twenty-nine genotypes from Algeria, Tunisia & Egypt was done using 0.1M Tris-HCl buffer in 4°C for two hours (Gottlieb, 1981). Then, the homogenates were centrifuged at 14.000 rpm for 20 minutes at 4°C using Centrion K3 centrifuge (Centrion, UK). Electrophoretic separation of the extracts was carried out in 10 % native PAGE (Laemmli, 1970). The peroxidase enzyme was stained as described by Soltis et al. (1983) as follows: gels were incubated in 100 ml staining solution containing 0.05 M acetate buffer (pH = 5.0) and 65 mg benzidine. Two ml of 0.1 M $CaCl_2$ were added as a catalyst. Finally, two ml of H_2O_2 were added as the substrate and the gels were kept in refrigerator until dark brown bands appeared. All isozyme bands were assessed according to their relative distances.

Table 1. Places, names and country of origin of the barley cultivars and landraces

Serial	Place of origin or cultivar name	Country of origin
1	Sidimehdi	Algeria
2	Temasine	Algeria
3	Kasrmegarine	Algeria
4	RasEllouche	Algeria
5	Saida	Algeria
6	Tichedielt	Algeria
7	Nailia	Algeria
8	Rihone-03	Algeria
9	Azrir	Algeria
10	Tozeurt	Tunisia
11	Tozeur2	Tunisia
12	Manel	Tunisia
13	SidiBeozid	Tunisia
14	Kibilliz	Tunisia
15	Tomban	Tunisia
16	Gabes	Tunisia
17	KairooaA	Tunisia
18	Rihan	Tunisia
19	Jerba	Tunisia
20	Arish (Sinai)	Egypt
21	Giza123	Egypt
22	Giza 126	Egypt
23	Giza 129	Egypt
24	Giza 125	Egypt
25	Giza 2000	Egypt
26	Giza 127	Egypt
27	Matrooh (Awama)	Egypt
28	Giza 131	Egypt
29	Giza 130	Egypt

2.3 DNA extraction and RAPD analysis

DNA isolation of the different barley cultivars, genotypes and landraces was performed using the CTAB method of Doyle and Doyle (1990). For RAPD-PCR analysis, 2 random 10-mer primers OPA3 and OPG3 were used. The random primers used in our study were ordered from SNEF medical, Germany. PCR reactions were conducted in a total volume of 20 µl reaction mix containing 2 µl of 10× reaction buffer, containing 2 mM $MgCl_2$, 2 µl of 0.2 mM dNTP, 0.1µl (0.5 U) of Taq DNA polymerase (Sigma, USA), 40 ng of genomic template DNA, and 10 pmol primer in a preheated thermocycler (Biometra, Germany) under the following conditions: 3 min at 95°C, followed by 44 cycles of 2 min at 92°C, 1 min at 37°C, and 2 min at 72°C. The reaction was finally incubated at 72°C for 10 min. For selecting the optimal conditions of the RAPD PCR, different optimization experiments were performed.

The PCR products were separated by electrophoresis on a 1 % agarose gels using 1× TAE buffer. Then, gels were stained with ethidium bromide (10 mg/ml) and visualized under UV light. A 100 bp DNA ladder (Axygen, USA) was used as a molecular DNA standard.

2.4 Resistance sequences and primers selection

Forty-six sequences showed resistance to different pathogens were retrieved from NCBI gene bank. These sequences were coded for different proteins included definsin (maize and wheat), superoxide dismutase (maize and wheat), catalase (barley and *Cynodon*). Six primer pairs were designed using Primer3 software. After primer selection with the Primer3 program, the complementarity of the primer pairs (primer dimmer and internal complementarity) was checked and the expected annealing temperatures were manually calculated. Table 2 contains the primer sequences, primers length and the expected PCR products in bp for the six primer pairs that were selected from resistance sequences, which were obtained from the NCBI gene bank.

Table 2: Oligonucleotide primers used for RT-PCR amplification

Primer name	Sequence	Primer length	Sequence/plant	Expected PCR fragments
AB089942P1 AB089942P2	GGTGTGAAGCGAGCAAGC CAGTGGCATCGTTATTACATCA	18 bp 22 bp	Definsin/wheat	522 bp
AF092524P1 AF092524P2	CTACGTCGCCCACTACAACAAG CCAACAGCGGGAAACTCAAG	22 bp 20 bp	SOD/wheat	565 bp
AJ849917P1 AJ849917P2	GGCCACAACGCTAGTACAATCTT CATGCGTATCACTCAATCTGCC	23 bp 22 bp	Definsin/*Zea*	433 bp
CV064086P1 CV064086P2	CGGCCATGGATCCCTACAAG CTCCTGCATGTTGGTCTTCGG	20 bp 21 bp	CatIso1/barley cDNA	509 bp
DN988165P1 DN988165P2	GTATCTTCATGTCATTGCTCGC CTCCGGCTGGTTCCTTTC	22 bp 18 bp	CatIso3/*Cynodon*	153 bp
X17564P1 X17564P2	AGGGCACCATCTTTTTCACC GCGACGCTCTTATTTCACGA	20 bp 20 bp	SOD/*Zea*	516 bp

The primers were ordered from Metabion International AG

2.5 RNA isolation

For RNA isolation from the two barley genotypes, leaves of the 7 day old seedlings of the barley genotypes were used. To avoid any contamination with RNase, all solutions were treated overnight with DEPC at a final concentration of 0.1 %. All non-disposable equipment, like glass and porcelain, was cleaned with 0.5 % SDS/0.5 M NaOH and rinsed with DEPC treated water and wiped with 70 % ethanol.

Total RNA was isolated using TRIzol reagent of the TriFast (PeqGOLD) extraction kit. In this method, 0.2 - 0.5 g of fresh leaves was ground to fine powder in liquid nitrogen. The powder was added directly to an Eppendorf tube containing 0.5 ml TRIzol reagent. The homogenate was incubated at room temperature (RT) for 5 min. After adding 0.2 ml chloroform, the tube was repeatedly inverted by hand for 15 sec. The mixture was incubated for 15 - 20 min at RT. Then, the upper aqueous phase was separated by centrifugation at 12000 rpm for 15 min at 4°C. The RNA was precipitated using 1 vol. isopropanol for 10 min at RT and afterwards by centrifugation at 12000 rpm for 10 min at 4°C. The pellet was washed with 70 % ethanol and resuspended in DEPC treated water (0.943 g/ml, BioBasic INC). To check the quality and quantity of the isolated RNA, RNA agarose gel electrophoresis was performed. Electrophoresis was carried out on 1.5 % agarose gel for 1h at 70 V.

2.6 RT-PCR analysis

RT-PCR was carried out using Ready-To-Go RT-PCR Beads kit (Amersham Biosciences). Each bead is optimized to allow the first-strand cDNA synthesis and PCR reactions to proceed sequentially (One-step Protocol for RT-PCR). In the One-step protocol, primers for the first-strand cDNA synthesis and PCR were added along with the template to an RT-PCR Bead.

First, for each reaction tube, 39 µl DEPC-treated water was added. The tube was taped to mix the water with the bead. To dissolve the bead, the tube was incubated on ice for 5 min. and the tube contents were gently pipetted up and down. After that, to each bead (contains 2 units of *Taq* DNA polymerase, 10 mM Tris-HCl, 60 mM KCl, 1.5 mM $MgCl_2$ 200 µM dNTPs, MuLV reverse transcriptase, RNAguard^{TM} Ribonuclease Inhibitor and stabilizer when brought to a final volume of 50 µl) 1 µl of the first-strand primer (0.5 µg), 5 µl of 10 pmol PCR primer pairs (resistance sequences based primers) and 5 µl of the template RNA were added.

For positive control reaction, 50 µl of DEPC-treated water were added to control mix bead and then the entire contents of the control tube were transferred into a tube containing a RT-PCR Bead. The reaction tubes were incubated at 42°C for 15-30 min in heat block or thermal cycler. Then, the reaction tubes were incubated at 95°C for 5 min in order to inactivate the reverse transcriptase. The PCR analysis was carried out in MiniCycler^{TM} (MJ Research) thermocycler for 30 cycles with a 1 min denaturation step at 94°C, 1 min annealing at 58°C and 1 min extension at 72°C. After RT-PCR amplification, the PCR products were resolved on 2 % agarose gel electrophoresis and the amplification profiles of the primer pairs were analyzed.

2.7 Data analysis

Peroxidase loci were labeled sequentially with those migrating closest to the anodal end designated as number 1, while alleles at each locus were labeled alphabetically from the most anodal band. A tree illustrating the genetic diversity among barley landraces and varieties, using unweighted pair-group method with arithmetic average (UPGMA), was generated based on peroxidase isozymes data using NTSYS-pc version 2.1 (Rohlf, 2000). The RAPD amplicons (bands) were recorded as 0 (absence) or 1 (presence) and were also analyzed by cluster analysis using the UPGMA method using the NTSYS-pc version 2.1. The goodness of fit of the cluster analysis, for both of isozymes and RAPD data, was determined by calculating the cophenetic value matrix from the tree matrix in order to carry out the Mantel test (Mantel, 1967) to determine the agreement between the two matrices.

3 RESULTS

3.1 PER isozyme analysis

Only three loci (PER 1, PER 2 and PER 3) were obtained from peroxidase enzyme analysis in the 29 accessions and varieties of barley. Out of these three loci, two loci (PER 2 and PER 3) were polymorphic in all accessions obtained from Algeria and Tunisia. All the three loci (PER 1 “one band”, PER 2 “2 bands” and PER 3 “one band”) were monomorphic in Egyptian varieties and landraces, where three accessions (Giza 129, Matrouh-Awama and Giza 131) showed one band with slower electrophoretic mobility. PER 1 and PER 3 were monomorphic with only one band shown in the Egyptian accessions and varieties. PER 3 displayed three polymorphic isozymes (bands) in the Algerian and Tunisian landraces. However, in case of PER 1 and PER 2, two polymorphic bands were observed in Algerian accessions. Nevertheless, in the Tunisian accessions, PER 1 (one band) was monomorphic and PER 2 (one band) was also monomorphic. Three Tunisian accessions (Tomban, Gabes and KairooaA) showed one unique allele in locus PER 2.

Based on the profiles of peroxidase isozymes, cluster analysis grouped all Egyptian barley varieties into one main cluster group at a distance of 1.5 (Fig. 1). However, only one landrace (Matrouh) was contained into a separate subgroup with two accessions (Manel and Tozeurt) from Tunisia and one accession (Sidimehdi) from Algeria. Similarly, Algerian and Tunisia accessions were grouped into one main at the same distance (1.5). Two accessions (Azrir: Algeria and Gabes: Tunisia) were found in one of the subclusters with two Egyptian barley varieties. A unique main cluster contained only one Algerian accession (Temasine), see Fig. 1. Cophenetic correlation based Mantel t-test was r = 0.800, which indicated a good fit to the dendrogram obtained with the cluster analysis.

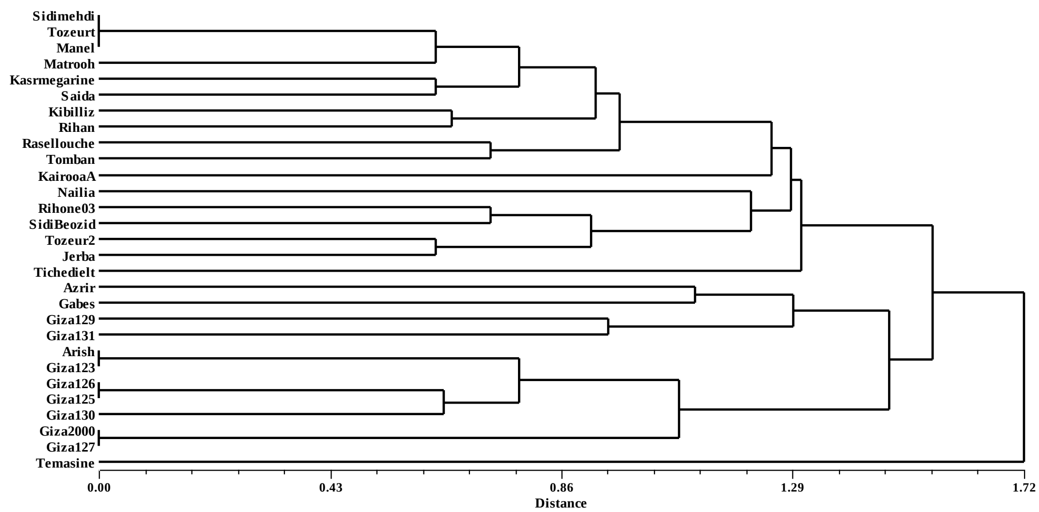

Figure 1: Cluster analysis of the isozymes-derived data of 29 barley accessions collected from three North African countries

3.2 RAPD analysis

RAPD analysis based on two random primers (OPA3 and OPG3) showed that about 60 % of amplified bands were polymorphic. The number of bands (alleles) ranged from 3 to 12 per primer, with an average of 6 per primer. Cluster analysis based on RAPD data revealed that the two accessions (Sidimehdi and Temasine) from Algeria were clearly separated at a distance of 2.47 as two identical genomes from all other barley accessions. The Egyptian accessions were grouped into two obvious groups; one contained 4 accessions (Giza126, Giza2000, Giza127 and Giza125) and the second contained two accessions (Giza123 and Giza129). However, only one accession (Giza130) was out of these two groups. Moreover, it was obvious that the accessions of each group were genetic invariable (showed high similarity). The Tunisian accessions showed no distinct clustering. The most high dissimilarities were revealed by the Algerian accessions (Fig. 2). Cophenetic correlation based on Mantel t-test, used to measure the goodness of fit of RAPD cluster analysis, was r = 0.977, which showed a very good fit to the generated dendrogram.

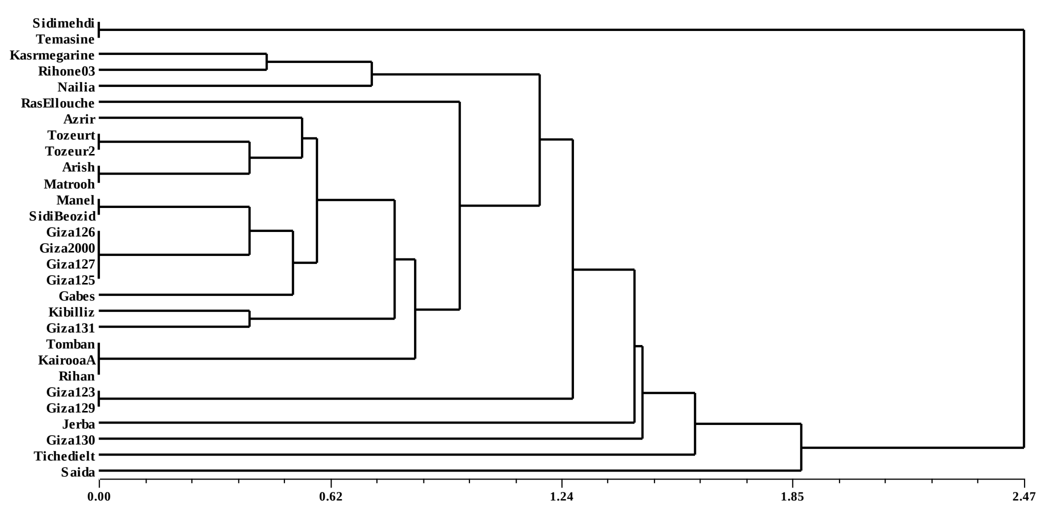

Figure 2: Cluster analysis of the RAPD-derived data of 29 barley accessions collected from three North African countries

3.3 RT-PCR and cDNA analysis

The total RNA was isolated with TriFast method, which ensured high quality total RNA in sufficient amount. RNA was checked for quality and quantity using agarose gel electrophoresis. To amplify the selected resistance gene-sequences from the Egyptian barley genotypes, which may represent candidate sequences for the resistance gene analogs of barley; comparative RT-PCR amplifications with each primer pair (Table 2) were performed. The total RNA isolated from two Egyptian barley genotypes (El-Awamah and Awlad-Ali) was used as template for RT-PCR analysis. The genomic DNA of the barley genotypes was also used to check and compare the amplification profiles when using RNA and DNA as templates. In case of DNA template, ordinary PCR analysis was performed.

Figure 3 showed that the primer combinations AB089942P1P2 and CV064086P1P2 amplified 3 weak amplicons in both barley genotypes (El-Awamah and Awlad-Ali). The primer pair AF092524P1P2 successfully amplified two PCR amplicons of approximately (340 & 220 bp) and (360 & 270 bp), respectively in both barley genotypes (Fig. 3). However, primer pair DN988165P1P2 gave only one amplicon in both barley genotypes (El-Awamah and Awlad-Ali) of 250 and 270 bp, respectively. The primer pairs AJ849917P1P2 and X17564P1P2 failed to amplify any fragments from both barley genotypes.

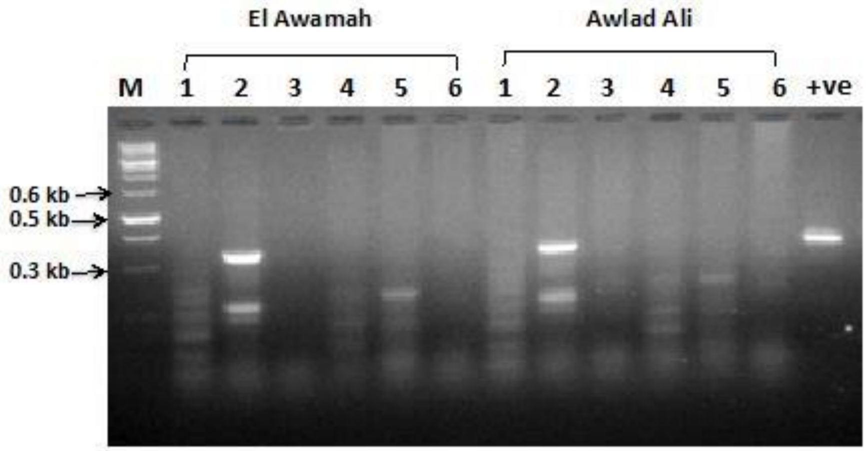

Figure 3: PCR amplification of specific DNA markers based on primers derived from resistance gene ortholog sequences, cDNA was used as template. +ve: positive control

Although, the primer combinations AB089942P1P2 and CV064086P1P2 have amplified three faint bands in the One-Step RT-PCR, they were successful to amplify only one specific PCR fragments when genomic barley DNA (El-Awamah) was used as template. The two PCR fragments were of approximately 230 bp and 200 bp, respectively (Fig. 4). Similarly, primer pair DN988165P1P2 amplified only one specific fragment of 700 bp. The X17564P1P2 primer pair failed to amplify any PCR product, while primer pairs AF092524P1P2 and AJ849917P1P2 amplified very faint fragment of 450 bp.

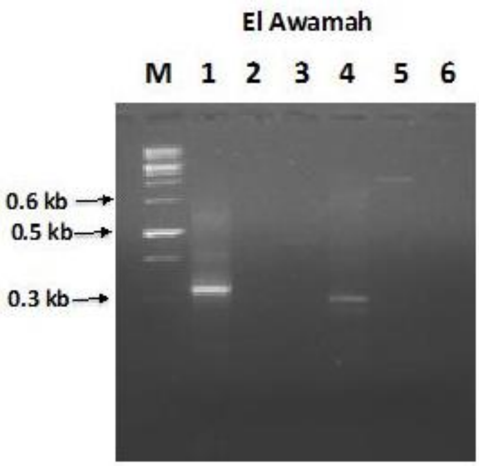

Figure 4: PCR amplification of specific DNA markers based on primers derived from resistance gene ortholog sequences, genomic DNA extracted from El-Awamah landrace was used as template

4 DISCUSSION

In this study, high genetic variability was observed among the barley landrace accessions and varieties collected from the three North African countries (Algeria, Egypt and Tunisia). However, relatively low genetic diversity was shown among the landrace accessions and varieties from the same region. In addition, development of molecular markers based on pathogen-resistance gene sequences was successful and two gene-based primer pairs amplified specific DNA fragments, which could be used in barley crop improvement.

The possible explanation of the low genetic diversity among varieties (collected from one country) is that they have a relatively narrow gene pool. Similar finding has been observed in the polish old oat cultivars, where low genetic variation of the old cultivars collection was noticed, which has been related to the entirely limited gene pool of such cultivars (Boczkowska et al., 2014).

Evaluation of the degree of genetic diversity within cultivated barley and its related wild germplasm is necessary for barley crop improvement and for the conservation of barley genetic resources (Boczkowska et al., 2014; Gepts, 2006). Therefore, estimation of genetic diversity of the North African barley landrace accessions and varieties were examined using isozymes and RAPD molecular markers.

RAPD markers provide a powerful tool for studying all aspects of genetic variability and genetic structure of the populations. Genetic data derived from isozymes are more robust this due to isozymes are codominant markers. However, isozyme analysis is restrained because fewer number of loci are generated. RAPDs are dominant markers. Therefore, there is less information per band. However, RAPD analysis has several advantages over other marker types: it has more loci that can be tested with RAPD, it is simple, has low cost, and it needs little amount of plant DNA (Garcia Mas et al., 2000).

It is well known that genetic variability of the wild populations is essential for plants to be adapted to environment (Nevo et al., 1997). Canadian durum wheat cultivars, which were analyzed by AFLP markers, showed that the cultivars that had been bred from landraces were characterized by higher genetic heterogeneity compared to those that were derived from commercial cultivars (Soleimani et al., 2002).

The current results indicated that it is possible to use specific primers based on the resistance gene sequences to amplify PCR products using cDNA or DNA as templates. Also, the amplified PCR products would help in identification of RGAs from barley. Wild barley and Middle Eastern landraces have already proven to be a very beneficial source of genes for modern crop improvement (Ellis et al., 2000). The most obvious example is the development of barley varieties that have *mlo* allele, which showed resistant to powdery mildew (Thomas et al., 1998).

Similar approach was used to develop gene-based markers, which were used to construct a dense linkage map in yellow fever mosquito *Aedes aegypti* (Linnaeus in Hasselquist, 1762). In this approach, cDNA sequences were downloaded from GenBank and primers were set to amplify PCR fragments of about 500 bp (Fulton et al., 2001). The identification of QTL and in special cases, gene cloning, are steps in the process of building a program for the genetic manipulation of abiotic stress tolerance (Ellis et al., 2000) without using transformation.

Fourmann et al. (2001) reported the development and mapping in *Brassica napus* L. of a series of resistance-gene analogs based on existing sequences of nucleotide-binding resistance genes. Some of the sequences could be amplified in *B. oleracea* L. and *B. rapa* L. and were employed as helpful markers, which were linked to disease resistance in the three major cultivated *Brassica* species.

Recently, barley genetic resources were mined for genes and alleles relevant for non-specific resistance (NR) to powdery mildew, which is caused by *Blumeria graminis* (DC.) Speer.f. *hordei* (*Bgh*). In that study, eleven candidate genes were identified, where they showed significant SNP or haplotype associations with the *Bgh*-phenotypes in a worldwide collection of spring barley (Spies et al., 2012).

Marker assisted selection (MAS) signifies that DNA markers, which are tightly-linked to target loci are exploited as a substitute for assist population phenotyping (Collard et al., 2005). In our study, some of the sequences amplified specific DNA fragments (Fig. 4), which could also be used for marker assisted selection in the breeding program of barley crop improvement.

Similarly, Giovanelli et al. (2002) developed gene-based markers, which were associated with cotyledon-stage downy mildew (*Hyaloperonospora parasitica* (Pers.) Constant.) resistance in broccoli. They proposed that such markers could be used for marker-assisted selection to generate downy mildew resistant varieties.

In conclusion, genetic diversity analysis revealed that relatively low genetic variability was found among the landraces and varieties from the same region. Using RT-PCR-based approach, it was successful to amplify gene-based DNA marker bands, which could be employed in the breeding of disease-resistant barley genotypes.

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Effects of organic fertilizers on growth and biochemical characteristics of Fenugreek

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ABSTRACT

Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb used as organic (green) manure and has medicinal applications. Organic fertilizers are used in sustainable agriculture of vegetables. Sources of organic manure and their effects on growth and yield characteristics of plants need to be determined. Effects of vermicompost and vermiwash were determined on qualitative and quantitative factors of chemical content, development and yield of fenugreek (*Trigonella foenum-gaecum* L.) from May to July 2012 at Agriculture College of University Guilan. A completely randomized block design with 3 replications was used. Treatments included 7 t/ha of cow manure, vermicompost, vermiwash (obtained from 7 t/ha vermicompost); 7 t/ha of leachate vermicompost + vermiwash and a control (no fertilization). Use of organic fertilizers beneficially affected plant height, pod length, pod fresh and dry mass, 1000-seed mass, plant fresh and dry mass, internode length and percents of leaves protein and nitrogen. Use of organic fertilizers may increase yield and yield components of fenugreek and its yield efficiency.

Key words: *Trigonella foenum-graecum* L.; yield components and their chemical composition; organic fertilizer; sustainable agriculture; vermicompost

IZVLEČEK

UČINKI ORGANSKIH GNOJIL NA RAST IN BIOKEMIJSKE LASTNOSTI SABLJASTEGA TRIPLATA

Sabljasti triplat (*Trigonella foenum-graecum* L.) je enoletno zelišče, ki se uporablja kot organsko (zeleno) gnojilo in je uporabno tudi v medicini. Organska gnojila se uporabljajo v trajnostni pridelavi zelenjave. Vire organskih gnojil in njihove učinke na rast in pridelek rastlin je potrebno še preučiti. Učinki komposta deževnikov (vermikomposta) in njihovih izcedkov (vermiwash) na kakovostne in količinske parametre kemijske sestave, razvoja in pridelka sabljastega triplata so bili preučevani od maja do julija 2012 na Agriculture College of University Guilan. Uporabljen je bil popoln naključni bločni poskus s tremi ponovitvami. Obravnavanja so obsegala: 7 t/ha kravjega gnoja, vermikompost, vermiwash (pridobljen iz 7 t/ha vermikomposta); 7 t/ha izcedka vermikomposta + vermiwash in kontrola (brez gnojenja). Uporaba organskih gnojil je ugodno vplivala na višino rastlin, dolžino strokov, suho maso, maso 1000 semen, svežo in suho maso rastlin, dolžino internodijev in na odstotek beljakovin in dušika v rastlinah. Uporaba organskih gnojil lahko poveča učinkovitost pridelka in njegovih komponent pri sabljastem triplatu.

Ključne besede: *Trigonella foenum-gaecum* L.; pridelek in njegova kemijska sestava; organska gnojila; trajnostno kmetijstvo; vermikompost

1 INTRODUCTION

Fenugreek (*Trigonella foenum-gaecum* L.) is an annual herb used as organic (green) manure and has medicinal applications (Kaviarasan et al., 2007; Bukhari et al. 2008; Haouala et al., 2008). Seed of fenugreek contains lysine and L-tryptophan, proteins, mucilaginous fiber and saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline (Bukhari et al., 2008). Polyphenol content in vegetables can be affected by environmental factors, cultivation, and conditions at, and after, harvest (Suthar, 2008). Organic fertilizers improve soil pH and cation exchange capacity (CEC) and increase micro-organism diversity

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and activity (Renato et al., 2003). Excessive applications of chemical fertilizers reduce plant performance due to soil acidification, reduced soil biological activities, degradation of soil physical features, and lack of micronutrients (Adediran et al., 2004). Use of compost materials results in improved soil fertility (Kasthuri et al., 2011). The garbage earthworm (*Eisenia fetida* (Savigny, 1826)) digests organic waste and converts it to materials beneficial for plant growth; the final product of its activity is called vermicompost (Gunadi et al., 2002). Materials produced by earthworms contain several nutritional substances easily available to plants (Suthar, 2008; Taylor et al., 2003). Vermicompost has high porosity, high potential of uptake and storage of mineral elements, which are gradually released. It also has high water holding capacity, and is used in sustainable agriculture of vegetables (Arancon et al., 2004a; Atiyeh et al., 2002). This research was conducted to evaluate effects of sources of organic manure on growth and yield characteristics of fenugreek to identify which is the best for natural antioxidants, total phenol and protein content in seed.

2 MATERIAL AND METHODS

For producing vermicompost, 25 g/kg or 2.5 kg/m^2 earthworm were added to the cow manure bed and vermicomposted for 2 months (Peyvast et al., 2008). For producing vermiwash, 50 l of water was added to 100 kg vermicompost and after 48 hrs extracts were purified by passage through a textile filter. The experiment was conducted at the University of Guilan Campus, Agriculture Faculty, Rasht, Iran (altitude 7 m below mean sea level, 37°16′ N, 51°3′ E), from May to July 2012. Annual average temperature is 15.9 °C. Thermal amplitude is 35.3 °C in summer and 6.6 °C in winter. Average rainfall is about 1359 mm per year with most occurring during late summer. The loam soil was plowed, disked and clods broken. Raised seedbeds, 0.3 m wide and 15 cm high, were prepared. Seed were planted in rows 15 cm apart at a depth of 0.5-1.0 cm on 23 April 2012. The bed surface was covered with a 1 cm of vermicompost or soil. Before spreading fertilizers, 3 mixed samples of soil were selected from 0 to 30 cm depth with 3 replications. Samples were dried in the air, and contents determined (Table 1). Furrow irrigation was used at 2 day intervals.

Table 1: Chemical and physical characteristics of soil (Greenhouse building at University of Guilan, 2012)

Soil texture	Clay %	Silt %	Sand %	Available K (mg/l soil extract)	Available P (mg/l soil extract)	Total nitrogen %	Organic carbon %	Total saturation acidity %	EC (exchange cation)×10^3
loam	19	30	51	219	65.6	0.168	1.08	7.44	1

A completely randomized block design with 3 replications was used. Plots were 4 m^2. Treatments included 7 t/ha of vermicompost; vermiwash (obtained from 7 t/ha vermicompost); vermicompost leachate + vermiwash from 7 t/ha vermicompost and a no fertilizer control (levels of nutrition in the soil sufficient for the crop which the recommended nutrition levels may not be correct and we are testing the additional fertilizer to determine if it provides a benefit). Vermicompost and leachate vermicompost were spread over beds and vermiwash applied 3 times at 7 day intervals. At flowering plant height, plant fresh and dry mass, pod fresh and dry mass, number of nodes, number of lateral branches, internode length, number of pods, pod length, number of seed per pod, total phenol, percent of antioxidant activity, fresh yield, dry yield, and contents of nitrogen and protein in leaves were determined. The Bradford protocol (Bradford, 1976) was used to determine protein amount in seed. The DPPH ability for scavenging free radicals was determined based on the method of Du et al. (2009) with minor modifications. Briefly, 50 µl of fenugreek extracts were added to 950 µl of a 6.25×10^{-5} M solution of DPPH in methanol. A control sample containing the same amount of solvent in place of extract was used for measuring maximum DPPH absorbance. The reaction took place in the dark for 30 min; absorbance at 517 nm was measured to determine the concentration of remaining DPPH. The percent DPPH, which was scavenged (% DPPHsc), was calculated using:

% DPPHsc = Acont - Asamp/Acont × 100

where Acont is absorbance of the control, and Asamp is absorbance of the sample. Total phenols were determined spectro-photometricaly using Folin-Ciocalteau colorimetric method with modifications as described by Singleton et al. (1999). Data were subjected to ANOVA in SAS (ver. 9.1, SAS Institute, Inc., Cary, NC) and means separated using the Tukey test.

3 RESULTS AND DISSCUSSION

The organic fertilizers did not affect numbers of pods/plant, number of seed/pod, number of nodes, number of lateral branches, antioxidant capacity, total phenols, and dry yield (Tables 2 - 4). Fertilizer affected plant height, pod length, pod fresh mass, 1000-seed mass, plant fresh mass and internode length; there were no differences between vermicompost types (Tables 2 - 4). In no fertilizer control the soil nutrient content is sufficient for some, but not all, benefits to the crop. However the lowest value was obtained from the unfertilized control of many traits. Organic fertilizers gave the lowest 1000 seed mass, plant fresh mass, plant dry mass, protein and nitrogen content of seed were obtained from control plants; fertilization decreased pod dry mass (Table 2 - 4). Plants treated with vermicompost were the tallest, as reported by Yadav et al. (2003), Arguello et al. (2006) and Almulla et al. (2012) in other crops. Organic fertilizer enhanced plant height what could be due to the plant hormone auxin (Muscolo et al. 1999). Organic fertilizer can affect soil properties and suppress plant diseases and improve plant health. Pod length was increased by vermicompost treatment and pod length may be related to seed yield via indirect effects of vermicompost on seed yield via pod length. The highest pod fresh mass was due to the vermicompost treatment. The 1000-seed mass were different between vermicompost and vermivash treatments. Plants treated with vermicompost had the highest total yield/ha (Table 4). It may be that chemical and physical properties of humic acid in vermicompost increase uptake of hormones and nutrients resulting in improved growth and yield (Arancon et al., 2005), increased soil microorganism activity (Arancon et al., 2004b), improved nitrogen accumulation, and increased total yield. Plants treated with vermiwash + vermicompost had the longest internodes. Renato (2003) reported that organic fertilizers in the soil can increase element uptake. Organic manure increases soil aggregation, aeration, water holding capacity, and supply roots with an extended source of nutrients (Rani and Nishana, 2012). Vermiwash increased fresh and dry mass; it may be that organic fertilizers increased water absorption through distribution and development of roots. Mucus deposit of epidermal cells and coelomic fluid produced by worms contain plant hormones and chemical exudates (Rani and Nishana, 2012). Antioxidant capacity was not affected by organic fertilizer which contrasts to those of Haghighi (2011) using sewage sludge on leafy vegetable growth. High antioxidant capacity was recorded in the vermicompost treatment. Haghighi (2011) indicated that a deficit of nutrient availability and uptake in the control induced antioxidant activity. Pant et al. (2009) claimed that low plant growth and N concentration caused high level of antioxidant activity in leafy vegetables. Amounts of phenolic compounds in plants grown under organic conditions are higher than those grown under inorganic conditions (Asami et al., 2003; Dixon, 2001) which contrast with our results. Higher total phenolic compounds can cause the decrease in environmental stress to plants (Asami et al., 2003). Higher levels of total phenolic content was found in plants treated with vermicompost compared with those grown with Osmocote (a type of trade fertilizer) and this was attributed to a gradual release of available nutrients in plants from vermicompost (Asami et al., 2003; Pant et al., 2009; Wang and Lin, 2002). Protein and nitrogen contents were affected by fertilizers as also reported by Kasthuri et al. (2011) who found that Municipal Solid Waste Compost affected protein content of fenugreek. The protein and nitrogen contents increased with vermiwash + vermicompost leachate treatment. It may be that soil microorganism activity under high manure (Arancon et al., 2004b) results in nitrogen accumulation in plants due to increases in protein and organic manure affected soil amylase, invertase activity and cellulose and increased microbial biomass (Kasthuri et al., 2011). Vermicompost (Peyvast et al., 2008) and vermiwash (Suthar, 2010) were proposed as organic fertilizers but there is no information on effects of the combined application of vermiwash and vermicompost on vegetables. The main problem that can arise from excessive application of vermicompost is toxicity due to high salt content. With leaching, negative effects related to high salinity decreased and continuous application of this material may be possible. Vermiwash and leachate vermicompost can be used as fertilizers for cultivation of organic fenugreek. Application of organic fertilizers may help alleviate salinity and sodium problems that develop as a result of excessive chemical fertilizers and irrigation (Almulla et al., 2012). Using sustainable and environmentally friendly organic materials can increase fertility without negative effects on human health and the environment. Organic fertilizers effects especially vermicompost, vermiwash and vermicompost leachate were continued during next year and their beneficially effect should be examined.

Table 2: Mean comparison of some measured characteristics in fenugreek (Greenhouse building at University of Guilan, 2012)

Treatment	Means					
	Plant height (cm)	Number of pods per plant	Plant fresh mass (g)	Pod length (cm)	Pod fresh mass (g)	Pod dry mass (g)
ermicompost	51a	73.33a[a]	11.67a[a]	13.2a	8.41a	1.02b
Vermiwash	48a	68.67a	11a	12.81a	7.57a	1.35ab
Vermiwash+vermicompost	45.67a	74a	11.33a	12.53a	7.74a	1.15ab
Control	35.67b	64.33a	10.33a	10.36b	5.78b	1.45a

[a] values in columns followed by the same letter are not significantly different, $P<0.05$, Tukey test.

Table 3: Mean comparison of some measured characteristics in fenugreek (Greenhouse building at University of Guilan, 2012)

Treatment		Means				
	1000-seed mass (g)	Number of nodes	Number of lateral branches	Plant fresh mass (g)	Plant dry mass (g)	Internode length (cm)
Vermicompost	1.49a	9.33a[a]	9.33a[a]	49.36b	23.69ab	6.18a
Vermiwash	1.49a	9a	9.33a	57.12a	26.04a	5.89a
Vermiwash+vermicompost	1.28b	8.33a	9.33a	52.27ab	22.57ab	6.22a
Control	0.78c	8.33a	9.67a	43.86c	19.44b	4.24b

[a] values in columns followed by the same letter are not significantly different, $P<0.05$, Tukey test.

Table 4: Mean comparison of some measured characteristics in fenugreek (Greenhouse building at university of guilan, 2012)

Treatment	Means					
	Total yield (g)	Dry yield (g)	Total phenol (mg galic acid /100 g)	Antioxidant capacity (% of inhibition)	Seed protein (%)	Nitrogen in seed (%)
Vermicompost	2453.9a[a]	298.43a	4.9a[a]	39.02a	24.35ab	4.60ab
Vermiwash	2076.9ab	370.35a	4.66a	38.31a	23.83ab	4.50ab
Vermiwash+vermicompost	2280.6ab	331.76a	4.58a	38.22a	24.65a	4.65a
Control	1490.4b	372.13a	4.34a	37.23a	20.83b	3.93b

[a] values in columns followed by the same letter are not significantly different, $P<0.05$, Tukey test.

4 CONCLUSIONS

According to the data the addition of the fertilizer provided improvement to some measured variables but a cost:benefit analysis will have to be done to determine if the cost of the fertilizer justifies its use. Further study is needed to confirm these results under field conditions. Use of organic fertilizers beneficially affected plant height, pod length, pod fresh and dry mass, 1000-seed mass, plant fresh and dry mass, internode length and percents of leaves protein and nitrogen. Use of organic fertilizers may increase yield and yield components of fenugreek and its yield efficiency.

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Assessment of morphological variability and chemical composition of some local pepper (*Capsicum annuum* L.) populations on the area of Kosovo

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ABSTRACT

Seven local pepper populations (*Capsicum annuum* L.) from different geographical regions of Kosovo, were evaluated for morphological traits, chemical composition, and antioxidant contents using standard analytical techniques. All local peppers populations were characterized for different morphological traits from seedling emergence to crop maturity. The total genetic variation for plant height (PH) was 11.72 cm or expressed in relative values was 27.94 %. Average of leaf area (LA) per plant was 2308.38 cm^2, while the lowest value for LA was 1136 cm^2. Yield per plant ranged from 265 to 691 g $plant^{-1}$. The acidity level was ranging from 1.44 to 1.61 %, carbohydrates varied greatly from 4.21 to 6.07 %. Vitamin C (as ascorbic acid) content in fresh fruit ranged from 65.544 to 520.51 mg $100g^{-1}$ of fresh mass. Minerals were of reasonable levels with Fe (15.31 mg kg^{-1}), Ca (216.71 mg kg^{-1}), Na (406.01 mg kg^{-1}), K (1851 mg kg^{-1}), and Zn (5.74 mg kg^{-1}).

Key words: *Capsicum annuum* L.; genetic diversity; antioxidant content; mineral composition

IZVLEČEK

OVREDNOTENJE MORFOLOŠKE SPREMENLJIVOSTI IN KEMIČNE SESTAVE NEKATERIH POPULACIJ PAPRIKE (*Capsicum annuum* L.) NA KOSOVU

S standardnimi analitskimi tehnikami so bile ovrednotene morfološke lastnosti, kemijska sestava in vsebnost antioksidantov sedmih lokalnih populacij paprike (*Capsicum annuum* L.) iz različnih geografskih območij Kosova. Za vse populacije so bile ovrednotene različne morfološke lastnosti od vznika do zrelosti plodov. Celokupna genetska spremenljivost za višino rastlin (PH) je bila 11.72 cm, izražena v relativnih vrednostih je bila 27.94 %. Povprečna listna površina na rastlino (LA) je bila 2308.38 cm^2, najmanjša 1136 cm^2. Pridelek na rastlino je bil med 265 in 691 g na rastlino. Vsebnost kislin je bila med 1.44 in 1.61 %, ogljikovih hidratov med 4.21 in 6.07 %. Vsebnost vitamina C v svežih plodovih je bila med 65.544 in 520.51 mg $100g^{-1}$ sveže mase. Vsebnosti mineralov so bile v običajnih območjih in sicer Fe, 15.31 mg kg^{-1}, Ca, 216.71 mg kg^{-1}, Na, 406.01 mg kg^{-1}, K, 1851 mg kg^{-1}, in Zn, 5.74 mg kg^{-1}.

Ključne besede: *Capsicum annuum* L.; genetska raznolikost; vsebnost antioksidantov; mineralna sestava

1 INTRODUCTION

In the world, several hundred types of peppers are cultivated. Chili pepper (*Capsicum* spp.) is a solaneceous plant, whose centre of origin in Middle America and Mexico is centre of genetic diversity and domestication (Pickersgill, 1971). Csillery (2006) indicates that the first component description of *Capsicum* was given in Hungarian herbal by Dioszegi and Fazekas (1807) cited by Bozokalfa et al., (2009). Pepper (*Capsicum annuum* L.) is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent source of natural colours and antioxidant compounds (Conforti et al., 2007; Deepa et al., 2007). Peppers are one of the main vegetables planted in Kosovo regarding production area and economic importance. The dominate type of pepper production is open field cultivation. Also, the local pepper genotypes in Kosova are rich in diversity with different populations where it has been cultivated for centuries and which is very useable for human food (Aliu et al., 2012; Kaciu et al., 2010). Almost indispensable food, salads, condiment of every Kosovar cuisine is pepper. In Kosovo from total area planted with vegetables, 4449 ha or expressed in

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percentage is 30.68 % are planted with pepper (MAFRD, 2014). Most pepper cultivars currently grown in Kosovo are open pollinated. Some local populations are still grown on many small farms due to consumer demand. For decades, these have been cultivated in different environments and growing techniques. Almost all the cultivars grown are of landraces types which are characterized by a wide range of observable variability. Since their introduction into the world, peppers are cultivated in various environments and a number of different populations were developed (Govindarajan, 1986). In general, they are genetically diverse and well adapted to the locations where they have been developed (Votava et al., 2005). Estimating genetic diversity and determining the relationships between germplasm collections helps ensure germplasm is efficiently collected and managed (Bozokalfa et al., 2009). Data on the level of genetic diversity of a germplasm collection may also increase the efficiency of efforts to improve this crop (Geleta et al., 2005). Pepper fruits are also source of vitamins A, complex B1 and B2, C and minerals such as dietary calcium, iron, magnesium etc. (Bosland,1992). A number of studies report that hot pepper seeds are rich in minerals content (Zou et al, 2015; Jarret et al., 2013; Park et al., 2006). The content of vitamin C in the pepper fruit is higher than in *Citrus* (Finger et al., 2010). The pepper fruit is a rich source of vitamin A, E, C and P in green chilli (Hosmani, 1993; Howard et al., 2000; Marin et al., 2004). They have a high level of vitamins C and E as well as the total of antioxidants is completed by phenolic compounds, which occur in peppers in connection with sugars (Materska et al., 2003; Shotorbani and Jamei, 2013). Antioxidants are beneficial because of their protective roles against multiple diseases such as cancer, anemia, diabetes and cardiovascular diseases. The compounds perform their function by counteracting the oxidizing effects on lipids by scavenging highly reactive oxygen free radicals, the major oxidizing factors for the oxidative modification of low density lipoprotein and nucleic acids (Perucka and Małgorzata, 2007). However, there is little information available about nutritional constituents of hot pepper seeds grown in Kosova. Therefore, the specific objective of this study was to evaluate morphological traits, and antioxidant content in local pepper populations.

2 MATERIALS AND METHODS

2.1 Plant material and collection of samples

Local pepper populations were collected from different bio-climatic regions throughout Kosovo. Kosovo has a central geographic position on the Balkan Peninsula. It lies between latitude 41°50'58 "and 43°51'42" north and 20°01'30" and longitude 21°48'02" east. Seven local peppers populations (LPP) were collected from various agro-climatic regions. The localities of the sampling sites for the Kosova region were: Krusha, Shtime, Lipjan, Viti, Mitrovica, Istog and Dečani. The altitudes of the sampling sites ranged from 306 to 649 m above sea level. The overall climate of Kosovo is a modified continental type, with some elements of a sub-mediterranean climate in the extreme south. Summers are hot with extreme temperatures of up to 37°C. The average annual rainfall is 720 mm (HMIK, 2008). These were selected to represent various geographical areas. (Figure 1).

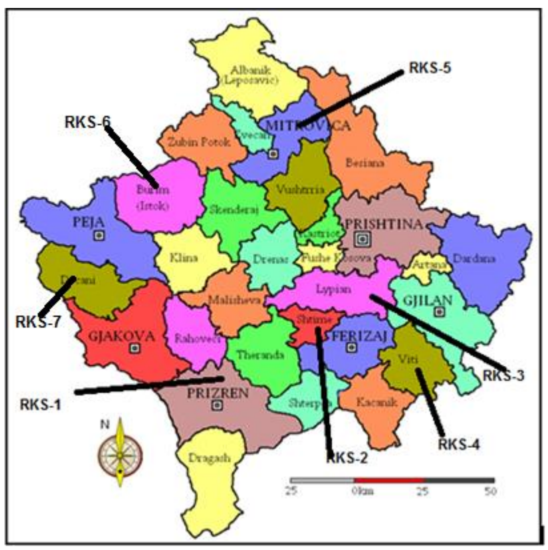

Figure 1: Geographical areas of local pepper populations

A total of seven genotypes within 7 geographical regions were studied under different field conditions over summer 2014. Plant material used in this research was coded as; RKS-A, RKS-B, RKS-C, RKS-D, RKS-E, RKS-F and RKS-G.

Table 1: Pepper populations (*Capsicum* spp.*)* analyzed within their geographical origin

Code	Longitude	Latitude	Elevation	Geographical origin	Local name
RKS-A	020º39´19"	42º18'29"	310	Krusha	Babure
RKS-B	021º32'23"	42º27'27"	577	Shtime	Somborka
RKS-C	021º10'43"	42º29'04"	564	Lipjan	Somborka
RKS-D	021º24'02"	42º21'47"	494	Viti	Dolma
RKS-E	021º54'10"	42º51'47"	521	Mitrovica	Somborka
RKS-F	21°04'05"	42°36'39"	479	Istog	Somborka
RKS-G	20°17'48"	42°31'42"	649	Decan	Somborka

All accessions were characterized for different agro-morphological traits from seedling emergence to crop maturity. All characteristics were measured in the field and laboratory at the normal harvest time. The methodology used to record qualitative values from seedling to harvest was obtained from the descriptor for *Capsicum* from International Plant Genetic Resources Institute (IPGRI 1995). The experimental design was a split plot with randomized complete block with three replications. Plants from local pepper populations were collected in farmer's fields during the period when full maturity of the plants were reached. Local paper populations were collected from different bio-climatic regions throughout Kosovo. To investigate the different traits which were including in our study we collected per location 30 plants x 7 locality = 210 plants (for each location = 3 repetitions x 10 plants/repetition = 30 plants/populations).The following characteristics were measured in Plant breeding laboratory, Faculty of Agriculture, Department of Crop science: plant height (PH), fruit diameter (FD), plant mass without fruits and root (PM), root mass (RM), number of flowers (FN), leaf number (LN), number of fruits (NF), fruit mass (FM), and yield per plant (YP). Leaf area (LA) per plant was measured by planimeter.

*Ascorbic acid extraction***:** The vitamin 'C' as ascorbic acid content (AAC) in fresh pepper fruits was determined by the titratability of 5 g of the blended pulp homogenized with 50 ml of oxalic acid at a concentration of 12 %. The titratable solution consisted of 2.6 sodium indophenol dichlorophenol. Results were expressed in mg of ascorbic acid per 100 g of pulp.

pH: was measured by using a digital pH meter with the application of the electrode directly in to the blended pulp.

The carbohydrates were determined by equipment of refractometer type R 200 (Reichert technologies, USA) precision placing a small sample of blended pulp on the reading prism. Results were expressed as percentage (%).

Water content: 5 g of fruit were taken from each replication, cut into pieces dried in a temperature 105^{o}C in equipment type AD-MF50 until constant mass (90 minutes). Results were expressed in percentage.

Mineral composition: Mineral elements including Fe, Ca, Zn, Mg, Na, K and Cu in (mg kg^{1}) were determined through burning and mineralizing of samples of fruit at 550 ^{o}C for 4 to 6 hours. Samples were digested in HCl and subsequently element concentrations were estimated using an atomic absorption spectrophotometer (1100 B Perkin-Elmer, Germany).

Statistical analyses: all statistical analyses were performed with the SPSS software (version 15.0, SPSS Inc., 2006) to investigate the difference between the populations. Effects of the studied traits were evaluated by ANOVA. Mean separation within columns was done by Duncan's Multiple Range test. In order to assess the differentiation of local pepper populations (LPP's) based on all variables that were measured, the Canonical Discriminant Analyses (CDA) were applied. CDA is a technique for classifying set of observations into predefined classes.

3 RESULTS AND DISCUSSION

The collection of local pepper populations investigated from Kosovo showed considerable variation in morphological and biometric fruit parameters. Plant height (PH) varied from 32.16 to 46.89 cm. The differences between them are 14.73 cm or expressed in percentage is 35.12 %. Results are presented in Table2. According to the analysis of variance (ANOVA) the differences for fruit diameter (FD) between populations were significant. The genetic variation for FD among them was with maximum of 4.73 cm and a minimum of 3.41 cm or differences among populations was 1.32 cm or 31.42 %. Similar range of FD in different pepper genotypes (2.74 to 4.57 cm) was reported by Bozokalfa et al. (2009) and Bassiony et al. (2010) (5.75 till 6.97 cm). The genetic variability for root mass (RM) ranges from 149.30 g plant^{-1} to 100.31 g plant^{-1} which was significant at level of probability of LSD (p = 0.01). Differences for these two populations were 48.99 g plant^{-1} or expressed in relative value was 47.79 %. The significantly higher value for number of flowers (NF) was characterized for population RKS-A with 73.55 flowers per plant, while lower number of flowers was recorded at RKS-C and RKS-E (38.01 flowers per plant). The differences between them were 35.54 flowers per plant or 70.45 %. The leaf number (LN) per plant also showed significant differences among populations. This trait segregated in a manner similar to plant yield. The variability varied from 217.01 to 125.88 leaves per plant, and had significant difference of 91.13 per plant or 52.45 %. Obtained results were in accordance with those obtained by Bassiony et al. (2010), obtaining LN from 158.5 to 191.67 leaves per plant. The leaf area is a one of the crucial factors in photosynthesis. The population RKS-B produced a higher (2308 cm^2 plant^{-1}) leaf area per plant (LA), while lower value was determined for population RKS-E, namely 1136 cm^2 plant^{-1}. The differences between two populations were 1171.56 cm^2 plant^{-1} or expressed in percentage 64.92 %. The results concerning LA are given in Table 2. Also other characters varied substantially. The number of fruits (NF) across pepper populations ranged from 9.44 to 19.66. In our case this trait had an effect an on yield per plant. Plant high yields depend on many factors, the most important factors are the structure of genotypes and environments. Production capacity is a complex character, a result of few morphological components, number of flowers, fruit size, and fruit mass (Madosa et al., 2008). The highest significant contribution (691.44 g) in production (yield) per plant was determined at population RKS-D, followed by RKS -A, a value of 540.66 g plant^{-1}. The major difference among populations is 425.96 g plant^{-1} or 91.34 % respectively. Results are presented in Table 2.

Table 2: Morphological and agronomic traits recorded in different pepper populations

Populations	PH (cm)	FD (mm)	RM g plant^{-1}	PM g plant^{-1}	FN plant^{-1}	LN plant^{-1}	LA cm^{2} plant^{1}	NF plant-1	FM g plant^{-1}	YP g plant^{-1}
RKS-A	46.89±0.16^{a}	4.42±0.005^{a}	138.21±0.005^{a}	116.23±0.015^{a}	73.55±0.015^{a}	162.33±0.025^{bc}	1392±0.473^{b}	15.11±0.055^{ab}	35.71±0.015^{ab}	540.66±0.050^{b}
RKS-B	42.44±0.10^{a}	3.96±0.010^{b}	113.65±0.020^{b}	86.71±0.010^{b}	57.44±1.73^{b}	217.01±0.026^{a}	2308.±0.451^{a}	10.66±0.020^{cd}	38.01±0.040^{a}	402.21±0.412^{c}
RKS-C	46.55±0.02^{a}	4.47±0.010^{a}	135.36±0.010^{b}	111.76±0.020^{a}	38.01±0.040^{c}	178.33±0.015^{ab}	2175±0.666^{a}	13.33±0.015^{bc}	32.83±0.055^{ab}	431.91±0.196^{bc}
RKS-D	41.66±0.05^{a}	4.73±0.18^{a}	149.30±0.005^{a}	120.14±0.011^{a}	45.220.043^{bc}	185.11±0.066^{ab}	2009±0.100^{a}	19.66±0.020^{a}	35.09±0.035^{ab}	691.44±0.294^{a}
RKS-E	32.16±0.02^{b}	3.41±0.005^{b}	100.31±0.015^{b}	77.73±0.010^{b}	38.01±0.040^{c}	125.88±0.043^{c}	1136±0.252^{b}	9.44±0.015^{d}	27.68±0.11^{b}	265.48±0.247^{d}
RKS-F	33.26±0.02^{b}	3.89±0.010^{b}	101.21±0.010^{b}	79.85±0.017^{b}	37.21±0.015^{c}	120.25±0.164^{c}	1212±0.100^{b}	9.78±0.035^{d}	25.21±0.09^{b}	425.96±0.230^{bc}
RKS-G	40.25±0.025^{a}	4.41±0.010^{a}	115.25±0.011^{b}	78.56±0.020^{b}	44.52±0.010^{bc}	124.78±0.066^{c}	1354±0.950^{bc}	13.24±0.025^{bc}	31.25±0.020^{ab}	425.96±0.225^{bc}
Mean	40.45±0.025	4.2±0.005	121.89±0.010	95.854±0.017	47.708±0.016	159.09±0.035	1655.54±0.0.493	13.031±0.037	32.25±0.010	454.8±0.133
LSDp=0.05	8.58	1.03	20.86	12.23	10.86	85.05	1239.04	3.37	9.32	132.79
0.01	12.49	1.50	30.36	17.79	15.81	90.34	1802.69	4.90	13.56	193.20

* Values are given as means of three replicates ± SD. Means with different superscript letters within a column are significantly different ($P < 0.05$).
Notes: PH - plant height; FD - fruit diameter; RM-root mass; PMW - plant mass ; FN - flower number; LN - leaf number; LA - leaf area; NF- number of fruits; FMW- fruit mass; YP - yield per plant

The acidity level is from 1.44 to 1.61 % at populations RKS-E and RKS-B, respectively (Table3). Apart from this, these acids make up the energetic reserves and the metabolic reactions that involve the synthesis of pigments, enzymes and other materials and degradation of pectins and celluloses, which are essential in different processes (Antoniali et al., 2007).

Table 3: Average values for some fruit parameters of local pepper populations

Population	Acidity mg 100 g^{-1}	pH values	Carbohydrates %	*AAC content mg 100 g^{-1}	Water content %
RKS-A	1.52±0.005^{b}	5.03±0.037^{b}	4.21±0.010^{c}	65.12±0.011^{a}	91.33±0.147^{b}
RKS-B	1.61±0.006^{a}	5.12±0.010^{b}	6.07±0.068^{a}	58.54±0.045^{d}	90.56±0.168^{b}
RKS-C	1.52±0.010^{b}	5.27±0.020^{a}	4.21±0.015^{c}	62.21±0.121^{b}	93.26±0.0921^{a}
RKS-D	1.53±0.010^{b}	5.24±0.020^{a}	6.14±0.015^{a}	52.51±0.010^{e}	89.71±0.134^{b}
RKS-E	1.44±0.005^{bc}	5.07±0.041^{b}	5.59±0.016^{b}	60.91±0.071^{c}	90.76±0.157^{b}
RKS-F	1.54±0.005^{b}	5.02±0.046^{b}	5.51±0.032^{b}	59.54±0.623^{d}	87.85±0.735^{b}
RKS-G	1.51±0.004^{b}	5.11±0.456^{b}	4.38±0.274^{c}	57.65±0.231^{d}	92.35±0.257^{b}
Average	1.52±0.057	5.15±0.015	5.24±0.041	59.86±0.219	91.12±0.166
LSD *p* = 0.05	0.13	0.11	0.39	0.17	1.33

* AAC- ascorbic acid content. Values are given as means of three replicates ± SD. Means with different superscript letters within a column are significantly different ($P < 0.05$).

The content of carbohydrates varied greatly within and among LPP’s, with values ranging from 4.21 to 6.07 %. The difference among populations for dry fruit mass is 1.86 % or expressed in percentage values was 35.49% higher. AAC is the least complex vitamin found in plants and is synthesized from glucose or other carbohydrates (Kays, 1991). Vitamin C (as ascorbic acid) content in fresh fruit ranged from 65.544 to 520.51 mg 100 g^{-1} of fresh mass. The difference among pepper populations for AAC was 12.61 mg 100 g^{-1} or genetic variation was 21.06 % (Table 3). It was found, that hot pepper cultivars were richer in vitamin C, than the sweet ones. As other studies have shown, the highest or the lowest values of vitamin C in *C. annuum* are dependent on the variety and the maturity stage of the fruits (Khadi et al., 1987; Howard et al., 2000). For example, in the *C. annuum* pepper grown in Turkey, a variation that ranged from 15.2 to 64.9 mg 100 g^{-1} fresh fruit was reported (Topuz and Ozdemir, 2007). And another study conducted in India with the same species showed a variation that ranged from 48.23 to 192.63 mg 100 g^{-1} as reported by Deepa et al. (2006). One of the factors affecting the production of plant biomass is the concentration of mineral elements. The differences among local pepper populations (LPP) are presented in Table 4.

According to the results the highest average values of zinc (Zn) (7.86 mg kg^{-1}) and copper (Cu) (13.9 mg kg^{-1}) was determined LPP coded for population RKS-A. While with the greatest accumulation of iron (Fe) was recorded for RKS-C population with the average value of 23.16 mg kg^{-1}. In our study the other elements including; Ca, Na, K and Mg were on the higher significance results. Results are given in |Table 4.

Table 4: The average mineral content (mg kg^{-1}) in pepper fruits

Populations	Zn[1]	Cu[1]	Fe[1]	Ca[1]	Na[1]	K[1]	Mg[1]
RKS-A	7.86±0.043[a]	13.9±0.17[a]	14.33±0.13[b]	236.76±0.59[ab]	225.76±0.10[d]	5337.06±4.1[a]	280.3±0.63[b]
RKS-B	7.06±0.072[ab]	0.82±0.21[e]	14.66±0.13[b]	216.13±0.40[b]	238.86±0.24[c]	2360.83±2.81[ab]	271.9±0.35[bc]
RKS-C	4.93±0.077[bc]	3.26±0.025[b]	23.16±0.05[a]	230.2±0.95[ab]	982.13±0.14[a]	572.16±1.15[e]	323.6±0.28[a]
RKS-D	2.93±0.066[c]	1.04±0.141[d]	13.33±0.10[c]	319.01±0.69[a]	670.66±0.55[b]	898.16±0.35[d]	322.53±0.085[a]
RKS-E	5.63±0.0921[b]	1.31±0.005[c]	13.01±0.095[c]	83.46±0.13[c]	247.2±0.17[c]	1998.76±0.27[c]	176.01 ±0.41[c]
RKS-F	4.75±0.0945[bc]	3.11±0.015[b]	13.78±0.141[c]	210.11±0.011[b]	228.78±0.66[cd]	895.63±0.78[d]	275.56±0.092[bc]
RKS-G	7.02±0.0461[ab]	0.98±0.016[e]	14.89±0.21[b]	221.3±0.10[b]	248.7±0.53[c]	901.23±0.51[cd]	285.23±0.15[b]
Mean	5.74±0.034	3.490.055±	15.31±0.061	216.71±0.11	406.01±0.45	1851.98±1.24	276.45±1.03

* Values are given as means of three replicates ± SD. Means with different superscript letters within a column are significantly different ($P < 0.05$).

[1]Elemental nutrient composition of local pepper populations (mg kg^{-1})

The canonical discriminant analysis (CDA) of the traits is presented in Figure 2. The first canonical functions described 93.4 % and a second canonical function is 5.6 % of the existing variance. The CDA analysis reported here differentiates LPP's on the basis of similarity. The first group is consisted by RKS-A and RKS-B, second group is consisted by RKS-C and RKS-G. The other populations are separated as individuals, RKS-D, RKS-E and RKS-F. The main elements that can affect the classification to the different groups were the genotype and environment which had influenced on the content of mineral composition and quality traits. The genotype and environmental interaction effects tend to be large when there is a wide variation among genotypes for different traits (Kanf, 1998).

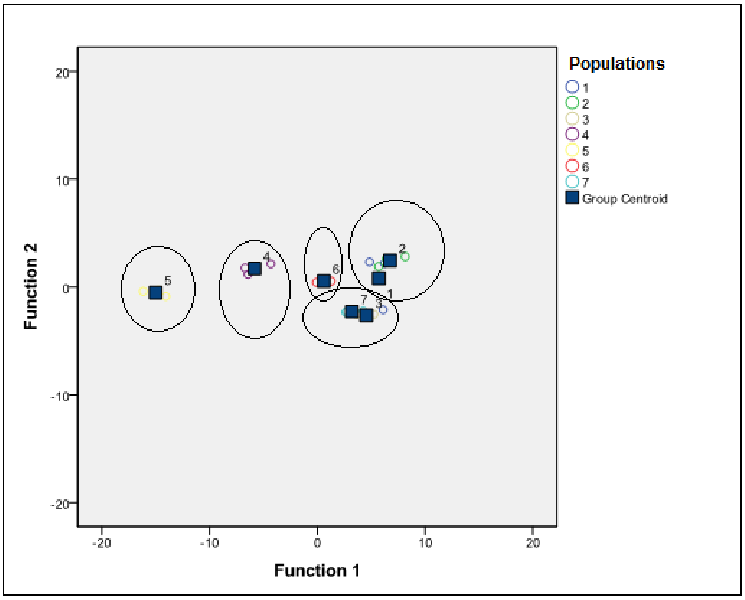

Figure 2: Canonical discriminant function at local pepper populations

4 CONCLUSIONS

The study showed that there was a significant morphological variability among local pepper populations. Some populations are interesting for quantitative traits including leaf area and yield per plant. A high variability was also determined for vitamin C, carbohydrates and mineral composition. The cluster analysis shows that there is an obvious diversity among the populations collected in different geographical regions. The observed morphological diversity among pepper populations is helpful for breeding programs aimed in selecting superior genotypes. These local pepper populations should be included in broader genetic analyses and should be considered as a source of new genetic variability used for the development of inbred lines in the Kosovo breeding program. The evaluation of plant genetic resources has been considered of prime importance, especially in those species having economic importance.

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Changes in essential oil and morpho-physiological traits of tarragon (*Artemisia dracuncalus* L.) in responses to arbuscular mycorrhizal fungus, AMF (*Glomus intraradices* N.C. Schenck & G.S. Sm.) inoculation under salinity

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ABSTRACT

This study aimed to evaluate the arbuscular mycorrhizal fungi (AMF) (*Glomus intraradices* N.C. Schenck & G.S. Sm.) inoculation and salinity effect on qualitative and quantitative changes in tarragon yield. Treatments included inoculation, and non-inoculation of AMF, and five salinity levels of irrigation water (with the electrical conductivity of 0, 2, 4, 6, and 8 dS m^{-1}). The results showed the plant height, SPAD value, number of leaves, dry mass of leaves and shoot per plant were reduced under salinity condition. The various levels of salinity decreased the content of tarragon essential oil and some its components consist of *α*-pinene, limonene, *Z*-ocimene, *E*-ocimene, and methyl chavicol while, it increased the content of bornyl acetate, eugenol, methyl eugenol, caryophyllene, germacrene, and *α*-farnesene. AMF inoculation without salinity had the greatest positive effect on the evaluated traits of tarragon. Also, it improved the morpho-physiological traits under salinity due to alleviation of the harmful effects of salinity. Although the essential oil content was reduced with the AMF inoculation, the methyl chavicol amount was increased by the AMF inoculation under salinity condition.

Key words: arbuscular mycorrhizal fungi; *Artemisia dracunculus* L.; electrical conductivity; essential oils; *Glomus intraradices*; methyl chavicol; morpho-physiological traits; salinity

IZVLEČEK

SPREMEMBE V VSEBNOSTI ETERIČNIH OLJ IN MORFOLOŠKO-FIZIOLOŠKIH LASTNOSTI PEHTRANA (*Artemisia dracuncalus* L.) KOT ODZIV NA INOKULACIJO Z ARBUSKULARNO MIKORIZNO GLIVO (*Glomus intraradices* N.C. Schenck & G.S. Sm.) V RAZMERAH SLANOSTI

Namen te raziskave je bil oceniti vpliv inokulacije z arbuskularno mikorizno glivo (*Glomus intraradices* N.C. Schenck & G.S. Sm.) in učinek slanosti na spremembe v kakovosti in količini pridelka pehtrana. Obravnavanja so obsegala inokulacijo in brez inokulacije z AMG ter pet stopenj slanosti vode za namakanje (z električno prevodnostjo 0, 2, 4, 6, in 8 dS m^{-1}). Rezultati so pokazali, da so se vrednosti znakov kot so višina rastlin, SPAD vrednost, število listov, suha masa listov in število poganjkov na rastlino zmajšale v razmerah slanosti. Različne slanosti so zmanjšale vsebnosti eteričnih olj , med njimi *α*-pinena, limonena, *Z*-ocimena, *E*-ocimena in metil kavikola medtem, ko so se vsebnosti bornil acetata, eugenola, metil eugenola, kariofilena, germakrena in *α*-farnezena povečale. Inokulacija z AMG v razmerah brez slanosti je imela največje pozitivne učinke na vse ovrednotene lastnosti pehtrana. Izboljšala je tudi morfološke in fiziološke lastnosti v razmerah slanosti. Čeprav je bila vsebnost steričnih olj zmanjšana ob inokulaciji z AMG, se je vsebnost metil kavikola povečala v razmerah slanosti.

Ključne besede: arbuskularne mikorizne glive; *Artemisia dracunculus* L.; električna prevodnost; eterična olja; *Glomus intraradices*; metil kavikol; morfološko-fiziološke lastnosti; slanost

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1 INTRODUCTION

Tarragon (*Artemisia dracunculus* L.) is an herbaceous, perennial plant with alternate leaves of linear shape and light green color (Fernandez-Lizarazo et al., 2011). It is native to Russia and Siberia, alluvial valley areas, but today is spread also in the western areas of North America. However, tarragon is widespread also in parts of temperate Asia, as well as Central Asia and Eastern Europe (Fernandez-Lizarazo et al., 2011; Obolskiy et al., 2011). The tarragon leaves contain about 0.3 % essential oil of which methyl chavicol comprise approximately 70 % of its component. (Chopra et al., 1986; Verma et al., 2010). This plant possesses a wide range of health benefits, therefore, it widely used in traditional medicine & pharmaceutical industry. It's health benefits that can be pointed include appetizing, stomach tonic, diuretic, anti-scurvy, mild anti-worm and induce of menstruation. Tarragon is used in traditional medicine for the resolution of joint pain, hiccups and treatment of painful menstruation. Despite contradictory statements, it was recently reported the tarragon extract is anticancer agent for some cancer cells line such as human leukemic cancer cells, human endometrial cancer cells, human breast cancer cells, etc. (Obolskiy et al., 2011).

Saline soil and saline irrigation are one of the most important environmental stresses, particularly in arid and semi-arid regions (Greenway and Munns, 1980; Kuznetsov and Shevyakova, 1997). Salt stress during vegetative and reproductive stage reduces crops biomass and yield (Aslam et al., 1993). Salinity affects plant growth and metabolism through disturbing several physiological processes of plants (Levitt, 1980; Hoshida et al., 2000). The first effect of salinity on plants is reducing water uptake even when the soil is completely wet, due to the decreased soil solution potential. Even more, cell membrane function may be damaged due to intracellular ion homeostasis disruption in plants exposed to salinity. Under these circumstances, some physiological disorders occur and programmed cell death imposed ultimately (Fan et al., 2013). Moreover, salt stress in plants leads to nutrients imbalance, reduction of the photosynthesis efficiency, increasing free radicals production such as superoxide anion ($O_2.^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^.$) and singlet oxygen ($^1O_2$), and metabolic toxicity which causes the destruction of the cell membrane (Greenway and Munns, 1980; Fan, et al., 2013; Sankar, et al., 2007; Agarwal and Shaheen: 2007).

Plants responses to salinity stress are complex thus, salinity tolerance mechanisms are not clear yet. During the evolution and adaptation to salinity stress, various molecular mechanisms have been developed in plants for confronting the salinity. Some plants mechanisms cope with salinity by regulating ion homeostasis with accumulation of compatible solutes for osmotic adjustment, free radical scavenging, alterations in membrane structures, and phytohormone biosynthesis stimulation. It was also reported that the ion homeostasis was regulated via selective accumulation of ions, ion exclusion as well as limited ions uptake by root, ion transport to the leaves and that distribution at cellular and plant level (Fan et al., 2013; Hasegawa et al., 2000; Jaleel et al., 2007).

Some microorganisms such as plant growth promoting rhizobacteria and fungi, especially arbuscular mycorrhizal fungi (AMF), can improve the plant growth and crop yield in saline soils via alleviating destructive effects of salinity stress (Cho et al., 2006). Arbuscular mycorrhizal fungi inhabit the rhizosphere and have a symbiotic association with the roots of most terrestrial plant species (Gini et al., 2003; Smith and Read 1997). The most common AMF in saline soil are species from the genus *Glomus* (Ho, 1987; Wang et al., 2004). Molecular studies revealed that about 80 % of fungi spores in saline soil belong to a single species, *Glomus geosporum* (T.H. Nicolson & Gerd.) C. Walker (Wilde et al., 2009). Wu et al. (2010) reported that root inoculation of *Citrus tangerina* Tanaka by *G. geosporum* and *Paraglomus occultum* (C. Walker) J.B. Morton & D. Redecker could improve growth, photosynthesis rate, root architecture, and ionic balance under 100 mM NaCl. Kapoor et al. (2002) reported AMF diminished the adverse effects of salinity and thereby increased coriander (*Coriandrum sativum* L.) growth. However, the root inoculation of sour orange (*C. aurantium* L.) and sweet orange (*C. sinensis* (L.) Osbeck) by *G. intraradices* under the 30, and 60 mM salt had no effect on salinity tolerance, but the Cl^- concentration increased in root (Zou et al., 2013).

AMF can significantly improve resistance of host plants to varied biotic and abiotic stresses. Arbuscular mycorrhiza leads to better nutrients mobility in nutrient poor soils (Marschner and Dell, 1994). Thus, the growth of plants coexisted with AMF is enhanced due to the increased nutrients uptake, especially N and P (Marschner and Dell, 1994). However, the role of AMF in stress conditions and salinity may not be limited only to this nutritional effect (Ruiz-Lozano and Azcon, 2000). The tolerance mechanisms to salinity in plant symbiosis with AMF is consisted with improved osmotic potential adjustment, enhanced water use efficiency, production of plant growth hormones, reduced detrimental effect of oxidative stress, mitigation of toxic ion effects or improved nutritional status (Auge, 2001). According to reports on the alleviating detrimental salinity effects via the AMF root inoculation, this study was aimed for evaluation the qualitative and quantitative changes of tarragon yield under AMF (*Glomus intraradices*) inoculation and salinity condition.

2 MATERIALS AND METHODS

2.1 Plant preparation and growth conditions

This study was conducted in an experimental greenhouse of the Medicinal Plants Institute (MPI) affiliated with the Academic Center for Education, Culture and Research (ACECR) in Karaj as a factorial experiment in the randomized complete block design (RCBD) with 4 replications during 2015. The research station is located at 1472 m a. s. l., 35˚ 54′ N and 50˚ 53′ E. The same rooted transplants of tarragon (*Artemisia dracunculus* L. var. *sativa*) were provided from MPI seed bank (1096-MPISB), and were transferred into pots. Plants were grown in a plastic greenhouse for 3 months (from March 21 to June 20, 2015) with a photon flux density about 1300 µmol m^{-2} s^{-1}, 16 h light and 8 h dark period and the average temperature of 21/16 °C for day/night. The soil texture was loam-silt, its physio-chemical properties contains 0.08 % nitrogen, 36.2 ppm phosphorus, 49.8 ppm potassium, 7.9 pH, and electrical conductivity (EC) 1.2 dS m^{-1}.

2.2 Treatments

Treatments included inoculation and non-inoculation with AMF (*Glomus intraradices* N.C. Schenck & G.S. Sm.) as the first factor, and five salinity levels of irrigation water (with the EC of 0, 2, 4, 6, and 8 dS m^{-1}) as the second factor. The leaching fraction equal 0.5 was used in irrigation practices in order to less salt accumulation. The electrical conductivity of the solutions was measured by EC meter (HI9811, Hanna, USA) and the salinity levels were kept constant throughout the experiment period for irrigation water. Same amount of irrigation water were applied for each treatment during the growing period. The salinity treatments began 30 days after transplanting via irrigation water with an interval every two days until the harvest time. The treatment solutions were made with saline water and distilled water depending on target salinity, while the control treatment of salinity was prepared with double distilled water. Natural saline water was obtained from Hoz-e-Soltan Lake in Qom, Iran. The major ions of the saline water were: 128 g l^{-1} Na^+, 218.7 g l^{-1} Cl^-, 1.23 g l^{-1} K^+, 19.5 g l^{-1} Mg^{2+}, 0.086 g l^{-1} Ca^{2+}, and 48.8 g l^{-1} SO_4^{2-}.

For this experiment, 40 uniform plastic pots (20 cm upper diameter × 15 cm bottom diameter × 18 cm height) as experimental plots were divided into subgroups with or without AMF inoculation. Inocula consisted of soil possessing fungal spores, hyphae and mycelium. According to the method of Tommerup (1992), fungus identification was checked using light microscope (AXIO Imager; Carl Zeiss, Jena, Germany). AMF inoculum was multiplied in the open pots culture of sweet corn (*Zea mays* L. convar. *saccharata* var. *rugosa* Bonaf.) as a host and after six months of plant growth, the shoots were eliminated and the underground parts were stored for two months in polyethylene bags at 5 °C. Thirty grams of the AMF inoculum (root fragments with 85 % of colonized roots length) was added to 3.0 kg of autoclaved (121°C, 0.11 MPa, 1 h) soil for AMF inoculation (Carretero et al., 2008). Non-mycorrhizal treatments received the same amount of autoclaved AMF inoculum.

2.3 Essential oils analysis

The harvested plant materials of tarragon were air-dried in a shaded place at a convenient temperature (24 ± 2 °C) during 6 days. Essential oils of the aerial parts were extracted by hydro-distillation method for 3 h using Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulfate and kept on 4 °C until further analysis (British Pharmacopoeia, 1988). The extracted essential oils were identified by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS) analysis. GC/MS analysis was carried out on an Agilent instrument coupled with a Agilent 5973N Mass system equipped with flame ionization detector (Hewlett-Packard Company, USA) and a SGE BPX5 capillary column (30 m × 0.25 mm; 0.25 µm film thicknesses, Kinesis Ltd., UK). Temperature program included: an oven temperature held for 5 minutes at 50 ºC and enhanced to 240 ºC with 3 ºC per min rate. Then, enhancement of temperature was programmed up to 300 ºC with 15 ºC per min rate and this temperature was held for 3 minutes. Other operating conditions include: carrier gas was He with a flow rate of 0.5 ml min^{-1}; injector and detector temperatures were 290 ºC, and split ratio, 1:25. Mass spectra were taken at 70 eV (Socaci et al., 2008). The components of the essential oils were identified by comparison of their mass spectra and retention indices with those published in the literature and presented in the MS computer library (Adams, 2001).

2.4 Measurements and statistical analysis

The sampling was conducted in the onset of flowering stage as all plants were harvested 93 days after transplanting. For future accuracy and to reduce errors, samples were picked in four replicates randomly from the separate experimental plot. The analyzed morpho-physiological traits were plant height, the number of leaves, SPAD value, leaf dry mass, shoot dry mass. The SPAD values were recorded using a SPAD-502 meter (Konica-Minolta, Japan). All the data were subjected to statistical analysis (one-way ANOVA) using SAS software (Ver. 9.2). The difference between treatments means was compared by Duncan's multiple range test at 5 % confidence interval.

3 RESULTS AND DISCUSSION

Results showed that the AMF inoculation had a significant effect on the plant height ($p \leq 0.05$), number of leaves, SPAD value, and leaf and shoot dry mass ($p \leq 0.01$). Also, analysis of variance showed that different levels of salinity had a significant ($p \leq 0.01$) effect on these traits. Between the salt stress and AMF inoculation was observed a significant interaction in the number of leaves ($p \leq 0.05$), leaf and shoot dry mass ($p \leq 0.01$) (Table 1).

Table 1: Analysis of variance for the effects of arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation and salinity on morpho-physiological traits of tarragon

S.O.V.	Df.	Mean Square				
		Plant height	Number of leaves	Leaf dry mass	Shoot dry mass	SPAD value
Replication	3	8.99 ^{ns}	91.6 ^{ns}	0.005 *	0.005 *	9.76 ^{ns}
AMF inoculation	1	190.14 *	11262.4 **	0.22 **	0.51 **	106.27 **
Salinity (S)	4	109.9 **	1828.4 **	0.03 **	0.075 **	48.92 **
AMF × (S)	4	47.68 ^{ns}	154.4 *	0.026 **	0.139 **	16.69 ^{ns}
Error	27	26.07	51.37	0.0016	0.0078	7.22
CV (%)		8.84	6.47	7.22	10.02	7.34

ns: non-significant differences; *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$.

The plant height and SPAD value were increased for 7.8, and 9.3 percentage by AMF inoculation, respectively (Figure 1), but their amount were decreased under salinity condition. In comparison with the control treatment, the plant height and SPAD values were significantly reduced with increasing salinity to 4 and 6 dS m^{-1}, what indicated that the leaf chlorophyll was more susceptible to raise of salinity (Figure 2, 3). These results also confirm the finding of other studies (Bernstein et al., 2010; Dolataadian et al., 2011; Amira and Qados, 2011; Mukhtar balal et al., 2011). Reduced osmotic potential in salinity condition is resulted in arrest of cell division and elongation (Jacoby, 1994). In addition, Na^+ and Cl^- accumulation have toxic effects on the cell division and photosynthetic system, a reason for reduced plants growth. Also, salinity stress reduced biosynthesis and transport of cytokinin and gibberellin, but ABA biosynthesis was increased. These factors are contributing to the reduction of plant height under salt stress compared to control (Jacoby, 1994). Destruction of chloroplasts, chlorophyll photo-oxidation and prevented chlorophyll biosynthesis are the main reasons for the decline in content of photosynthetic pigments under salinity conditions (Sultan, 2005). It has also been reported that reduced chlorophyll amount is a consequence of increased chlorophyllase activity under salt stress (Reddy and Vora, 2005). Glutamate is a precursor for proline and chlorophyll biosynthesis. Thus, increased proline production in salt stress decreases glutamate availability in the chlorophyll biosynthesis (Drazkiewicz, 2000).

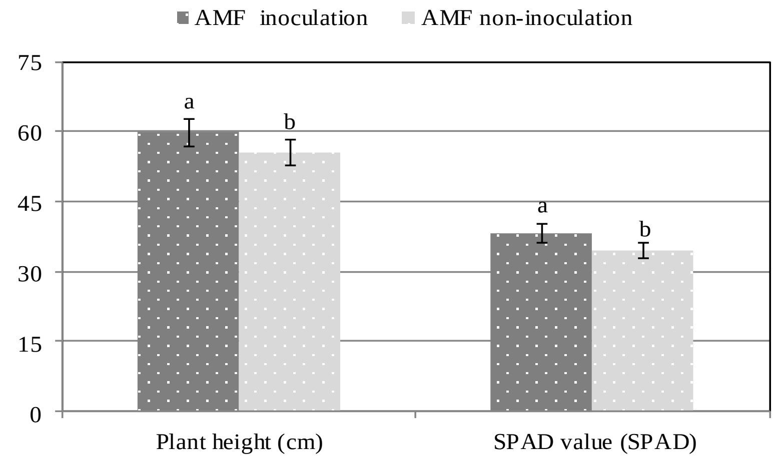

Figure 1: Effect of arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation on plant height (cm), and SPAD value (SPAD). The vertical bars represent standard errors of the means

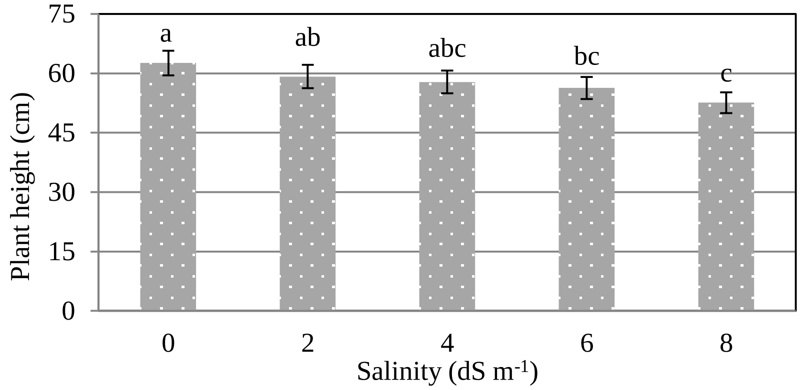

Figure 2: Effect of irrigation water salinity on plant height (cm). The vertical bars represent standard errors of the means

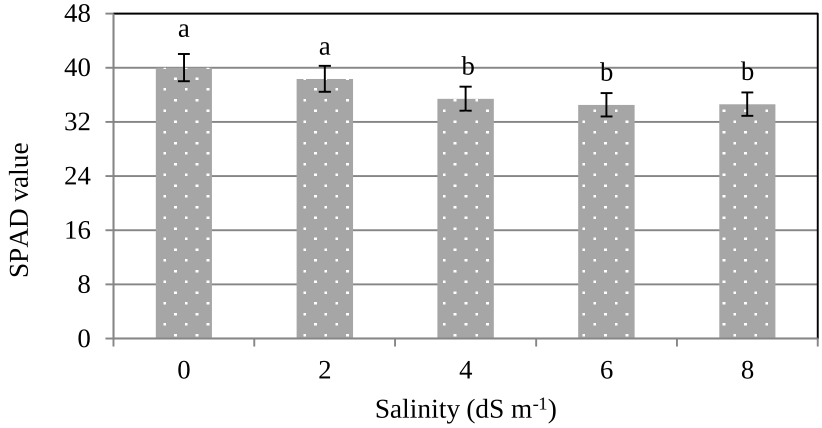

Figure 3: Effect of irrigation water salinity on SPAD value (SPAD). The vertical bars represent standard errors of the means

The number of tarragon leaves was increased by AMF inoculation. However, the number of leaves was reduced through increasing salt concentration under either inoculation or non-inoculation of AMF. The highest number of leaves (with an average of 146.28) was observed in AMF inoculation without the salinity. However, the lowest of it (67.91) was obtained at the 6 dS m^{-1} salinity without using the AMF (Figure 4). These findings confirm the results of other studies (Bernstein et al., 2010; Amira and Qados, 2011; Mukhtar balal et al., 2011). During salinity stress, plant leaf area was reduced due to smaller leaves formation and leaf abscission. Thus, the photosynthetic capacity diminished and the supply of assimilates for optimal growth was reduced. In addition, the rapid leaves senescence under salt stress caused the reduction of the leaf area durability (Munns, 1993).

Use of the AMF could ameliorate the tarragon leaf and shoot dry mass so that AMF inoculation in non-saline condition produced the greatest leaf dry mass (0.76 g) and its minimum (0.39 g) was observed in the 8 dS m^{-1} salinity without AMF inoculation (Figure 5). Similar to leaf dry mass, the highest shoot dry mass was obtained when the AMF inoculation was used in no-salinity stress (1.33 g) conditions and it's the least amounts was gained in the 8 dS m^{-1} salinity without AMF inoculation (0.62 g). Also, leaf and shoot dry mass were increased with increasing salinity to 4 dS m^{-1} withour AMF inoculation. In contrast, increasing salinity to more than 4 dS m^{-1} led to reduced leaf dry mass compared to the control (Figure 6). These findings are consistent with other studies (Kapoor et al., 2002; Ben Khaled et al., 2003; Rabie and Almadini, 2005; Gupta and Rutaray, 2005; Saleh and Al-Garni, 2006; Porras-Soriano et al., 2009).

Plant dry mass reduction in saline conditions is a response to spent metabolic energy for coping with the salt stress (Parida and Das, 2005). Main factors that influenced the plant dry mass consisted of reduced leaf area, increased chlorophyll destruction, reduced photosynthesis rate, toxic effects of Na^+ and Cl^- accumulation, decreased water uptake, and imbalance in nutrients (Sankar et al., 2007; Agarwal and Shaheen, 2007; Verma and Mishra, 2005). However, some authors reported that phosphorous nutrition can reduce the detrimental effects of salinity stress on plant growth. Therefore, AMF with increased phosphorous uptake can ameliorate for the harmful effects of salinity stress. On the other hand, the potassium content was increased in AMF inoculated plants. Thus, it protected host plant against adverse effect to sodium through enhanced potassium to sodium ratio (Marschner and Dell, 1994; Ruiz-Lozano and Azcon, 2000; Jeffries et al., 2003). Also, it was reported that use of AMF in lettuce is the reason for the roots extension. In addition, the photosynthesis rate and water use efficiency were improved, while the evapotranspiration was reduced (Ruiz- Lozano et al., 1996).

The tarragon essential oil content in this study was linearly reduced by the increased salinity. The AMF inoculation in various salinity levels reduced the essential oil content more than when AMF was not used, especially under the 8 dS m^{-1} salinity. Therefore, the greatest content (1.15 %) of essential oil was obtained in the AMF inoculation under no salinity condition, while its minimum (0.2 %) was acquired in the AMF inoculation along with the 8 dS m^{-1} salinity (Figure 7).

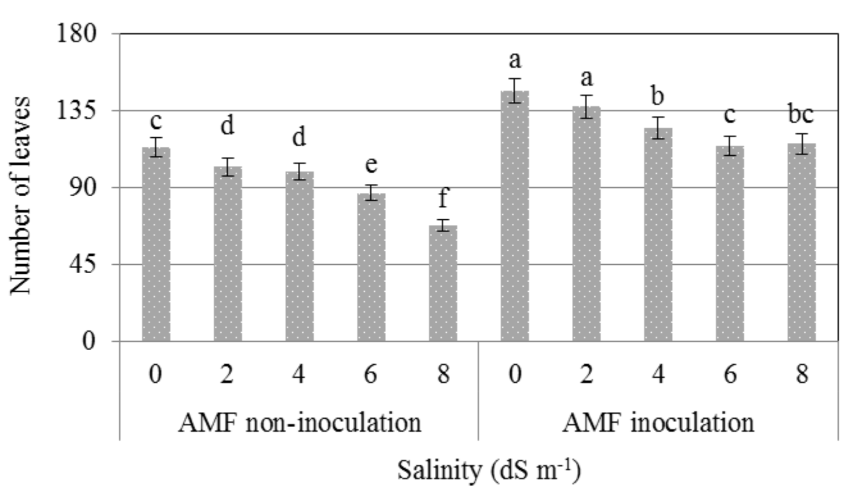

Figure 4: Effect of irrigation water salinity and arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation on number of leaves. The vertical bars represent standard errors of the means

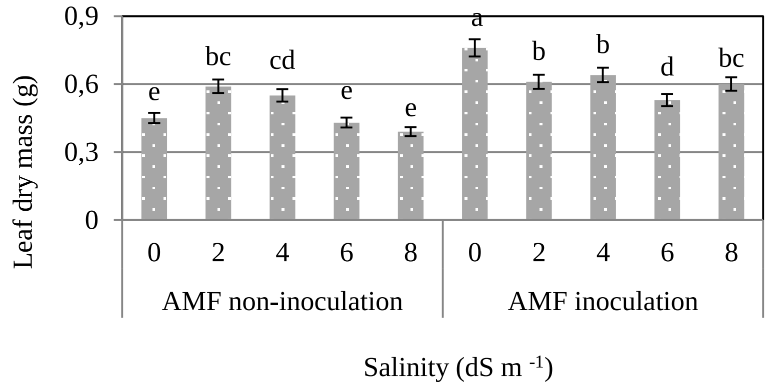

Figure 5: Effect of irrigation water salinity and arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation on leaf dry mass (g). The vertical bars represent standard errors of the means

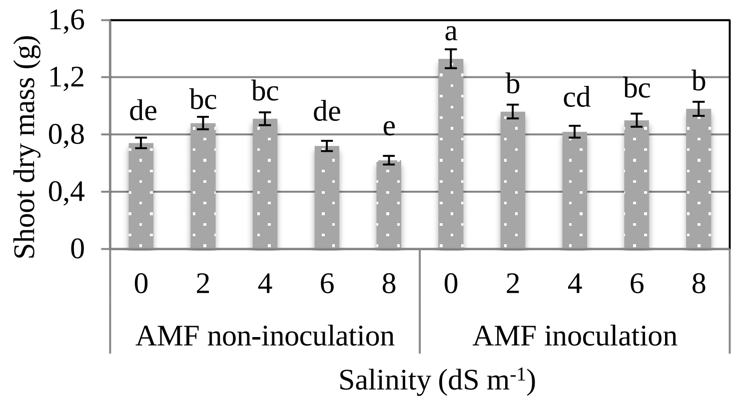

Figure 6: Effect of irrigation water salinity and AMF inoculation on shoot dry mass (g). The vertical bars represent standard errors of the means

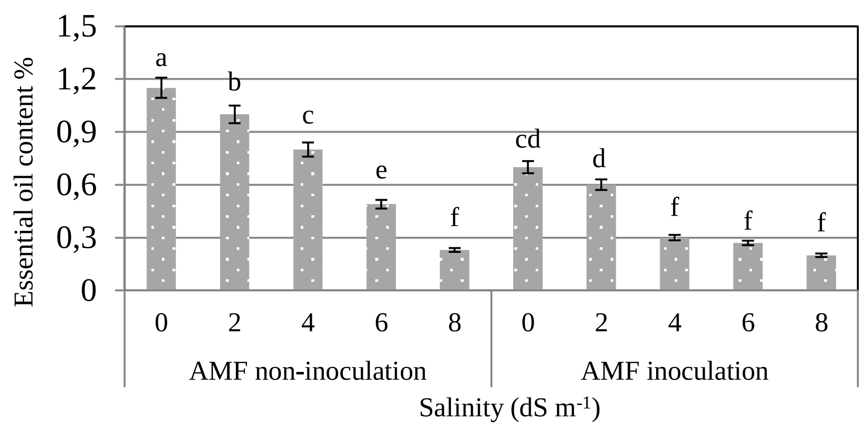

Figure 7: Effect of irrigation water salinity and arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation on essential oil content (%). The vertical bars represent standard errors of the means

Results showed that the effect of salinity on essential oil components was also dependent on the salt concentration. The content of *α*-pinene, limonene, *Z* and *E*-ocimene were increased at 2 and 4 dS m^{-1} salinity, but their content decreased when the salt concentration increased to over 4 dS m^{-1} compared to the control treatment. The amounts of these compounds were increased by AMF inoculation in non-salinity stress, but using AMF in 2 and 4 dS m^{-1} salinity reduced their content. Use of AMF had no significant difference with AMF non-inoculation in 6 and 8 dS m^{-1} salinity. The highest content of *α*-pinene (1.03 %), limonene (3.22 %), *Z* and *E*-ocimene (6.35 and 7.31 %) were obtained under 2 dS m^{-1} salinity without AMF inoculation and the lowest amount of these compounds were obtained in the 4 dS m^{-1} salinity with AMF application (0.1, 0.47, 0.91, and 0.77 %, respectively) (Table 3).

The content of methyl chavicol had no significant difference with the control up to 6 dS m^{-1} salinity, but it was significantly reduced at the 8 dS m^{-1} salinity. AMF application in control and under 8 dS m^{-1} salinity increased methyl chavicol content. However, in the other grades of salinity there was not a significant difference between AMF inoculation and non-inoculation. The maximum and minimum methyl chavicol content were gained in AMF inoculation and control treatment (88.8 %), and non-application of AMF under 8 dS m^{-1} salinity (64.3 %) (Table 3).

The amount of bornyl acetate was increased under salinity with AMF inoculation. Its highest content (0.73 %) was observed in the AMF inoculation with the 8 dS m^{-1} salinity and its minimum content (0.08 %) was obtained in AMF non-inoculation with control treatment of salinity. The content of eugenol, methyl eugenol, caryophyllene, germacrene, and *α*-farnesene was increased with increasing the level of salinity. Thus, their maximum content (0.9, 4.24, 1.33, 2.54, and 2.64 %, respectively) was observed under the 8 dS m^{-1} salinity. Moreover, their minimum content (0.11, 0.61, 0.13, 0.41, and 0.06 %, respectively) was obtained in the control without AMF inoculation. The amounts of these compounds were elevated with increasing the salinity up to 6 dS m^{-1} when AMF inoculated. But their content was significantly reduced when the salinity increased to the 8 dS m^{-1} (Table 3).

In summary, the various salinity levels decreased the tarragon essential oil content and the content of *α*-pinene, limonene, *Z*-ocimene, *E*-ocimene, and methyl chavicol, while the content of bornyl acetate, eugenol, methyl eugenol, caryophyllene, germacrene, and α-farnesene was increased. Similar results were obtained also by other authors (Ashraf and Orooj, 2006; Tabatabaie and Nazari, 2007; Aziz et al., 2008; Belaqziz et al., 2009; Baatour et al., 2010). Salinity could directly influence essential oil content and its components via changing the activity of the enzymes which are responsible for the terpenoids or phenylpropanoid biosynthesis and by altering the abscisic acid to cytokinins ratio. Furthermore, impaired photosynthesis and carbohydrate production indirectly affect the essential oil content and its components (Safarnejad et al., 2006; Turkan, 2011). It is reported that under the salinity stress, the biosynthesis of monoterpene biosynthesis is more affected than sesquiterpenes due to energy shortages thus, monoterpene biosynthesis is more vulnerable to salinity stress (Turner and Croteau, 2004). However, that the reduced ratio of oxygenated monoterpene to sesquiterpenes is caused by changes in cell bioenergetics under environmental stress. Another factor involved in this issue is the difference in the position of the compounds biosynthesis in using oxygen and energy resources (Dudareva et al., 2004).

The AMF inoculation in various levels of salinity resulted in reduced content of essential oils and a number of its major components, while the content of methyl chavicol, bornyl acetate, and caryophyllene was increased. Ahmadi-Khoei et al. (2013) reported similar results that the stimulation and changes in essential oil and phenol biosynthesis through fungal inoculation are possible.

Table 2: Analysis of variance for effects of arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation and salinity on phytochemical traits of tarragon

S.O.V.	Df.	Mean Square					
		Essential oil content	*α*-pinene	limonene	*Z*-ocimene	*E*-ocimene	methyl chavicol
Replication	3	0.002 ns	0.007 **	0.026 **	0.18 ns	0.018 ns	5.92 ns
AMF inoculation	1	1.02 **	0.67 **	1.3 **	37.71 **	42.47 **	306.42**
Salinity (S)	4	0.68 **	0.12 **	0.35 **	16.58 **	24.94 **	194.9 **
AMF × (S)	4	0.074 **	0.28 **	0.81 **	21.31 **	26.8 **	160.51 **
Error	27	0.005	0.0008	0.0038	0.067	0.066	27.68
CV (%)		12.39	3.97	5.18	7.71	7.33	6.74

ns: non-significant differences; *: significant at $p \le 0.05$; **: significant at $p \le 0.01$.

Table 2: Continued

S.O.V.	Df.	Mean Square					
		germacrene	caryophyllene	methyl eugenol	eugenol	bornyl acetate	*α*-farnesene
Replication	3	0.108 **	0.005 ns	0.004 ns	0.0085 **	0.0008 ns	0.011 ns
AMF inoculation	1	0.526 **	0.251 **	2.04 **	0.083 **	0.34 **	1.42 **
Salinity (S)	4	4.38 **	0.665 **	16.06 **	0.642 **	0.24 **	6.14 **
AMF × (S)	4	0.78 **	0.075 **	2.19 **	0.118 **	0.004 **	0.93 **
Error	27	0.022	0.002	0.011	0.0015	0.0009	0.024
CV (%)		11.15	5.8	5.11	9.089	7.57	10.95

ns: non-significant differences; *: significant at $p \le 0.05$; **: significant at $p \le 0.01$.

Table 3: Comparison of the means percentage in essential oil components under the salinity and arbuscular mycorrhizal fungi (AMF), *Glomus intraradices* inoculation of tarragon

AMF	Salinity	*α*-pinene	limonene	*Z*-ocimene	*E*-ocimene	methyl chavicol	bornyl acetate	eugenol	methyl eugenol	caryophyllene	germacrene	*α*-farnesene
Non-inoculation	0	0.72 c	2.16 c	4.98 c	5.5 c	75.9 bc	0.08 g	0.11 g	0.61 f	0.13 f	0.41 f	0.07 f
	2	1.03 a	3.22 a	6.35 a	7.31 a	83.4 ab	0.21 f	0.11 g	0.65 f	0.3 e	0.57 ef	0.8 d
	4	0.95 b	2.57 b	6.29 ab	6.38 b	76.4 bc	0.28 e	0.13 g	0.75 f	0.31 e	0.72 e	0.59 de
	6	0.58 d	0.71 e	1.33 e	1.11 f	75.9 bc	0.42 d	0.68 b	2.72 c	0.92 bc	1.81 c	1.99 b
	8	0.29 f	1.34 d	2.63 d	2.38 d	64.3 d	0.5 bc	0.9 a	4.24 a	1.33 a	2.54 a	2.64 a
Inoculation	0	0.88 b	2.54 b	5.96 b	6.65 b	88.8 a	0.23 f	0.18 f	0.61 f	0.21 ef	0.55 ef	0.51 e
	2	0.13 g	0.44 f	0.93 f	1 f	80.8 b	0.42 d	0.34 e	1.45 e	0.59 d	0.99 d	1.14 c
	4	0.10 g	0.47 f	0.91 f	0.77 f	83.4 ab	0.49 c	0.5 d	1.96 d	0.88 c	1.67 c	1.79 b
	6	0.39 e	0.92 e	1.64 e	1.57 e	71. cd	0.54 b	0.73 b	4.13 a	1.43 a	2.2 b	2.58 a
	8	0.3 ef	1.35 d	2.43 d	2.38 d	79.5 b	0.73 a	0.62 c	3.08 b	1.02 b	1.78 c	1.96 b

Means with the same letters in each column indicate no significant difference between treatments at the 5 % level of probability.

4 CONCLUSION

The morpho-physiological characteristics and essential oil content of tarragon were reduced under salinity stress, but some content of essential oil components were increased such as bornyl acetate, eugenol, methyl eugenol, caryophyllene, germacrene, and *α*-farnesene. In general, the AMF inoculation in no salinity condition had the most positive effect on tarragon morpho-physiological traits and methyl chavicol amount. Although, the essential oil content was reduced with the AMF inoculation, but methyl chavicol amount as a major tarragon essential oil component was increased by the AMF inoculation under salinity condition. Therefore, we can conclude that the AMF inoculation caused to alleviate salinity stress harmful effects.

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Combining ability × environment interaction and genetic analysis for agronomic traits in safflower (*Carthamus tinctorius* L.): biplot as a tool for diallel data

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ABSTRACT

Combining ability × environment interaction is considerable to identify the effect of environment on the combining ability and gene action of the traits to select appropriate parents for safflower hybrid production. The 36 genotype (28 F_2 progenies of eight-parent half-diallel crosses across 8 parental genotypes) of safflower were studied to investigate the mentioned parameters across different geographical regions of Iran. The results indicated significant differences among parents for general and specific combining ability, except for seeds per capitulum across three environments. The overall results indicated that K_{21} and Mex.22-191 were excellent parents with greater general combining ability for the improvement of seed yield in safflower. The K_{21} × Mex.22-191 hybrid could be, therefore, employed for the production of high seed yield in safflower breeding. The estimates of genetic variance components recommended the importance of additive- dominance genetic effects that contributed to variation in yield per plant. Such gene action expression for seed yield needs auxiliary methods based on hybridization and selection for seed yield advancement in safflower.

Key words: combining ability; heritability; oil seed; and variance component

IZVLEČEK

ANALIZA INTERAKCIJE OKOLJA IN KOMBINACIJSKE SPOSOBNOSTI IN GENETSKA ANALIZA AGRONOMSKIH LASTNOSTI ŽAFRANIKE (*Carthamus tinctorius* L.): BIPLOT KOT ORODJE ZA ANLIZO DIALELNIH KRIŽANJ

Preučevanje kombinacijske sposobnosti križancev in interakcije okolja je pomembno pri določanju učinkov okolja na uspešnost križancev in delovanja genov na določene lastnosti pri izbiri primernih starševkih rastlin žafranike pri pridobivanju hibridov. Preučevanih je bilo 36 genotipov žafranike (28 F_2 potomcev osmih starševskih pol dialelnih križanj z 8 starševskimi genotipi), da bi preučili odziv analiziranih parametrov v različnih geografskih območjih Irana. Izsledki so pokazali značilne razlike med starševskimi rastlinami za splošne in specifične kombinacijske sposobnosti razen za število rožk na košek v treh preučevanih okoljih. Celokupni rezultati so pokazali, da sta bila K_{21} in Mex.22-191 odlična starša z večjo splošno kombinacijsko sposobnostjo za izboljšanje pridelka semena žafranike. Hibrid K_{21} × Mex.22-191 bi lahko bil uporabljen pri žlahtnjenu žafranike za doseganje velikih pridelkov semena. Ocenjene komponente genetske variance kažejo na pomembnost aditivno-dominantnih genetskih učinkov, ki vplivajo na spreminjanje pridelka na rastlino. Preučevanje takšnega izražanja genov, ki vpliva na pridelek semena žafranike potrebuje dodatne metode, ki temeljijo na hibridizaciji in selekciji za povečanje pridelka.

Ključne besede: kombinacijska sposobnost; heritabilnost; oljna semena; komponente variance

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1 INTRODUCTION

Safflower (*Carthamus tinctorius* L.) (n = 12) is one of human' s oldest crops that has been traditionally grown for the production of vegetable oils, fabric dyes, food coloring, industrial and medicinal properties (Dajue and Mundel 1996; Weiss 2000). After oil extraction, the resultant meal is used for animal feed as it is rich in proteins (Singh 2007). It may have some production potential under arid and semi - arid regions (Kizil et al 2008). Cultivars with increased content of seed yield are required for safflower production in comparison with other oilseeds (Mohammadi and Pourdad, 2009; Golkar 2014). Iran and Mediterranean region are considered as rich centers for safflower (Knowles 1969) with diverse agro-ecological regions contributing to the increase in area and production of safflower (Golkar 2014). Annual yield of safflower production is about 700 tha^{-1} in Iran. Crop yield improvement requires adequate knowledge regarding the nature of parents combining ability that are available for hybridization programs and the nature of gene action included in the expression of quantitative traits of economic importance (Nassimi et al 2006; Milic et al 2011; Acquaah 2012; Gouda et al 2013). The variance for General Combining Ability (GCA) includes the additive portion of the total variance, whereas that for Specific Combining Ability (SCA) involves the non-additive portion of the total variance, which arises from dominance and epistatic deviations (Sincik et al 2011; Acquaah 2012). Specific combining ability is an important indicator of the potential of inbred lines for generating superior hybrid combinations (Singh and Pawar 2005; Zhang et al 2015).

Diallel analysis is the most common genetic design that is used for determining combining abilities (Singh and Pawar 2005; Bocanski et al. 2011). Some methods are available to analyze diallel crosses, such as the method developed by Griffing (1956). Biplot method for analysis of diallel data reports the inter-relationships and combining ability of parents based on graphical presentation, using PC_1 and PC_2, which are derived through principle component (PC) analysis of environment-centered yield data (Yan and Hunt 2002). In this relation, the knowledge of genetic control and combining abilities can be useful for improvement of seed yield, allocation of new genetic resources and construction of selection indices in safflower (Milic et al 2011; Suresh et al 2013). Reports on the combining ability and the inheritance of some agronomic traits in safflower are available (Mandal and Banerjee 1997; Gupta and Singh 1988; Singh et al 2005; Golkar et al., 2012), but literature review has shown that there is no information regarding the effects of different environments on the magnitude of general and specific combining ability and their interaction with environments for important agronomic traits in safflower, especially in the Middle east region. The genotype × environment interaction is a major source of bias that affects general and specific combining ability testing (Suresh et al 2013). Improvement of new hybrids adapted to world climate modification is an important breeding stage to sustain safflower production in Iran. The objectives of the present research were 1) to determine genetic parameters and the mode of inheritance for seed yield and its components in safflower, and 2) to identify best combinations for seed yield and its components in safflower hybrids across different geographical environments.

2 MATERIALS AND METHODS

2.1 Plant material and experimental design

The seeds of 36 genotypes (8 parents and 28 F_2 hybrids) were originated by a 8×8 half-diallel design in F_2 generation**.** The cultivars were hand-crossed with emasculation in a diallel mating design. Eight safflower genotypes including six local Iranian genotypes: C_{111} (selected from Kouseh landrace), C_{4110} (selected from Kouseh landrace), ISF_{14} (selected from Isfahan), A_2 (selected from Azarbayejan), K_{21} (selected from Kordestan) and IL.111 (selected from Auromyeh), with two exotic genotypes from Mexico (Mex.22-191) and Germany (GE_{62918}). Iranian genotypes were selected on the basis of their differences in geographical origin, adaptability and morphologic characters. In the F_2 generation, evaluation was made in three geographical environments. The seeds of 36 genotypes (8 parents, 28 F_2 hybrids) were sown at three different research farms that were located in different regions of Iran including (Isfahan: 51º 32´ E and 32º 32´ N, 1630 m asl, dry and hot climate), Maragheh (46º 12´ E and 37º 30´ N, 1477.7 m asl, cold and wet climate) and Shahrekord (49º 22´E and 32 º 20´N, 2060 m asl, cold and dry climate) in spring of 2015 by using a randomized complete block design with three replications. The mean of annual temperature at Isfahan, Shahrekord and Maragheh were recorded 19.5˚C, 11.5˚C and 13.5˚C, respectively. The soil at three location farms were silty clay loam, typical Haplargids of the arid tropic with pH 7 (Maragheh) to 7.5 (Isfahan and Shahrekord). Plots of parents consisted of 1 rows of 2.5 m length, whereas F_2 progenies consisted of 3 rows of 2.5 m length. The plants were spaced 50 cm and 5 cm between and within rows. To raise the crop, plot was fertilized with 200 kg ha $^{-1}$ N and 200 kg ha $^{-1}$ P_2O_5 previous of sowing and 100 kg ha $^{-1}$ N was applied bout one month after planting. The standard agronomic practices for safflower were followed during the growing season. From each plot, 30 competitive progenies were selected randomly for recording observations on studied traits including: capitula per plant (CP), seeds (achenes) per capitulum (SC), 1000-seed mass (g) (SW) and yield per plant (g) (YP).

2.2 Statistical analysis

The data were first subjected to the Analysis of variance (ANOVA) by using General Linear Model of SAS program (SAS, 2011).The mean of each plot was used for statistical analysis.

The least significant Difference (LSD $_{5\%}$) test was used for mean separation. Combining abilities (general and specific) were estimated following Method 2 Model1 described by Griffing (1956) via SAS program (Zhang et al., 2005) based on the following statistical model:

$$X\text{ijk} = m + g_i + g_j + s_{ij} + e_{ijk} \quad (1)$$

in each location, where, X_{ijk} is the observed value for a cross between the i_{th} and j_{th} parents in the k_{th} replication, m is population mean; g_i and g_j are GCA values of the i_{th} and j_{th} parents, respectively; s_{ij} is the SCA value for the hybrid between the i_{th} and j_{th} parents and e_{ijk} is the residual. Combined analysis over locations for combining ability analysis, genetic parameters (δ^2_A and δ^2_D) and heritability estimates (broad sense and narrow sense heritability) was conducted according to Perkins (1970) by the following formulae:

$$h_b^2 = \sigma_G^2 / (\sigma_G^2 + \frac{\sigma_{GE}^2}{s} + \sigma^2{}_e) \quad (2)$$ and

$$h_n^2 = \sigma_A^2 / (\sigma_G^2 + \frac{\sigma_{GE}^2}{s} + \sigma^2{}_e) \quad (3)$$

The genetic components were estimated according to Perkins (1970):

$$\sigma_G^2 = 2\sigma_{GCA}^2 + \sigma_{SCA}^2 \quad (4)$$

$$\sigma_{G\times E}^2 = 2\sigma_{GCA\times Env}^2 + \sigma_{SCA\times Env}^2 \quad (5)$$

The dominance ratio (Singh & Pawar, 2005) which explains the ratio of dominance to additive gene effects was calculated using following equation:

$$\sqrt{\frac{2\sigma^2{}_D}{\sigma^2{}_A}} \quad .(6)$$

2.3 Biplot interpretations

GGE biplot analysis is expressed as: $Y_{ij} - \beta j = \lambda_1\varepsilon_{i1}\eta_{j1} + \lambda_2\varepsilon_{i2}\eta_{j2} + \varepsilon ij$ Where, Y_{ij} is genotypic values of the combination (F_2 hybrids) between genotypes i and tester j for a given trait; βj average value of all combinations with tester j, λ_1 and λ_2 are singular values for PC1 and PC2. The ε_{i1} and ε_{i2} are PC_1 and PC_2 eigenvectors for genotypes i (entry), respectively; η_{j1} and η_{j2} are PC_1 and PC_2 eigenvectors for tester j, respectively; εij is the residual of model for inbred i and tester j (Yan & Hunt 2002). In diallel crosses, a parent is both an entry and a tester (Yan & Hunt 2002). Biplot analysis for seed yield per plant done by GGE biplot software (Yan 2001).

3 RESULTS AND DISCUSSION

3.1 Combining ability analysis in individual locations

The analysis of variance for the combining ability of evaluated traits for each individual location (Isfahan, Maragheh and Shahrekord) is presented in Table 1. Analysis of variance revealed significant genotypic differences for all studied traits in Isfahan (Table 1a). Combining ability analysis also showed that GCA and SCA mean squares were significant for all traits studied. The combining ability analysis in Maragheh revealed that the genotypes showed a significant difference for all studied traits (Table 1b). The mean squares of GCA were significant for all studied traits (Table 1b). The SCA mean squares were not significant for seed mass (Table 1b). The analysis of variance in Shahrekord revealed a significant difference between genotypes for all studied traits (Table 1C). The mean squares of GCA were significant for capitula per plant, seeds per capitulum and yield per plant (Table 1 C). The SCA mean squares were also significant for capitula per plant, seeds per capitulum and yield per plant (Table 1C). The total comparison of GCA and SCA mean squares presented different ratios of δ^2_{gca}/ δ^2_{sca} (Table 1). Genetic differences were more pronounced under high temperatures (Isfahan) than the low ones (Shahrekord and Maragheh). Also, such other factors as location altitude and relative rainfall might have played a role in the expression of genotypic differences in each location. According to the obtained results, under cold and dry climates (Shahrekord), genotype differences were less pronounced for traits; so, intercrossing of candidate genotypes at this location would result in flat breeding (Barten et al 1993).

Table 1: Analysis of variance for combining ability for agronomic traits in safflower under different environment

		Mean squares of studied traits			
Isfahan	df	CP	SC	SW	YP
Replication	2	9.39**	27.47**	1.5	45.52**
Genotype	35	11.66**	194.73**	49.52**	172.33**
GCA	7	37.99**	833.52**	194.32**	594.14**
SCA	28	5.08*	35.04**	13.32**	66.88**
Error	70	2.95	2.09	5.9	2.94
δ^2_{GCA}		1.10	26.62	6.03	17.58
δ^2_{SCA}		0.71	10.98	2.47	21.31
δ^2_{A}		2.19	53.23	12.07	35.15
δ^2_{D}		0.71	10.98	2.47	21.31
Maragheh	df	CP	SC	SW	YP
Replication	2	6.52	42.14	66.61	148.34**
Genotype	35	7.52**	39.4**	60.07**	77.38**
GCA	7	19.14**	35.07	153.12**	237.39**
SCA	28	4.59*	40.47**	36.78	37.35*
Error	70	2.86	18.52	23.58	22.6
δ^2_{GCA}		0.49	0	3.88	6.67
δ^2_{SCA}		0.58	7.32	4.40	4.92
δ^2_{A}		0.97	0.00	7.76	13.34
δ^2_{D}		0.58	7.32	4.40	4.92
Shahrekord	df	CP	SC	SW	YP
Replication	2	23.06**	49.03*	99.59**	3.88
Genotype	35	14.41**	130.03**	60.92**	159.37**
GCA	7	25.83**	532.35**	243.21**	514.26**
SCA	28	11.55**	29.43**	15.33	70.35**
Error	70	2.83	14.09	14.85	4.16
δ^2_{GCA}		0.48	16.76	7.60	14.80
δ^2_{SCA}		2.91	5.11	0.16	22.06
δ^2_{A}		0.95	33.53	15.19	29.59
δ^2_{D}		2.91	5.11	0.16	22.06

* and ** significant at $P<0.05$ and $P<0.01$, respectively;
CP: capitula per plant, SC: seeds per capitulum, SW: 1000-seed weight, YP: yield per plant.

3.2 Combining ability across all locations

Information on the relative importance of general and specific combining ability can be helpful in the analysis and interpretation of the genetic basis of important traits in safflower. Analysis of variance combined over environments showed that different environments had a significant effect on all studied traits (Table 2). The results obtained from the combined analysis of variance indicated a significant genotypic difference in F_2 diallel experiment for all traits studies (Table 2). Combined analysis of variance for combining ability over three locations revealed that GCA mean squares were highly significant for all the traits (Table 2). The SCA mean square was highly significant, except for SCA of seeds per capitulum (Table 2). This result implied that selection for the improvement of seeds per capitulum could be delayed to later generations until the non-additive portion would be mitigated to the additive portion as this trait showed the higher magnitude of SCA variance, as compared to GCA variance in the respective location. The proportion of δ^2gca to δ^2sca varied from trait to trait. On the other hand, the proportion of δ^2_{gca} :δ^2_{SCA} showed that most total genetic variability for seeds per capitulum and seed mass was more associated with GCA (fixable genetic portion) than SCA (non- fixable genetic portion) effects (Table

2); thus simple selection would confer the rapid improvement of these traits.

The genotype× environment interaction was significant for seeds per capitulum and yield per plant (Table 2), thereby suggesting significant variation among F_2 genotypes for the mentioned traits in their sensitivity to the geographical location. The conditions in this experiment were favorable for detecting genotype × environment interactions, since different temperatures in three locations represented extreme environmental conditions under which high seed yield safflower genotypes could be commercially grown (Suresh et al 2013). Thus, for population improvement of F_2 hybrid combinations, evaluation of capitula per plant and seed mass should be sufficient in one location.

With considering to combining abilities (GCA and SCA) interaction with different locations, GCA × location interaction was significant for all studied traits, except for seed mass (Table 2), and the SCA × location interaction was significant for seeds per capsule and yield per plant (Table 2). These significant interactions suggested that the selection of different parental genotypes and specific hybrids was required for developing populations specific to each location.

3.3 Genetic components for Seed yield per plant and its components across different environments

Genetic components for seed yield and its components (capitula per plant, seeds per capitulum and 1000- seed mass) are presented in Table 3. The higher proportion of δ^2_D (1.66) to δ^2_A (1.1) and dominance ratio (1.73) implied the predominance role of dominance gene action, rather than the additive one, for the genetic control of capitula per plant (Table 2). This finding was similar to those obtained in the previous reports (Shahbazi and Saeidi 2007; Golkar et al., 2012; Deedawat et al 2015). According to the obtained results, the large proportion of δ^2_A to δ^2_D and dominance ratio (less than unity) indicated the predominance role of additive gene effects, rather than the dominance ones, in the genetic control of seeds per capitulum and seed mass in safflower. Our findings were consistent with previous reports (Mandal and Banerjee 1997; Shahbazi and Saeidi 2007; Nakhaei et al 2014).The comparison of the magnitude of δ^2_{GCA} (12.61) and δ^2_{SCA} (0.0017) and dominance ratio (1.01) for seed yield per plant indicated the additive - dominance genetic control of this trait (Table 2). The previous studies observed the predominant role of dominance gene action for seed yield per plant (Singh et al 2008; Deedawat et al 2015) and additive gene action in salinity stress (Nakhaei et al 2014). So, this new type of genetic control for the seed yield of safflower per plant could be utilized by both breeding methods based on selection and hybridization.

Table 2: Analysis of variance for combining ability for agronomic traits in safflower under different environment

Source of variation	df	Mean squares of the studied traits			
		CP	SC	SW	YP
Environment	2	120.9**	13515.1**	1017.4**	4213.7**
Rep (Environment)	6	12.99	39.55	55.9	65.9
Genotype	35	27.84**	227.28**	137.54**	365.1**
GCA	7	70.85**	931.97**	531.23**	1282.7**
SCA	28	17.09**	51.1	39.11**	135.71**
Gen× Environment	70	2.88	68.44**	16.48	21.9**
GCA× Environment	14	6.07*	234.50**	29.72	31.5**
SCA× Environment	56	2.08	26.93**	13.18	19.5**
Error	210	2.88	11.57	14.78	9.9
Genetic parameters					
δ^2_{GCA}		0.55	7.48	5.28	12.6
δ^2_{SCA}		1.66	2.68	2.88	12.9
δ^2_{GCA}:δ^2_{SCA}		0.33	2.79	1.83	0.97
δ^2_{A}		1.1	14.96	10.56	25.2
δ^2_{D}		1.66	2.68	2.88	12.9
h^2_b (%)		0.87	0.70	0.86	0.93
h^2_n (%)		0.34	0.59	0.68	0.62
Dominance ratio		1.73	0.59	0.73	1.01

CP: Capitula per plant, SC: seeds per capitulum, SW: 1000-seed weight, YP: yield per plant.
* and ** significant at *P*<0.05 and *P*<0.01, respectively

3.4 Heritability of the traits

The efficiency of selection largely depends on the extent of genetic variability present in the population and the heritability of the concerned character (Mohammadi and Pourdad, 2009; Acquaah, 2012). The broad- sense heritability ranged from 73 (%) in seeds per capitulum to 93 (%) in seed yield per plant (Table 2). High estimates of broad-sense heritability for seed yield and its components in this study denoted that dominance or epistatic effects as other types of genetic effects might be contributed in their variation. Although, the higher value of broad – sense heritability proposed that selection would be more effective and improvement could be expected for the traits in future breeding programs (Singh and Pawar, 2005;Mohammadi and Pourdad, 2009). The high broad- sense heritability for the number of seeds per capitulum (about 70 %) was similar to that in the previous reports (Mohammadi and Pourdad, 2009). A low value for the broad – sense heritability (about 60 %) of seed yield per plant was reported (Shahbazi and Saeidi, 2007; Mohammadi and Pourdad, 2009) that was different from that found in this study. Different methods for heritability estimation resulted in different values of heritability for the same trait (Acquaah, 2012). In F_2 generation, the narrow-sense heritability ranged from 34 % for capitula per plant to 68 % in seed mass (Table 2). The ranges of narrow-sense heritability in low and medium values (13 % < h_n^2 < 68 %) implied that a moderate progress would be achieved through selection for these traits.

3.5 Parental GCA and SCA of the crosses

Selecting parents based on their genetic merit is a vital component in the utilization of genetic resources. The estimates on *gca* effects and the mean performance of the parents would help the breeder to understand the genetic architecture and the potentiality of the selected parents in F_2 and later generations (Suresh et al., 2013). The GCA effects for different traits across three environments are presented in Table 3. In the case of capitula per plant, GE_{62918}, K_{21} and Mex-22-191 possessed high positive GCA effects that were suitable for breeding programs aimed at increasing the number of capitula per plant in safflower (Table 3). The GCA effects for CP varied from 1.21 (GE_{62918}) to -1.03 (ISF_{14}) (Table 3). The mean of parental genotypes for CP varied from 26.20 (GE_{62918}) to 21.18 (A_2) (Table 3). The seeds per capitula showed wide variation for GCA effects, from -5.94 (GE_{62918}) to 3.07 (C_{4110}). The positive and significant GCA effects for C_{111}, C_{4110}, ISF_{14} and Mex.22-191 demonstrated the useful role of these genotypes in safflower breeding to improve the number of seeds per capitulum. The mean of seeds per capitulum for parental genotypes varied from 38.96 (C_{4110}) to 20.81 (GE_{62918}). The GCA effects for1000-seed mass ranged from -2.69 (C_{4110}) to 4.54 (IL.111). The highest mean for seed mass (41.09) was attributed to IL.111 (Table 3).

Table 3: General combining ability (GCA) effects and mean values for seed yield and different traits in safflower

Trait		GE_{62918}	C_{111}	C_{4110}	ISF_{14}	A_2	K_{21}	IL.111	Mex.22-191	(g_i)	LSD (mean)
CP	GCA	1.21	-0.36	-0.28	-1.01	-1.03	1.18	-0.25	0.53	0.16	
	Mean	26.20	23.54	22.20	22.41	21.18	26.17	24.04	24.20		1.57
SC	GCA	-5.94	1.62	3.07	1.43	-1.41	1.58	-3.21	2.84	0.33	
	Mean	20.81	35.43	38.96	35.91	28.40	30.07	26.27	35.53		3.16
SW	GCA	0.96	-2	-2.69	-2.36	0.24	-0.31	4.54	1.62	0.37	
	Mean	33.27	28.72	28.69	27.32	30.42	25.59	41.09	30.77		3.57

CP: capitula per plant, SC: seeds per capitulum (SC), SW: 1000-seed weight, YP: yield per plant.

3.6 Specific combining ability of the crosses

Estimates of SCA effects of the 28 crosses for CP, SC and SW across the combined data are given in Table 4.The scope exploitation of hybrids for further breeding cycles in any crop largely depends on 1) mean performance of the hybrids over a range of environments, and 2) the specific combining ability effects of the parents (Suresh et al., 2013). So, the hybrids identified based on SCA effects could be exploited in heterosis breeding (Zhang et al., 2015). The SCA for capitula per plant varied from -4.32(C_{111}×IL.111) to 3.06 (A_2×IL.111). However, the crosses of low × low GCA with the negative sign led to a superior hybrid with a positive sign indicating epistatic gene actions in controlling capitula per plant (Singh and Pawar, 2005). The specific combining ability for seeds per capitulum ranged from -5.09 (ISF_{14}×GE_{62918}) to 4.18 (A_2×GE_{62918}) (Table 4). The specific combining ability effects for seed weight varied from 3.19 (A_2×ISF_{14}) to A_2×Mex.22-191 (3.94) (Table 4). According to the obtained results, the hybrid genotype compromising A_2 with GE_{62918} and Mex.22-191 could be used in breeding projects for the improvement of seeds per capitulum and seed weight in safflower, respectively.

Table 4: Specific combining ability effects of different F_2 generations for agronomic traits in safflower

Crosses	CP	SC	SW
GE_{62918}×C_{111}	1.08	-1.65	-0.57
GE_{62918}×C_{4110}	-0.61	0.56	1.16
GE_{62918}×ISF_{14}	1.25	-5.09	1.43
GE_{62918}×A_2	-1.22	4.18	1.64
GE_{62918} ×K_{21}	0.09	-0.69	-1.78
GE_{62918}×IL111	-0.24	0.57	-1.70
GE_{62918}×Mex.22-191	-0.23	2.08	1.29
C_{111}×C_{4110}	1.48	1.47	-1.87
C_{111} × ISF_{14}	-0.46	0.82	1.60
C_{111}×A_2	-0.99	-1.75	0.69
C_{111}×K_{21}	1.32	-1.98	2.46
C_{111} ×IL. 111	-4.32	3.3	-2.92
C_{111}× Mex.22-191	1.00	0.76	-0.67
C_{04110} × ISF_{14}	-1.18	-1.35	-1.33
C_{4110}×A_2	-0.18	-4.77	-2.15
C_{4110}×K_{21}	0.57	0.38	0.26
C_{4110}×IL.111	1.04	-0.09	0.09
C_{4110}×Mex.22-191	0.96	3.56	-0.10
ISF_{14}× A_2	1.39	3.92	-3.19
ISF_{14}×K_{21}	-1.06	2.41	-1.35
ISF_{14}×IL.111	-0.19	-1.78	0.27
ISF_{14}×Mex.22-191	-0.92	0.37	2.63
A_2×K_{21}	-1.47	0.54	1.88
A_2×IL.111	3.06	1.54	1.50
A_2×Mex.22-191	0.58	-0.75	3.94
K_{21}×IL.111	-0.12	-2.25	1.58
K_{21}×Mex.22-191	0.68	1.27	0.68
IL.111×Mex.22-191	-0.68	-1.26	1.36
(S_{ij})±SE	0.44	0.89	1.01
$(S_{ij}-S_{kl})$±SE	0.71	1.43	1.62
$(S_{ij}-S_{ik})$±SE	0.76	1.52	1.71

CP: capitula per plant, SC: seeds per capitulum, SM: 1000-seed weight.

3.7 The Biplot graphs for seed yield per plant

Existing new superior inbred lines with enhanced combining abilities is one of the goals of safflower breeding programs. Seed yield is a complicated multigenic trait that its yield depends on the great influence of environmental situations (Bočanski et al., 2011). In this study, the GGE biplot technique allowed the effective and rapid view of GCA and SCA effects of the parental genotypes, best lines and tester and their performance in different crosses in this study. Even though GGE biplot analysis of diallel data is widely used to determine combining ability and heterotic responses in many crops for yields, such as oil crops [rape seed (Sincik et al., 2011), cotton (Hamoud, 2014), maize (Bočanski et al., 2011; Badu-Apraku et al., 2013)] and forages such as alfalfa (Milić et al. 2011). This method has rarely been reported for diallel data analysis for safflower at multi location trials.

3.7.1 GCA and SCA of the parents for seed yield per plant

The GCA and SCA of parental genotypes are presented in Figure 1. Biplot accounted for 87.5 % (PCA_1 = 66.1 %; PCA_2 = 23.3 %) of the total variance, which was partitioned, according to conventional analysis by Griffing, to GCA of parental genotypes and SCA of its crosses. General combining abilities of parents (entries) were increased in the direction of the arrow on ATC (Average Tester Coordinate) abscissa, ranking the genotypes by their GCA, as shown: G_8 (Mex.22-191) > G_6 (K_{21}) > G_3 (C_{4110}) > G_2 (C_{111}) > G_7 (IL.111) > G_4 (Isf-14)> G_5 (A_2) > G_1 (GE $_{62918)}$ (Figure 1). The highest (40.37) and lowest (23.22) means for yield per plant were observed at K_{21} and GE_{62918}, respectively. Therefore, it is necessary to select parents with high general GCA, so that it would benefit the offspring for seed yield and its components.

The specific combining ability effects for seed yield per plant ranged from -9.41 (C_{4110} × ISF_{14}) to 5.36 (K_{21}× Mex.22-191) and A_2 × Mex.22-191) (Figure 1). So, by considering the positive and significant GCA effects of parental genotypes of this cross, the K_{21} × Mex.22-191 cross with the highest positive SCA for yields per plant could the best cross combination for developing high yielding hybrid safflower varieties.

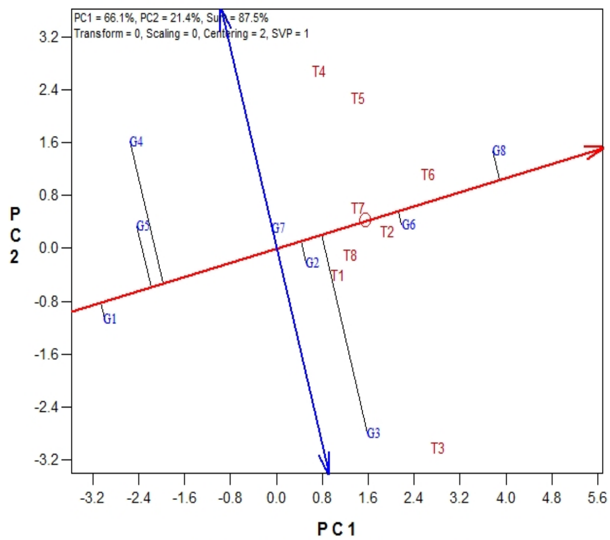

Figure 1: The biplot based on diallel data for seed yield per plant with the average tester ordination view, for representation of GCA and SCA of eight parental genotypes in safflower. Codes of the genotypes are: G_1 = GE_{62918}, G_2 = C_{111}, G_3 = C_{4110}, G_4 = ISF_{14}, G_5 = A_2, G_6 = K_{21}, G_7 = IL.111 and G_8 = Mex.22.191; codes of the testers are: T_1 = GE_{62918}, T_2 = C_{111}, T_3 = C_{4110}, T_4 = ISF_{14}, T_5 = A_2, T_6 = K_{21}, T_7 = IL.111 and T_8 = Mex.22.191.

3.7.2 Heterotic groups detection for seed yield per plant

Identifying, developing and retaining broad-range of heterotic germplasms should be considered as an important aim in safflower breeding. It is not be expected that heterosis could be occurred in highly recombinant germplasm either, since crossing populations that are very distant results to a loss of advantageous epistatic effects (complementary gene interactions) that exist within parental populations (Sakiroglu and Brummer, 2007; Milić et al., 2011). GGE biplot could discriminate the heterotic groups in this study. According to tester vector, the PCA_1 scores were positive for all testers. According to Figure 2, the genotypes of 4 (ISF_{14}), 5 (A_2) and 6 (K_{21}) were one group located above PC_2 guideline, and the genotypes of G_1 (GE_{62918}, G_2 (C_{111}), G_3 (C_{4110}) and G_8 (Mex.22-191) were the other group located below the PC_2 guideline. Moreover, testers of T_4, T_5 and T_6 interacted positively with genotypes G_4 (Isf_{14}), G_5 (A_2), G_6 (K_{21}), G_7 (IL.111) and G_8 (Mex.22-191), but they interacted negatively with G_1 (GE_{62918}), G_2 (C_{111}) and G_3 (C_{4110}). Similarly, testers T_1, T_2, T_3 and T_8 interacted positively with G_1 (GE_{62918}), G_2 (C_{111}) and G_3 (C_{4110}). This interaction pattern clearly indicated heterosis in twelve crosses, which was (4, 5, 6) × (1, 2, 3, 8) and defined as better than those in both parents. Heterosis could also occur between germplasms that are divergent and genetically distant, s something not necessarily dependent on geographic origin (Sakiroglu and Brummer 2007). Similar to this report, safflower genotypes with different geographical regions from Iran (Isfahan, Azarbayejan and Kordestan) and three genotypes with different country origins (Germany, Iran and Mexico) were grouped at the same heterotic groups. Tester 7 (IL.111), which was located near the ATC abscissa, did not seem to belong to any of the groups. An ideal tester should be discriminating of the entries and be highly representative of all testers (Yan & Hunt, 2002). So, the T6 was the best tester in this data set.

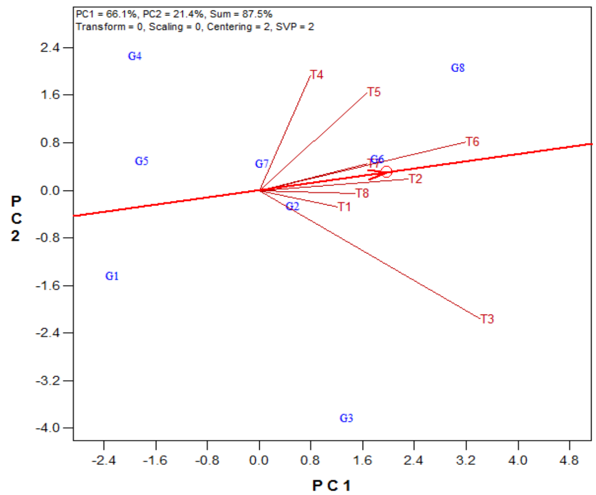

Figure 2: Determination of *heterotic* groupings by the average tester coordination for seed yield per plant in safflower. Codes of the genotypes are: G_1 = GE_{62918}, G_2 = C_{111}, G_3 = C_{4110}, G_4 = ISF_{14}, G_5 = A_2, G_6 = K_{21}, G_7 = IL.111 and G_8 = Mex.22.191; Codes of the testers are: T_1 = GE_{62918}, T_2 = C_{111}, T_3 = C_{4110}, T_4 = ISF_{14}, T_5 = A_2, T_6 = K_{21}, T_7= IL.111 and T_8 = Mex.22.191.

3.7.3 Polygon view of the biplot for seed yield per plant

According to Figure 3, the biplot was divided into four sectors, with genotypes G_8 (Mex.22-191), G_4 (Isf_{14}), G_1 (GE_{62918}) and G_3 (C_{4110}) as the corner genotypes. No testers fell in the G_1 (GE_{62918}) and G_4 (Isf_{14}) sectors, suggesting that genotypes of 1 and 4 were not the best mating parents with any of the genotypes. Moreover, this showed that these genotypes produced the poorest hybrids with some or all of the testers. As the single tester (T_3) fell in the G_3 (C_{4110}) sector, G_3 (C_{4110}) was the best mating partner with T_3. All of the testers, except T_3, fell in the G_8 (Mex.22-191)sector, indicating that the genotype 8 (Mex.22-191) was the best mating parent with the mentioned testers, but the crosses of G8 (Mex.22-191) by testers of 6 and 5 were better to some extent. According to the polygon view at Fig 3, the best crosses for seed yield were represented by G8 (Mex.22-191) × G5 (A_2) and G8 (Mex.22-191) × G6 (K_{21}).

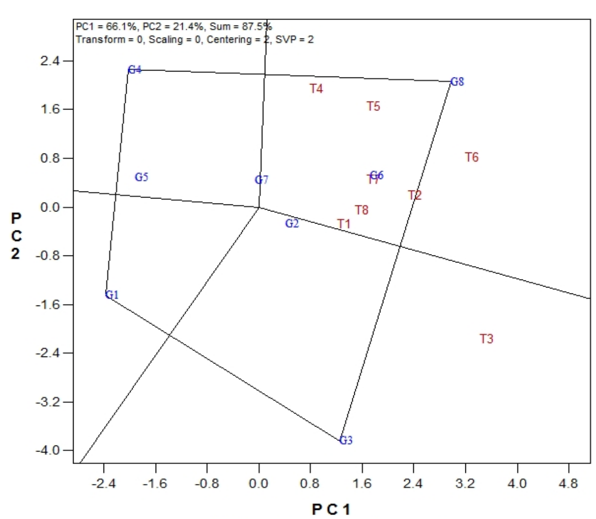

Figure 3: Polygon view of the biplot, showing the best crosses among all possible combinations for seed yield per plant in safflower. Codes of the genotypes are: G_1 = GE_{62918}, G_2 = C_{111}, G_3 = C_{4110}, G_4 = ISF_{14}, G_5 = A_2, G_6 = K_{21}, G_7 = IL.111 and G_8 = Mex.22.191; codes of the testers are: T_1 = GE_{62918}, T_2 = C_{111}, T_3 = C_{4110}, T_4 = ISF_{14}, T_5 = A_2, T_6 = K_{21}, T_7 = IL.111 and T_8 = Mex.22.191.

4 CONCLUSION

This study was undertaken to study combining ability × environment interaction in safflower by diallel design. Combining ability × environment interaction was carried out to regulate testing over more than one environment for population improvement purposes and the early testing of superior safflower new hybrids. High-yielding cultivars could be especially necessary to ensure success for safflower cultivation in arid and semi arid regions of Iran. So, this study revealed the importance of gaining knowledge about combining ability and the identification of superior genotypes across different environments. Considering the contribution of different genetic components in genetic control of a trait showed that an appropriate strategy for improvement of each desirable trait could be achieved. It may also be worthwhile to attempt bi-parental mating in the segregating generation among some selected crosses such as A_2 × Mex.22-191 and K_{21} × IL.111 and A_2 × IL.111 to permit superior recombination. The greater negative GCA values of IL.111 for seed mass, K_{21} for capitula per plant and yield per plant, and C_{4110} for seeds per capitulum imply the capacity of these parents in producing superior progenies when combined with another parent for improving the noted traits, respectively**.** In this study, the superior genotypes across three environments could be used in recombination breeding programs to accumulate suitable genes responsible for improving seed yield in safflower.

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UVA + B treatment affects antioxidant system and phytochemicals of parsley plant under different concentrations of Zn

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ABSTRACT

Decline in ozone layer that followed by enhanced solar UV radiation is a limiting factor for some plants. In this study the effect of UVA+UVB radiation on parsley plant was studied hydroponically at different concentrations of Zn (1.5 and 6.5 µm). UV radiation at both concentrations of Zn, slightly decreased the plant growth and significantly increased the carotenoids, flavonoids, total phenols and H_2O_2 contents, but had no effect on chlorophylls content. At concentration of 1.5 µm of Zn, UV radiation caused significant increases in the MDA and anthocyanin contents and the activities of POD and CAT enzymes, but decreased the soluble sugars and protein contents. At concentration of 6.5 µm of Zn, UV radiation caused significant increases in the CAT activity, but had no significant effect on other parameters. Results suggest that parsley plant tolerates UVA+UVB radiation particularly at concentration of 6.5 µmof Zn.

Key words: antioxidant enzymes; parsley; photochemicals; UVA + B; Zn

IZVLEČEK

OBRAVNAVANJE Z UVA + UVB VPLIVA NA ANTIOKSIDACIJSKI SISTEM IN KEMIZEM PETRŠILJA PRI RAZLIČNIH KONCETRACIJAH Zn

Tanjšanje ozonske plasti, ki mu sledi povečano UV sončevo sevanje sta omejujoči dejavnik za nekatere rastline. V tej raziskavi je bil preučevan učinek UVA+UVB sevanja na hidroponsko gojen peteršilj pri različnih koncentracijah Zn (1.5 in 6.5 µm). UV sevanje je pri obeh koncentracijah cinka rahlo zmanjšalo rast in značilno povečalo vsebnost karotenoidov, flavonoidov, celokupnih fenolov in H_2O_2, a brez učinka na vsebnost klorofilov. Pri koncentraciji 1.5 µm Zn, je UV sevanje povzročilo značilno povečanje vsebnosti MDA in antocianinov, povečalo je aktivnosti encimov POD in CAT, a zmanšalo vsebnost topnih sladkorjev in beljakov. Pri koncentaraciji 6.5 µm Zn je UV sevanje povzročilo značilno povečanje v aktivnosti CAT, a ni imelo značilnega učinka na ostale parametre. Rezultati nakazujejo, da peteršilj dobro prenaša UVA+UVB sevanje, še posebej pri koncentraciji 6.5 µm Zn.

Ključne besede: antioksidacijski encimi; peteršilj; kemizem rastlin; UVA + B; Zn

1 INTRODUCTION

Interruption of ozone layer as an outcome of human activities has resulted to enhanced intensity of UV radiation on the Earth surface. Plants have obligate requirement for sun light are more susceptible to this radiance. UV radiation increases the production of reactive oxygen species (ROS) that are extremely cytotoxic (Mahdavian et al., 2008; Czégény et al., 2016). The antioxidant system is one of the most important mechanisms responsible for detoxifying the free radicals. Non-enzymatic antioxidant system includes biochemicals such as carotenoids, flavonoids, ascorbic acid and glutathione. Flavonoids commonly absorb the light in the region of 280-320 nm and thus are capable to protect the plant from damage (Eichholz et al., 2011; Reshmi and Rajalakshmi, 2012). Carotenoids also have antioxidant properties and act as an internal filter against UV radiation (Nasibi and Kalantari, 2005). Enzymatic antioxidants such as catalase, peroxidase and superoxide dismutase can moderate the UV-induced injuries by protecting the photosynthetic pathway and cellular components (Wei et al., 2013). A wide range of morphological,

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physiological and biochemical responses of plants have been reported to elevate the UV resistance. Some plants are more tolerant to UV radiation than others because they activate a variety of mechanisms against stress (Fedina et al., 2010; Wei et al., 2013).

Zinc is an essential micronutrient and involves in the various metabolic pathways in plants (Alloway, 2008). The positive role of Zn in different environmental stresses such as salinity, drought and high irradiance was reported by several authors (Hassan et al., 2005; Weisany et al., 2012; Michael and Krishnaswamy, 2014). In this study the effect of UVA + B treatment on parsley plant antioxidant system and phytochemicals was investigated at two concentrations of Zn.

2 MATERIALS AND METHODS

The seeds of parsely plant (*Petroselinum crispum* Mill. var. *neapolitanum*) was achieved from the Agricultural Research Center of Tabriz, Iran.

2.1 Plants growth condition

Plants were grown hydroponically in a growth chamber with a temperature of 28/20 °C, 16 h photoperiod and relative humidity of 70 %. Seeds were germinated in petri-dishes and transferred to plastic containers with 2 l of Cooper nutrient solution (50 %) and pre-cultured for 7 days. After pre-culturing period plants were transferred to the full strength nutrient solution, containing two levels of Zn (1.5 and 6.5 µm) as zinc sulphate. Applied UV doses that were received by one-half of plants were 20.5 and 176 kJ m^{-2} day^{-1} for UVA and UVB respectively (supplied with 30 W, UV lamps, Philips; UVA 30 %, UVB 5 %). 20 days after treatments, the plants were harvested and stored in -80 °C for further analyses.

2.2 Photosynthetic pigments and phytochemicals assays

Fresh leaf tissues were homogenized with 80 % aqueous acetone. The extracts were centrifuged for 10 min at 4000 g. Chlorophylls and carotenoids contents were determined spectrophotometrically at 470, 646.8 and 663.2 nm using equations described by Lichtenthaler (1987).

Anthocyanins were extracted with acidified methanol (methanol: HCl, 99:1, v/v) solution on a shaker in the dark at 4 °C per 48 h. After filtering, the absorbance of samples were measured spectrophotometrically at 550 nm and calculated using an extinction coefficient of 33000 $mol^{-1}cm^{-1}$ (Wagner, 1979).

Total phenolic of shoots was extracted by 80 % aqueous methanol for 20 min using ultrasonic bath. The mixture was centrifuged at 14000 g for 5 min.To 0.5 ml of supernatant, 1.5 ml (1:10 v/v diluted with distilled water) Folin-Ciocalteau reagent was added and allowed to stand for 5 min at 22 °C. After 5 min, 2 ml of 7.5 % of sodium carbonate was added. These mixtures were incubated for 90 min in the dark with intermittent shaking. After incubation, development of blue color was measured at 725 nm. The phenolic content was calculated on the basis of standard curve of gallic acid (Fletcher and Kott, 1999).

The total flavonoid content of shoots was determined using the aluminum chloride assay through colorimetry. An aliquot (1 ml) of extracts were taken in different test tubes then 6 ml of distilled water was added followed by the addition of 0.3 ml of sodium nitrite (5 % $NaNO_2$, w/v) and allowed to stand for 6 min. Later 0.3 ml of aluminum trichloride (10 % $AlCl_3$) was added and incubated for 6 min, followed by the addition of 2 ml of sodium hydroxide (NaOH, 4 % w/v). After 15 min of incubation the mixture turns to pink and its absorbance was measured at 510 nm. The total flavonoid content was calculated on the basis of standard curve of quercitine (Toor and Savage, 2005).

2.3 Antioxidant enzymes assays

To obtain the crude extract, 0.1 g parsley leaves were homogenized in 3 ml of 10 mmol l^{-1} potassium phosphate buffer (pH = 7), containing 0.2 % polyvinyl pyrrolidone. The homogenate was centrifuged at 21,000 g at 4 °C for 20 min. The resulting supernatant was used to measure the activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and protein content. The activity of SOD was measured according to its capacity to inhibit photochemical reduction of nitroblue tetrazolium. The reaction mixture contained 2.65 ml of 67 mmol l^{-1} potassium phosphate buffer (pH = 7.8), 0.2 ml of 0.1 mmol l^{-1} EDTA solution containing 0.3 mmol l^{-1} sodium cyanide, 0.1 ml of 1.5 mmol l^{-1} NBT, 50 ml of 0.12 mmol l^{-1} riboflavin and 50 ml enzyme extract. The amount of enzyme that catalyzed 50 % inhibition from photochemical reduction of NBT was defined as one unit (U) of SOD. Due to the possibility of auto-oxidation of the substrates, control assays were prepared in the absence of plant extract (Winterbourn et al., 1976).

Guaiacol POD was assayed in plant shoots, following the method of Chance and Maehly (1955). The reaction mixture contained 1.50 ml of 100 mmol l^{-1} citrate-phosphate - borate buffer solution (pH = 7.5), 50 µl of 15 mmol l^{-1} guaiacol, 25 µl enzyme extract and 50 µl of 3.3 mmol l^{-1} H_2O_2. The polymerization of guaiacol was initiated by adding H_2O_2 and an increase in absorbance at 470 nm was recorded for 3 min. POD activity was calculated using the extinction coefficient, 26.6 (mmol $l^{-1})^{-1}$ cm^{-1}, for guaiacol. The generation of 1 µmol of tetra guaiacol per min was catalyzed by the amount of enzyme that was introduced as one unit of POD.

The CAT activity was determined by monitoring the decrease in absorbance at 240 nm for 3 min due to dismutation of H_2O_2. The reaction mixture contained 1.50 ml of 100 mmol l^{-1} citrate-phosphate-borate buffer solution (pH = 7.5), 50 µl enzyme extract and 13 µl of 10 mmol $l^{-1}H_2O_2$. The amount of enzyme for dismutation of 1 µmol l^{-1} H_2O_2 per min was expressed as one unit. Extinction coefficient for H_2O_2 at 240 nm was considered 39.4 (mmol $l^{-1})^{-1}cm^{-1}$ (Obinger et al., 1997).

2.4 Total proteins and soluble sugars

Total protein content was measured by the method of Bradford (1976) using bovine serum albumin as a standard. The soluble sugars content was measured by DuBois et al. (1956) method.

2.5 Hydrogen peroxide and malondialdehyde assays

The hydrogen peroxide (H_2O_2) content was estimated according to the Harinasut et al. (2003). Samples were homogenized with 0.1 % (w/v) trichloroaceticacid (TCA). Mixture was centrifuged at 12000 g for 15 min. To 0.5 ml of the supernatant, 0.5 ml of 10 mM phosphate buffer (pH = 7.0) and 1 ml of 1 m potassium iodide (KI) was added. The mixture was incubated at 25°C for 15 min. The absorbance was measured at 390 nm. The H_2O_2 content was calculated from a standard curve prepared in a similar way.

Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture (Heath and Packer, 1968). Leaf tissues were homogenized in 0.1 % (w/v) (TCA). The homogenate was centrifuged at 10,000 g for 5 min. To 1 ml of the supernatant, 4 ml of 20 % TCA containing 0.5 % thiobarbituric acid was added. The mixture was incubated at 95 °C in a water bath for 30 min, and then quickly cooled on ice. The mixture was centrifuged at10,000 g for 15 min and the absorbance was measured at 532 nm. MDA levels were calculated from 1,1′,3,3′-tetra ethoxy propan standard curve.

2.6 Statistical Analysis

Experiment was conducted in complete randomized design with 3 replications. Analysis of variance was performed using InStat (3.0) software. The data were presented as the means ± SE for each treatment. Means were compared with Tukey's Multiple Range Test at the 5 % probability level.

3 RESULTS AND DISCUSSION

3.1 Growth parameters

In this study, UV radiation non significantly decreased the fresh and dry mass and lengths of parsley plants shoots and roots at both levels of applied Zn. Application of Zn at concentration of 6.5 µm significantly ($p < 0.05$) increased the dry and fresh mass of shoots (Fig 1), but non significantly increased the dry and fresh mass of roots (Fig 2) and plant length (Fig 3) in compared to plants received concentration of 1.5 µm of Zn at normal and UV radiation conditions. The effect of UV radiation on plants growth is varying among different species. In the wide range of species, plant growth decreases in response to UV radiation, but in some cases the growth is not affected or it is even promoted by this radiation (Fedina et al., 2010, Ravindran et al., 2010; Zlatev et al., 2012). The induced changes in the plant's growth regulators biosynthesis and transport by UV radiation are responsible for the decreased growth of plants (Toosi et al., 2009). Similar to results obtained from this study, the biomass and production of potato, clover, oat and barley plants did not dramatically affect by 24-33 % increases in UV radiation during the growing season (Hakala et al., 2002). The ability of plant in the prevention of growth reduction under UV treatment is an indicator for plant tolerance (Smith et al., 2000). In this study, the application of Zn at high concentration could improve the growth of parsley under normal condition and UV radiation. The beneficial effects of Zn on plants growth are related to its necessity for carbohydrate and protein metabolism, membrane integrity, auxin synthesis and reproduction (Alloway, 2008).

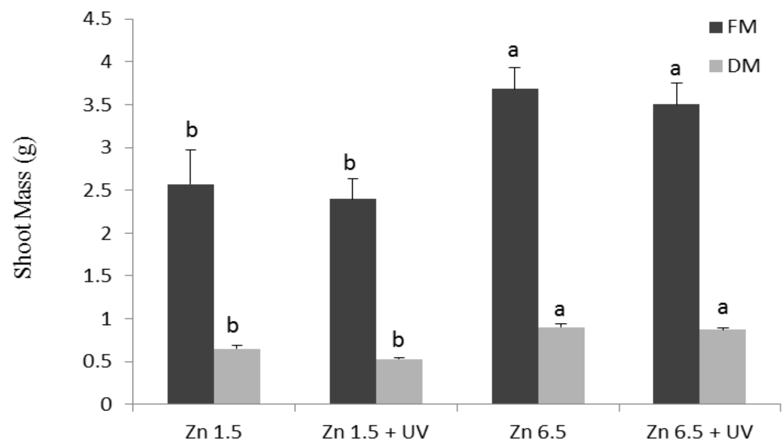

Figure 1: Effect of UV treatment on the fresh and dry mass of parsley shoots under different concentrations of Zn

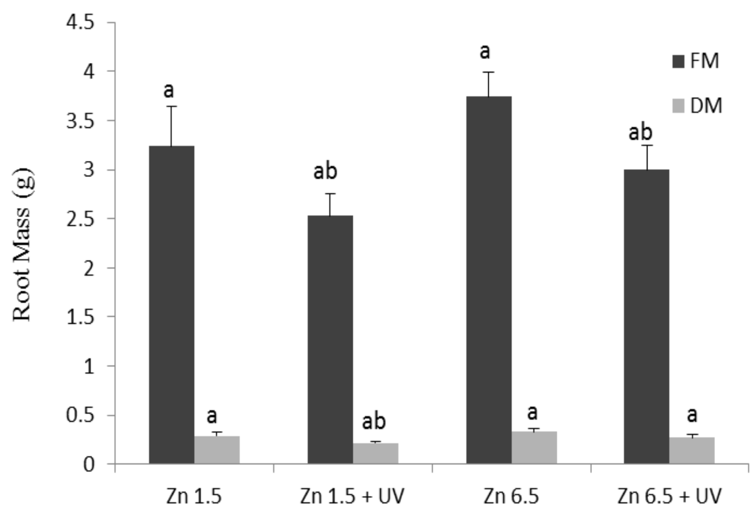

Figure 2: Effect of UV treatment on the fresh and dry mass of parsley roots under different concentrations of Zn

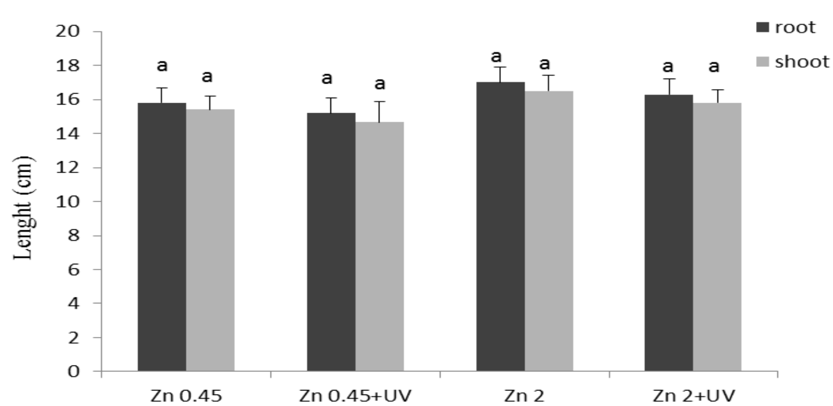

Figure 3: Effect of UV treatment on the length of parsley shoots and roots under different concentrations of Zn

3.2 Photosynthetic pigments

The concentrations of chlorophylls a, b and total were not significantly affected by UV treatment and Zn concentrations. But, carotenoids content and carotenoid/chlorophyll ratio of UV treated plants increased significantly ($p < 0.05$) in both concentrations of Zn. Application of Zn at concentration of 6.5 µm slightly increased the carotenoid content and carotenoid/chlorophyll ratio of plants compared to the concentration of 1.5 µm of Zn in both conditions (Table

1). According to results obtained from this study, parsley plant could effectively protects its own chlorophyll content against the enhanced UVA + B radiation by increasing the carotenoids content in both levels of Zn. Similar to this study, Salama et al. (2011) reported that in *Rumex vesicarius* L. the chlorophyll content of plant was not affected notably by UV treatment. The carotenoids are involved in the photosynthetic structures protection against the destructive effects of UV radiation (Nasibi and Kalantari, 2005). The efficacy of carotenoids in protecting the photosystems is likely due to their function as efficient quenchers of high energy of short wave radiation. The plant capacity in the protection of photosynthetic pigments content under enhanced UV conditions restores plant photosynthesis rate and tolerates plant against this stress (Levall and Bornman, 2006; Reshmi and Rajalakshmi, 2012). Zn application in this study did not affect the chlorophylls contents of parsley plant significantly. Similar to this result are those obtained for maize plants at different concentrations of Zn under the condition without stress (Saeidnejad and Kafi, 2013).

Table 1: Effect of UV treatment on photosynthetic pigments of parsley plant under different concentrations of Zn

treatment	Chlorophyll a (mg g^{-1}FM)	Chlorophyll b (mg g^{-1}FM)	Total chlorophyll (mg g^{-1}FM)	Carotenoid (mg g^{-1}FM)	Carotenoid/ chlorophyll
Zn 1.5 µm	0.14 ± 2.53 a	0.48 ± 3.07 a	0.15 ± 5.93 a	0.11 ±0.31 b	0.01 ± 0.05 bc
Zn 1.5 µm + UV	0.04 ± 2.63 a	0.09 ± 2.34 a	0.34 ± 5.15 a	0.97 ± 3.027 a	0.07 ± 0.48 a
Zn 6.5 µm	0.031 ± 2.59 a	3.33 ± 0.12 a	0.6 ± 5.95 a	0.19 ±0.54 b	0.09 ± 0.07 b
Zn 6.5 µm + UV	0.07 ± 2.55 a	0.27 ± 3.1 a	0.41 ± 5.64 a	0.65 ± 3.44 a	0.08 ±0.6 a

Each value represented as mean ± SE (n = 3); mean values followed by the same letter (s) are not significantly different ($p < 0.05$).

3.3 Phenolic compounds

UV treatment induced a significant increase in the anthocyanin content of parsley plants at concentration of 1.5 µm of Zn, but a slight increase was seen at concentration of 6.5 µm of Zn. The flavonoids concentration of plants significantly ($p < 0.05$) increased by UV treatment in both levels of applied Zn. There were some increases in the total phenols contents of plants treated with UV at both levels of Zn wich they were not significant. In normal conditions without UV radiation, application of Zn at concentration of 6.5 µm caused non-significant increase in these parameters compared to the concentration of 1.5 µmof Zn (Table 2). The increased level of UV absorbing phenolics is the common protective response to enhanced UV radiation in plant species (Reshmi and Rajalakshmi, 2012; Wei et al., 2013). It has been demonstrated that UV-B photoreceptor, UV RESISTANCE LOCUS8 protein (gene name: UVR8), absorbs UV-B light through conserved tryptophan residues (Mach, 2016). Absorbing UV-B causes the apparent UVR8 homodimer to dissociate into monomers, which interact with constitutively photomorphogenic1 (COP1), an E3 ubiquitin ligase (Rizzini et al., 2011). This interaction induces genes encoding protective factors such as phenylpropanoid biosynthesis pathway, and damage-repair factors such as photolyases (Fasano et al., 2014). Furthermore, the role of phenolic compounds as a product of phenyl propanoid pathway in the free radicals scavenging was also proved (Nasibi and Kalantari, 2005). It was proposed that plants with low levels of phenolic compounds are sensitive to UV radiation (Kim and Rodrigo, 2001; Zlatev et al., 2012). In this study, application of concentration of 6.5 µmof Zn increased the phenolic compounds compared to 1.5 µm. Our results about the positive effect of Zn application on biosynthesis of phenolic compounds is parallel to that reported for *Pistacia vera* L. by Tavllali et al. (2010) under saline condition.

3.4 Total proteins and soluble sugars

Total proteins and soluble sugars contents of UV treated plants decreased significantly ($p < 0.05$) in plants which received 1.5 µm of Zn, but the induced decreases in total proteins and soluble sugars in plants received 6.5 µm of Zn were not significant. At normal condition there was no main difference in these parameters between two levels of applied Zn (Table 2). The reductive effect of UV radiation on protein content is related to direct DNA injury, amino acid destruction and proteins and enzymes inactivation induced by UV radiation (Salama et al., 2011; Zlatev et al., 2012). Moreover, UV radiation causes the detrimental effects in the structure of RNA molecules and thus disrupts protein synthesis (Ulm and Nagy, 2005). According to this study, zinc application at high concentration in

UVA + B treated plants increased the protein content. The beneficial effect of Zn application on the protein content was reported for many species such as wheat plants under stress conditions (Morshedi and Farahbakhsh, 2010). Zn is necessary for the activity of the enzyme RNA polymerase and it protects the ribosomal RNA from attack by the enzyme ribonuclease. It is proposed that the most fundamental effect of zinc on protein metabolism is through its involvement in the stability and function of genetic material (Alloway, 2008).

The results attained for soluble sugars in this study were parallel to that obtained for total protein content. It has been proposed that UV radiation by inactivation the photosynthetic enzymes such as rubisco and some other Calvin cycle enzymes and damaging the photosystem II proteins adversely affects the photosynthesis and decreases the sugar synthesis (Zu et al., 2004; Zlatev et al., 2012). In this study, the induced reduction in soluble sugars by UVA + B treatment was not significant at sufficient amounts of Zn.The positive effect of Zn application on soluble sugar content probably is related to its role in protection of photosynthetic enzymes from UV damages and contribution in the structure of enzyme ribulose bisphosphat carboxylase (Alloway, 2008). The enhanced amounts of soluble sugars by sufficient Zn application was reported for different plant species such as *Cucurbita pepo* L. under stressful and normal conditions (Sorkhi Lalelou et al., 2013).

Table 2: Effect of UV treatment on anthocyanins, total phenolics, flavonoids, soluble sugars and total protein contents of parsley plant under different concentrations of Zn

treatment	Anthocyanins (mg g^{-1}FM)	Flavonoids (mg g^{-1}FM)	Total phenols (mg g^{-1}FM)	Soluble sugars (mg g^{-1}DM)	Total protein (mg g^{-1}FM)
Zn 1.5 µm	1.4 ± 0.08 b	1.35 ± 0.082 b	8.61 ± 0.7 b	109.8 ± 3.63 a	27.6 ± 0.59 a
Zn 1.5 µm + UV	1.94 ± 0.07 a	2.53 ± 0.32 a	14.32 ± 0.12 ab	79.1 ± 2.7 b	25.4 ± 0.21 b
Zn 6.5 µm	1.47 ± 0.06 b	1.39 ± 0.09 b	10.43 ± 0.7 ab	111.8 ± 4.23 a	28.7 ± 0.44 a
Zn 6.5 µm + UV	1.51 ± 0.045 ab	2.62 ± 0.22 a	16.11 ± 0.1 a	96.8 ± 6.3 a	26.53 ± 0.38 a

Each value represented as mean ± SE (n = 3); mean values followed by the same letter (s) are not significantly different ($p < 0.05$).

3.5 Antioxidant system

In this study, UV treatment significantly ($p < 0.05$) increased the activities of POD and CAT enzymes, but slightly decreased the activity of SOD enzyme at concentration of 1.5 µm of Zn. In plants received the concentration of 6.5 µmof Zn, UV treatment had no significant effect on the activities of SOD and POD, but significantly increased the CAT activity. In the conditions with no UV radiation, application of 6.5 µm of Zn could increase the SOD activity of plants compared to 1.5 µm Zn, but did not affect the POD and CAT activities considerably (Table 3).

H_2O_2 content of plants significantly ($p < 0.05$) increased in response to UV treatment in both levels of Zn. UV treated plants that received the concentration of 1.5 µm of Zn had the highest amounts of H_2O_2. Under condition without UV radiation, plants received 6.5 µm of Zn had slightly lower amount of H_2O_2 compared to plants which received 1.5 µm of this element. The MDH content of plants increased significantly only in the UV treated plants which received 1.5 µm of Zn. In the plants fed with concentration of 6.5 µm of Zn, UV could not enhance this metabolite considerably (Table 3). UV radiation induces oxidative stress in plants (Hakala et al., 2002; Tossi et al., 2009). The increased levels of ROS in plants damage biomolecules such as lipids and result to MDA formation as the breakdown product of polyunsaturated fatty acids of membranes. The effect of sufficient Zn application on controlling the production of these detrimental components was reported by authors in different full stress conditions (Tavallali et al., 2010; Weisany et al., 2012), but there is no available reference concerning role of this element under UV condition. Zinc plays a key role in controlling the generation and detoxification of free oxygen radicals and subsequent lipid membrane oxidation (Alloway, 2008). It has been demonstrated that Zn ions have strong inhibitory effect on membrane bound NADPH oxidase (Cakmak and Marschner, 1988; Kawano et al., 2002).

The activities of antioxidant enzymes POD and CAT in UV treated plants increased in this study. The enhancement in the activities of these enzymes in response to UV-B treatment were reported by several authors (Nasibi and Kalantari, 2005; Czégény et al., 2016). Antioxidant enzymes play a significant role in the dynamic equilibration between free oxygen radicals production and destruction. The responses of antioxidant enzymes to UV radiation vary among plants species (Tossi et al., 2009; Salama et al., 2011; Czégény et al., 2016). The increasing in the activities of antioxidant enzymes could be the indicator of build-up of a protective mechanism to reduce oxidative damages induced by stress (Harinasut et al., 2003; Chawla et al., 2013). According to results obtained from this study, the produced H_2O_2 effectively removed by POD and especially CAT in plants fed with concentration of 6.5 µm Zn. There was a small decrease in the activity of SOD enzyme in response to UV treatment in parsley plant in low level of applied Zn that was improved by application of 6.5 µm of Zn. Reduction in the activity of SOD enzyme as a result of UV-B radiation has been reported for sun flower plant (Costa et al., 2002). It has been proposed that a high amount of H_2O_2 is able to inhibit Cu-Zn-SOD via the reduction of Cu^{2+} to Cu^{+} (Casano et al. 1997). Zn is able to facilitate the biosynthesis of antioxidant enzymes (Cakmak, 2000) and its effects on improvement of antioxidant system of plants under various stresses have reported by several authors (Tavallali et al., 2010; Weiasany et al., 2012; Michael and Krishnaswamy, 2014).

Table 3: Effect of UV treatment on antioxidant enzymes activities that defined as unit (U) and MDH and H_2O_2 contents of parsley plant under different concentrations of Zn

treatment	SOD activity (U mg^{-1} pro min^{-1})	POD activity (U mg^{-1} pro min^{-1})	CAT activity (U mg^{-1} pro min^{-1})	H_2O_2 (µmol g^{-1}FM)	MDA (nmol g^{-1}FM)
Zn 1.5 µm	0.4 ± 0.03 ab	0.12± 0.09 b	0.45 ± 0.007 b	0.096 ± 0.005 c	1.74 ± 0.097 b
Zn 1.5 µm + UV	0.3 ± 0.015 b	0.25±0.04 a	1.3 ± 0.045 a	0.23 ± 0.023 a	5.57 ± 1.11 a
Zn 6.5 µm	0.47 ± 0.07 a	0.17±0.06 b	0.42 ± 0.09 b	0.088 ± 0.003 c	1.64 ± 0.017 b
Zn 6.5 µm + UV	0.5 ± 0.04 a	0.19 ± 0.02 b	1.5 ± 0.029 a	0.18 ± 0.01 b	2.7 ± 0.5 b

Each value represented as mean ± SE (n = 3); mean values followed by the same letter (s) are not significantly different ($p < 0.05$).

4 CONCLUSIONS

The results of this study showed the relative tolerance of parsley plant against applied doses of UV radiation at both concentrations of Zn, but it was more obvious at concentration of 6.5 µm. This plant could effectively increase UV absorbing phenolic compounds and carotenoids in response to UV radiation that are involved in photosynthetic apparatus protection. Moreover the induced increases in the activities of antioxidant enzymes in UV treated plants are responsible to moderate the ROS production in this plant. According to results obtained from this study, Zn application at concentration of 6.5 µm had positive effects on parsley resistance to UV radiation.

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Genetic relationships among okra (*Abelmoschus esculentus* (L.) Moench) cultivars in Nigeria

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ABSTRACT

This study was conducted on okra (*Abelmoschus esculentus* (L.) Moench) cultivars at the Teaching and Research Farm, University of Maiduguri, Nigeria. The objective was to evaluate the level of genetic divergence and heritability of eight characters in 2015 and 2016 dry seasons using irrigation. The results showed highly significant ($p<0.01$) differences in the ten okra cultivars for days to anthesis, plant height, fresh capsule length, fresh mass per capsule and fresh capsule diameter across the two years. A high genotypic coefficient of variation, heritability, and genetic advance were detected in all the characters except for days to anthesis and fresh capsule diameter. This implied that different genetic constitution and preponderance of additive effects governed these characters, thus presenting a significant opportunity for selection. The Mahanalobis D^2 analysis allotted the ten cultivars into four clusters. The highest was cluster I comprising four cultivars, followed by cluster II containing three cultivars, cluster III consisting two cultivars, and cluster IV with mono genotypic. The three most superior okra cultivars (Salkade, Y'ar gagure and Kwadag) for yield and related characters could be exploited directly or introgressed with other promising ones in future breeding programs.

Key words: diversity; genetic advance; heritability; okra; fruit yield

IZVLEČEK

GENETSKA RAZMERJA MED IZBRANIMI SORTAMI JEDILNEGA OSLEZA (*Abelmoschus esculentus* (L.) Moench) V NIGERIJI

Raziskava je bila opravljena na izbranih sortah jedilnega osleza oz. okre (*Abelmoschus esculentus* (L.) Moench) na Teaching and Research Farm, University of Maiduguri, Nigeria. Namen je bil ovrednotiti raven genetske pestrosti in dednost osmih lastnosti sort osleza ob namakanju v sušnem obdobju 2015 in 2016. Rezultati so pokazali značilne razlike ($p<0.01$) pri desetih sortah v dolžini obdobja (številu dni) do cvetenja, višini rastlin, dolžini svežih plodov ('strokov'), sveži masi plodov in v premeru svežih plodov v obeh vegetacijskih sezonah. Visoke vrednosti genotipskega koeficienta variabilnosti, dednosti in genetskega napredka, povezanega s selekcijo, so bile ugotovljene pri vseh preučevanih lastnostih, razen pri številu dni do cvetenja in premeru svežih plodov. To nakazuje veliko genetsko raznolikost in prevladovanje aditivnih učinkov pri genetski kontroli teh lastnosti, kar se lahko ugodno uporabi pri selekciji. Mahanalobisova D^2 analiza je razdelila deset sort v štiri skupine (klastre). Največja skupina I je vsebovala 4 sorte, tej sledi skupina II s tremi sortami, nato skupina III z dvema sortama in mono-genotipska skupina IV. Tri najboljše sorte (Salkade, Y'ar gagure and Kwadag) glede na pridelek in z njim povezane lastnosti bi lahko bile uporabljene neposredno ali v križanjih z drugimi obetajočimi sortami v bodočih žlahtniteljskih programih.

Ključne besede: diverziteta; genetska prednost; dednost; jedilni oslez (okra); pridelek plodov

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1 INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) usually referred to as Lady's finger belongs to the Malvaceae family (Kishor et al., 2016). Okra is proposed to originate from the Tropical Africa from where it extensively spread to Asia, America, Southern Europe and other countries (Muhammad et al., 2013). In 2008, the five top most okra producing countries were Iraq, Nigeria, Togo, Sudan and India (FAOSTAT, 2010). However, Nigeria ranks third in okra amid fruit vegetables based on production and consumption, succeeding pepper and tomato (Ibeawuchi, 2007). The okra local cultivars diverged in growth habits including leaf arrangement and size, fruits branching, height and maturity period. During the vegetative stage, okra growth patterns are similar, but those that were highly vigorous produced improved leaf area and accumulated dry matter (Akanbi et al., 2010).

The unripe green finger-like seed capsule of okra, usually called "pod" are processed and consumed as stews and salads, soups, sliced, boiled and fried vegetables (Akanbi et al., 2010; Daniela et al., 2012). The fruits contain effortlessly digestible fiber, fat-free contents and low calories (Kumar & Sreeparvathy, 2010; Reddy et al., 2013). Okra fruits are used for soups and stews thickening due to its mucilaginous and tender texture nature, (Ijoyah & Dzer, 2012; Das et al., 2013). The fruit contents comprises of 9.7 % carbohydrate, 86.1 % water, 1.0 % fiber, 0.8 %, 0.2 % fat and 2.2 % protein (Saifullah & Rabbani, 2009). Furthermore, the unripe pods are very rich sources of potassium, vitamins, calcium, and other minerals. Okra is tolerant to various climatic conditions and adaptable to the Nigeria agroecology.

The collection of desirable plant germplasm relies on the proven accession features and genetic divergence, which are essential in genetic resources utilization (Olaoye et al., 2009; AdeOluwa & Kehinde, 2011). Genetic diversity denotes the variability in different crop species, and its links with accessions identification, which is important in gene bank curators (Bello et al., 2012ab; Bello et al., 2011; Osekitar & Akinyele, 2008). Morphological characterization of plants has been recommended as the first step to be adopted prior to far-reaching molecular research and biochemical analyses (Akash et al., 2013). Many researchers reported a substantial morphological degree of variance in the West African okra varieties (Adeniji, et al., 2007; Akanbi et al., 2010; AdeOluwa & Kehinde, 2011). The improvement in plant breeding scheme leans on high genetic differences in the population and the magnitude of inheritance of favorable attributes (Olawuyi et al., 2015, Bello et al., 2014ab). The variability obtained in a population are apportioned into non-heritable and heritable parameters utilizing genetically related components including heritability, the genotypic coefficient of variation and genetic advance, which are the core for selection (Muluken et al., 2016; Seth et al., 2016). The expected response to selection and methods of selection are assessed on high heritability values of the characters. Cluster analysis is one of the powerful tools in determining genetic divergence among varieties of crops. The objective of this study was to evaluate the level of genetic divergence and heritability of ten okra cultivars in the stress-free irrigation conditions of Nigeria Sudan savannah, with the view to devising a breeding strategy for okra selection for further improvement.

2 MATERIALS AND METHODS

Field experiments were conducted on okra (*Abelmoschus esculentus* (L.) Moench.) cultivars at the Teaching and Research Farm, University of Maiduguri, Nigeria to evaluate the level of genetic divergence and heritability of eight characters in 2015 and 2016 dry seasons using irrigation. Ten okra cultivars were used; of which six cultivars (Salkade, Yar'gagure, Kwadam, Lai-lai, Yar'duwi and Y'ar kwami.) were obtained from Gagure, Gulani Local Government Area of Yobe State and four (Kwalpuku, Composite Kwadag and Mola Kwadag) were acquired from Borno State Agricultural programme, Maiduguri, Nigeria, and coded as described in Table 1.

Table 1: The morphological descriptions of ten okra cultivars studied

Code	Cultivars	Morphological descriptions
P1	Salkade	This cultivar is tall containing broad leaves, a red stem and few flowers, long fruit with a small diameter. The fruit is long, white and smooth with a small diameter.
P2	Y'ar duwi	It has short pale green stem, few flowers, and small slim fruit with no spine
P3	Composite	It has a dark green fruit of medium size with medium diameter. It also has a green stem and broad leaves with many flowers.
P4	Y'ar gagure	It has a pale green spiny fruit, broad diameter, and long stem. It also has red and sparsely flowers.
P5	Kwadag	It has a long stem with few flowers, big capsules with spine and a red stem.
P6	Kwalpuku	It has a short stem, small leaves with many flowers and spiny fruits.
P7	Y'ar kwami	It has a dark green fruit with many flowers and big capsules with spines.
P8	Mola Kwadag	It has a short green stem with small finger-like leaves. It also has many flowers with big capsules.
P9	Lai-lai	It is runner-like, short, dark green with medium capsule diameter. It also has a white stem and small leaves with many flowers.
P10	Kwadam	This cultivar has a short and white stem, medium leaves and spiny capsules.

The field experiment was based on Randomized Complete Block Design with three replications. The plot was 216 m^2, divided into 33 plots of 2 × 2 m with 1 m spacing between replications, and 0.5 m between treatments. Weeding was carried out manually at 3, 6, and 9 weeks after sowing (WAS). A compound fertilizer, N.P.K. 15:15:15 was applied at the rate of 60 kg N/ha in twice, first at three weeks after planting and then at flowering. Two milliliters of Ultracide 40EC insecticide in 15 liters was applied fortnightly to control insect pests. Light watering was applied using a watering can at every morning and afternoon. This was continued for a week for rapid and well establishment of the germinated seedlings.

For the evaluation of the eight studied characters; from each plot, ten (10) plants were randomly chosen. The eight studied characters include fresh capsule length, fresh capsule yield per plant (g), the number of primary branches per plant, days to anthesis, the number of capsules per plant, fresh capsule diameter (cm), fresh mass per capsule (g) and plant height (m). Individual year analysis of variance (ANOVA) was calculated, and then a combined ANOVA across the two years with the use of SAS PROC GLM model (Version 9.2, Volume 1), to determine the mean squares for every character (SAS Institute, 2011). A mixed model of the SAS PROC GLM model was utilized for the ANOVA. Cultivars were considered as fixed effects, while replication as a random effect. The degree of variation was estimated employing % coefficient of variation $p < 0.05$. Also, the variations in the character means were computed with the use of Least Significant Difference (LSD). However, genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were computed based on the formula proposed by Burton (1952) with the use of PROC GLM model of SAS (SAS Institute, 2011, Version 9.2, Volume 1). The broad sense heritability was determined as recommended by Johnson et al. (1955). The estimate of the expected genetic advance for each character was ascertained using the procedure of Allard (1960). The approach of Comstock & Robinson (1948) was followed for estimating the genetic advance in a percentage of the mean. To evaluate genetic divergences in the ten okra cultivars, the Mahalanobis' D^2 model (Mahalanobis 1936) and its auxiliary analysis were applied following the method of Rao (1952).

3 RESULTS AND DISCUSSION

The combined analysis of variance revealed exceedingly significant ($p < 0.01$) differences in the studied okra cultivars for days to anthesis, plant height, fresh capsule length, fresh mass per capsule and fresh capsule diameter across the two years (Table 2). It shows that the genetic parameters of the parental materials were quite dissimilar. This result corroborates with that of several earlier researchers (Akinyele & Osekita, 2006; Nwangburuka et al., 2011; Nwangburuka et al., 2012; AdeOluwa & Kehinde, 2013; Muluken et al., 2016). Again, the cultivars exhibited significant ($P<0.05$) differences for fresh capsule yield per plant and the number of capsules per plant. On the other hand, nonsignificant differences in the cultivars for the number of primary branches per plant revealed that the genetic parameters of the okra cultivars were very intact. The ANOVA also showed variations in the studied cultivars for almost all the characters. This variation could be used via selection to improve the okra studied characters. This result is supported by several previous researchers (Düzyaman, 2005; Salesh et al., 2010; Nwangburuka et al., 2012; Hazem et al., 2013; Amoatey et al., 2015). The first-order cultivar × year interaction was significant for all the okra characters. It signified that environment condition affected the wide variation of these characters, and is considered as a key parameter for the yield of crops. The okra yield potential is essential in producing many capsules per plant which could be attained by means of timely harvesting the fresh fruits to enable the development of more branches. This effort will undeviatingly increase the crop yield. This finding is also in conformity with the previous researchers (Akinyele & Osekita 2006; Mehta et al., 2006; Alade et al., 2008).

Table 2: Combined ANOVA for eight studied okra characters in Maiduguri, Nigeria across two years

Sources of variation	Days to anthesis	Primary branches per plant	Fresh capsule yield per plant	Capsules per plant	Plant height	Fresh capsule length	Fresh capsule diameter	Fresh mass per capsule
Year (Y)	8.22	22.88	9.67	11.54	6.61	20.92	6.25	6.63
Rep (Year)	19.27	18.63	12.63	4.86	4.44	6.54	14.74	19.22
Cultivars	862.29**	174.11**	2376.11**	39.19**	4286.82**	5.85**	67.66**	65.44**
Cultivar × Year	63.82*	54.65*	45,53	41.53*	72.97*	34.74*	38.37*	38.45*
Error	24.59	4.15	5.89	1.14	8.28	0.34	4.11	7.21

*,**, significant at P< 0.05 and P< 0.01 respectively

The mean performance of the studied okra cultivars showed a significant difference in days to anthesis (Table 3). The maximum number of days to anthesis (50.69 days) was recorded for ‘P4’, while the minimum number of days (42 days) was obtained in ‘P10’. The average number of days to anthesis was 46.82 days and five cultivars had higher days to anthesis than the average. It denoted that the assessed cultivars differed morphologically from one other especially on flower bearing habits, similar to the findings of Muluken et al. (2016). Besides, all the studied okra cultivars varied decidedly for plant height with ‘P5’ being the tallest while P3 cultivar was the shortest. The average plant height in ‘P5’ and ‘P4’ was 1.25m which was higher than the overall mean height. The number of primary branches per plant significant differed in the cultivars with ‘P1’ possessing the greatest number, whereas ‘P10’ exhibited the least. Subsequently, the greatest number of primary branches was obtained from ‘P1’, followed by ‘P7’, ‘P5’, and ‘P4’ which were more than the average. It is obvious that the number of primary branches varied significantly at the early growth of okra, as previously reported by Jagan et al. (2013). The average number of capsules per plant recorded was 28.03 with about half of the cultivars accomplishing a greater average. The ‘P4’ was outstanding with the greatest number of capsules per plant, followed by ‘P1’’, ‘’P5’, and ‘P9’. The highest number of fresh capsule length was attained in ‘P1’, while ‘P6’ had the lowest. This variation might be the differences in the number of bearing internodes and plant height in the cultivars. Half of the okra cultivars had an extended fresh capsule length than the average length. Fresh capsule length was reported to vary significantly from one accession to the other since it invariably articulates the distinctiveness (Nwangburuka et al., 2012; Hazem et al., 2013; Amoatey et al., 2015). The ‘P2’, ‘P4’, and ‘P5’ had fresh capsule diameter more than the average, while 40 % of the cultivars produced more than the average fresh capsule mass. The okra capsule length is at variance from one cultivar to the other, perhaps due to differences in days to anthesis and other morphological characters. However, ‘P1’, ‘P4’, and ‘P5’ also possessed capsule yield greater than the average.

Table 3: Mean performance for eight studied okra characters in Maiduguri, Nigeria across two years

Cultivars	Number of primary branches per plant (no.)	Days to anthesis	Fresh capsule yield per plant (g)	Number of capsules per plant (no.)	Fresh capsule length (cm)	Plant height at harvest (m)	Fresh capsule diameter (cm)	Fresh mass per capsule (g)
P1	4.23	50.33	598.65	33.75	14.88	1.44	1.22	16.23
P2	2.84	43.11	479.38	25.34	12.34	1.14	1.56	15.96
P3	1.89	44.23	428.62	22.54	12.52	1.11	1.48	14.73
P4	3.51	50.75	616.97	34.45	14.73	1.39	1.83	16.56
P5	3.75	49.31	622.67	34.35	14.37	1.49	1.84	16.83
P6	3.18	43.00	488.38	23.92	11.49	1.18	1.51	13.14
P7	3.33	45.34	431.63	25.18	13.37	1.18	1.52	14.34
P8	2.66	49.11	457.92	24.88	12.84	1.21	1.48	14.64
P9	2.12	47.93	532.85	28.64	11.59	1.16	1.52	14.98
P10	1.67	45.11	580.38	28.23	13.64	1.22	1.44	15.11
Mean	2.92	46.82	523.75	28.03	13.08	1.25	1.54	15.25
Range	2.56	7.33	194.05	11.91	3.39	0.38	0.62	3.68
SE±	2.759	11.849	11.73	6.654	6.149	6.111	11.234	10.171
LSD α 0.05	1.45	2.23	3.57	4.62	2.14	1.11	1.01	1.43*
CV %	6.39	4.36	6.83	4.92	10.52	7.82	7.45	7.18

Variability parameters estimated viz. GCV, PCV, heritability and genetic advance studied characters are depicted in Table 4. The large magnitude of PCV and GCV with a small difference between the two heredity parameters indicated a smaller amount of environmental influence on the phenotypic expression. Muluken et al. (2016) earlier buttressed this extrapolation. The GCV and PCV ranged between 1.1–33.3 % and 2.4 %–48.5 %, respectively, for fresh capsule yield and fresh capsule diameter. Several researchers reported the consistent differences of okra cultivars due to cultivars and environmental interactions (Thirupathi et al., 2012; AdeOluwa & Kehinde, 2013; Ehab et al., 2013; Adekoya et al., 2014). This statement also showed the level of productivity in crops, as statistical groupings of the cultivars were believed to be distinguished. Several researchers corroborate these discoveries for West African okra germplasm (Nwangburuka et al., 2012; AdeOluwa & Kehinde, 2013; Adekoya et al., 2014). The number of primary branches and fresh capsule diameter, on the other hand, were having the lowest estimate of below 10 % for PCV and GCV. This expressed a slight range of difference and hindered possibility for selection of these characters. Furthermore, the least GCV and PCV estimate of characters, implied higher impacts environmental conditions on these characters therefore; selection based on phenotypic basis will not be useful for the genetic progress of the crop (Chaurasia et al., 2011; Bharathiveeramani et al., 2012; Das et al., 2012; Sankara & Pinaki, 2012; Thirupathi et al., 2012; Ehab et al., 2013; Kishor et al., 2016]. Contrariwise, days to anthesis possessed medium GCV and PCV values (Chaurasia et al., 2011). It indicated that genetic effects influenced these characters. Therefore, these characters are responsive to selection for onward improvement. The number of capsules per plant, fruit length, plant height, the number of primary branches, fresh capsule length, fresh capsule yield per plant and fresh mass per capsule exhibited high values more than 20 % for both PCV and GCV with a considerably low degree of variation between the two. This result substantiates with the findings of Ehab et al. (2013). Many researchers nevertheless, noticed that high magnitude of GCV and PCV inferred a low environmental manifestation effects on the characters, which probably increase greater improvement prospects through selection scheme (Salesh et al., 2010; Bharathiveeramani et al., 2012; Nwangburuka et al., 2012; Swati et al., 2014; Kishor et al., 2016). Thus, selections of favorable characters by utilizing high phenotypic and genotypic estimates could be exploited in enhancing the characters during the breeding program.

Broad sense heritability estimates ranged from 25.84 % for the number of capsules per plant to 93.84 % for fresh capsule yield per plant (Table 4). As described by Robinson et al. (1955), heritability is categorized as least with a range of 0-30 %, fair (31-60 %) and best (> 60 %**)**. In the present research, a broad sense heritability of greater than 60 % was obtained for capsule yield, plant height, days to anthesis, capsule length, capsule diameter, and capsule mass. These agronomic characters seemed to respond effectively to the pressure of selection. Whenever heritability is up to 80 % or more of a character, selection would be easy for such character. Thus, selection for all these characters might result in an increase in capsule yield of okra. A great heritability also showed a great genetic base. A close association between the phenotype and cultivar could be the cause of small environmental interplay conditions (Jagan et al., 2013; Muluken et al., 2016). Fairly broad sense heritability estimate was observed for the number of primary branches. The least heritability value, however, was obtained for the number of capsules per plant. This alluded that these cultivars may not be improved via direct selection. Whenever a character is of a range between medium and high heritability, a selection due to specific performance can allow rapid progress. Fairly heritability implied improvement via selection. The least heritability also indicated ineffectual direct selection for the advancement of the characters owing to environmental masking effects (Nwangburuka et al., 2012; Bello & Olawuyi, 2015; Muluken et al., 2016).

As described by Johnson et al. (1955), genetic advance as percent mean were categorized as high (≥20 %), moderate (10 ≤ 20 %) and low (0 ≤ 10 %). Based on this ranking, the number of capsules per plant, the number of primary branches, capsule length, plant height, capsule yield per plant and capsule mass possessed the genetic advance of greater or equal to 20 % (Table 4). This revealed the predominance of additive genetic effects for these characters. Capsule diameter and days to anthesis showed low and moderate genetic advance, respectively. Appropriately, this result depicted that expected progress from the selection of the cultivars is between 16.1 % (days to anthesis) and 51.8 % (capsule yield). This substantiates with the findings of Olawuyi et al. (2015) and Hazem et al. (2013).

The relative amount of heritable variability is not enough to determine the GCV only, except with the aid of heritability and genetic advance. The high estimates of heritability coupled with genetic advance offered sufficient information on each character and indicated a genotypic response to selection (Pradip et al., 2010; Sibsankar et al., 2012). High heritability and genetic advance were observed for all the characters studied except days to anthesis and fresh capsule diameter (Table 4). This showed that differences in the genetic background would enable great opportunity for selection. Furthermore, this demonstrated the preponderance of additive gene effects for these characters, instead of the environmental influences. Thus, selection can be made based on the phenotypic expressions of okra characters for the improvement of yield (Muluken et al., 2016). As moderate heritability and high genetic advance were noted for the number of primary branches of okra, low heritability and high genetic advance estimates were detected for the number of capsules per plant. This could also be on high environmental influences controlling the expression of the characters. This, therefore, possibly hinders the opportunity for selection for crop improvement due to the prevalence of non-additive (dominant and/or epistatic) or non-fixable effects. Jagan et al. (2013) earlier noticed these findings. In a condition of low heritability and genetic advance for the characters, unique approaches such as hybridization and recurrent selection should be adopted (Bozokalfa et al., 2013; Jagan et al., 2013).

Table 4: Estimates of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and environmental coefficient of variation (ECV) for eight studied okra characters in Maiduguri, Nigeria across two years

Characters	GCV (%)	PCV (%)	Heritability H^2(bs) (%)	Genetic Advance as % of mean	Genetic Advance (GA)
Days to anthesis	17.7	18.1	86.7	16.1	8.7
Number of primary branches per plant	24.8	29.9	48.41	20.2	7.4
Plant height	18.9	24.2	72.54	24.3	20.8
Fresh capsule diameter	1.1	2.4	84.54	6.9	7.4
Number of capsules per plant	21.9	27.3	25.84	28.7	18.6
Fresh capsule length	20.7	22.5	79.98	37.5	6.8
Fresh capsule yield per plant	33.3	48.5	93.84	51.8	103.3
Fresh per capsule	20.9	22.5	71.30	41.6	16.1

The parameters of four different Clusters, respective cultivars, and their numbers are presented in Table 5. Cluster I possessed the highest with 4 cultivars followed by Cluster II with 3 cultivars and Cluster III with 2 cultivars, whereas Cluster IV had one cultivar (mono genotypic). The highly varied cultivars, P7, P8, P9, and P10 were obtained from Cluster I. These clusters outline showed that geographical variation had an indirect association with genetic diversity. Genetic diversity in okra germplasm with the use of cluster analysis had earlier been reported by several researchers (Akotkar et al., 2010; Das et al., 2012; Umrao et al., 2014; Seth et al., 2016). In general, the cultivars distribution patterns from diverse geographical regions into discrete clusters were at random. This result might be attributed to the free exchange and recurrent genetic constitution efforts by the farmers and plant breeders of the diverse agroecological zones in Nigeria. Besides, the disparity of selection pressure owing to regional okra favorites could improve the resemblance of the cultivars. Lack of relationship between the genetic diversity and geographical distance indicated that forces like natural and artificial selection, the exchange of genetic material, genetic drift and spontaneous mutation could lead to genetic diversity instead of geographical origin (Pradip et al., 2010; Seth et al., 2016). Therefore, selection for outcrossing of okra cultivars ought to base on genetic diversity instead of geographic diversity.

Table 5: Clustering form of 10 okra cultivars by Tocher's method

Cluster	Number of cultivars	Okra cultivars
I	4	P7, P8, P9, and P10
II	3	P1, P2, and P4
III	2	P5 and P6
IV	1	P3

Widely varied inter-cluster distances in the four clusters of okra cultivars are shown in Table 6. The inter-cluster distances were higher than the intra-cluster distances, signifying high genetic diversity among the cultivars of the varied groups. The intra-cluster distance in the ten studied cultivars indicated a low value between cluster IV and I (4.57), revealing similar relationships in the cultivars of these clusters (Pradip et al., 2010). The greatest intra-cluster estimate was observed between clusters III and I followed by between clusters IV and III. This depicts that the cultivars in these clusters diverged greatly. Therefore, outcrossing the cultivars featuring in these clusters could enhance transgressive segregations and generation progress. This has been as earlier opined by Umrao et al. (2014) and Seth et al. (2016). Conclusively, an involvement of highest yielding cultivars (Salkade, Y'ar gagure and Kwadag) could be exploited directly or hybridized to enhance novel recombinants and exploit transgressive segregates with high genetic yield potentials.

Table 6: Mean intra (bold) and inter-cluster D2 values of 4 clusters for 10 okra cultivars formed by Torcher's method

Cluster	I	II	III	IV
I	-			
II	8.54	-		
III	14.2	7.24	-	
IV	4.57	6.13	9.83	-

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Prostorska analiza kmetijskih zemljišč v zaraščanju v Sloveniji

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IZVLEČEK

V prispevku je predstavljena prostorska analiza razredov dejanske rabe kmetijskih zemljišč v zaraščanju (razred rabe 1410) in kmetijskih zemljišč, poraslih z gozdnim drevjem (razred rabe 1800), z namenom ugotoviti vpliv izbranih naravnih dejavnikov (delež gozda, nadmorska višina, naklon površja, ekspozicija, poplavnost) in statusa upravljanja območja na pojavljanje in razporeditev teh zemljišč v Sloveniji. Vsi ti dejavniki vplivajo na upravljavske odločitve lastnikov. Ugotovili smo, da so zemljišča razreda rabe 1410 razporejena po celotni Sloveniji z glavnino vzdolž osi od jugozahoda proti severovzhodu, zemljišč razreda rabe 1800 je največ v jugozahodnem delu države. Na občinski ravni nismo našli izrazite povezave med deležem gozda in deležem raziskovanih razredov rab. Z večanjem nadmorske višine in naklona se odstotek rabe 1410 in 1800 poveča. Ugotovili smo večjo pojavnost razreda rabe 1400 na pogosteje poplavljenih območjih. Zaraščanje je večje znotraj območij z različnimi statusi upravljanja (npr. Natura 2000). Za izboljšanje stanja in preprečevanje nadaljnjega zaraščanja bi bilo v statističnih regijah, kjer sta razreda rabe 1410 in 1800 najbolj prisotna, potrebno pospešeno izvajati zakon o kmetijskih zemljiščih, ki določa odpravljanje zaraščanja na območjih z večjo boniteto zemljišč.

Ključne besede: kmetijska zemljišča; prostorska analiza; zaraščanje; raba tal

ABSTRACT

SPATIAL ANALYSIS OF THE ABANDONMENT OF AGRICULTURAL LAND IN SLOVENIA

In this study we performed a spatial analysis to determine the influence of selected environmental factors (forests share, elevation a.s.l., slope, aspect, flooding) and the management status of an area on the spatial distribution of the abandoned agricultural land (land use class 1410), and agricultural land with forest trees (land use class 1800) in Slovenia. All these natural factors influence the management decisions of landowners. We found out that land use class 1410 is distributed over the entire country, along the south-west to the north-east axis. The majority of the land use class 1800 can be found in the south-western part of the country. On municipality level, the link between the forest and studied land uses was weak. The percentage of land use classes 1410 and 1800 grows with increasing altitude a.s.l. and slope. Land use class 1400 is more frequent in flooded areas. The percentage of the abandoned agricultural land is greater in areas with special management status (e.g. Natura 2000 areas). A strict, professional and fast implementation of the Agricultural Land Act is needed to improve the situation and prevent further agricultural land losses.

Key words: agricultural land; spatial analysis; abandonment; land use

1 UVOD

Na svetovni ravni delež kmetijskih površin narašča in površin gozda upada (FAOSTAT, 2013). Razlog je predvsem v krčenju gozdov za potrebe kmetovanja. Rahla rast gozdnih površin je opazna v Evropi, Aziji in tudi ponekod v Severni Ameriki. Izguba svetovnih gozdnih površin v obdobju od leta 1990 do 2000 je bila 8,3 milijonov ha/leto in v obdobju od leta 2000 do 2010 5,2 milijona ha/leto (FAO, 2010). Gozd obsega preko 4 milijarde ha oziroma 31 % površine kopnega, na drugi strani kmetijska zemljišča skoraj 5 milijard ha oziroma 38 %. Primarni gozdovi (brez vpliva človeka) predstavljajo 36 % površine, sekundarni (antropogeno preoblikovani) 57 % in gozdne plantaže 7 % (FAO, 2010). Največ gozda, glede na površino, ima Južna Amerika (49 %) in najmanj Azija (19 %). Največ kmetijskih zemljišč po površini ima Azija (53 %) in najmanj Evropa (21 %) (FAOSTAT, 2013).

Enako kot v Severni Ameriki in Evropi je tudi v Sloveniji opazen trend povečanja površin gozda in upadanja kmetijskih površin (Keenleyside in Tucker, 2010; FAO, 2010; ZGS, 2011; SURS-STAT, 2012; EU, 2013). Razlogi za zmanjšanje obsega kmetijskih površin so opuščanje in zaraščanje za kmetijstvo manj ugodnih

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zemljišč ter urbanizacija za kmetijstvo ugodnejših zemljišč (Cunder, 1998; Jankovič, 2003; Hladnik, 2005). V obdobju od leta 2002 do 2007 je bilo v povprečju urbaniziranih 11 ha kmetijskih zemljišč dnevno (Vrščaj, 2008). Stopnja prehranske samooskrbe se manjša, saj za zadovoljevanje potreb prebivalcev vedno več hrane uvažamo (Plut, 2011). Na državni ravni se s preoblikovanjem krajine in vse večjim uvažanjem hrane povečujeta izguba pridelovalnega potenciala tal in prebivalstva ter prehranska odvisnost od tujine.

Zaraščanje je proces, ki nastane po prenehanju kmetijske obdelave (npr. paše, košnje, oranja) in poteka na opuščenih kmetijskih zemljiščih (FAO, 2006). Zemljišča so prepuščena naravnemu razvoju – sukcesiji. Sukcesija je spreminjanje združb organizmov v času kot posledica vpliva rastlinstva na določeno okolje. Sukcesijski proces je usmerjen, sprva v združbo z malo vrst, z medvrstnimi povezavami se razvije kompleksna (klimaksna, pedoklimaksna) združba, ki je značilna za neko okolje. Primarna sukcesija se odvija na območjih, kot so npr. ozemlja po umiku ledenika, ozemlja, prekrita z magmo, novonastali ognjeniški otoki. Sekundarna sukcesija se odvija na območjih po določeni motnji (požar, poplava), ki le delno odstrani rastlinstvo s prizadetega območja, ali na območjih, kjer je bila opuščena določena dejanska raba (kmetijska zemljišča, urbana zemljišča) (Wilfing, 1993; Krebs, 2001).

Vzroke oziroma dejavnike, ki otežujejo in dražijo kmetijsko pridelavo ter privedejo do opuščanja kmetijskih zemljišč, delimo na naravno pridelovalne, strukturne, socio-ekonomske in agrarno politične in so običajno medsebojno prepleteni (Cunder, 1998). Naravno pridelovalni dejavniki, ki vplivajo na kmetijsko pridelavo, so naklon, nadmorska višina, usmerjenost zemljišč (ekspozicija površja), lastnosti tal in prisotnost drugih razredov rabe na območju. Strukturni vzroki izhajajo iz neugodne lastniške in posestne strukture, kot so velikost in oblika parcel, velikost kmetijskega gospodarstva, razdrobljenost parcel, njihova oddaljenost od lastnikov in dostopnost (Cunder, 1998; Gellrich in Zimmermann, 2007; Ilc, 2008; Corbelle Rico in sod., 2012; Zaragozi in sod., 2012). Socio-ekonomski vzroki so prepleteni in vezani na lastnosti kmetovalcev in družbene razmere, kamor prištevamo spremembe tržnih razmer, splošno nezanimanje za kmetovanje, možnost zaposlitve ob kmetovanju v neposredni bližini, selitev v urbane centre in staranje prebivalstva (MacDoland in sod., 2000; Khanal in Watanabe, 2006; Ilc, 2008, Diaz in sod., 2011). Med vzroke za opuščanje kmetovanja in spodbujanje zaraščanja sodijo tudi agrarnopolitične odločitve (plačilne pravice) ter finančna (neposredna plačila) in davčna politika (katastrski dohodek), ki vplivajo na stroške pridelave (Cunder, 1998; Khanal in Watanabe, 2006). Renwick in sod. (2013) ugotavljajo, da lahko v primeru opustitve neposrednih plačil in ukrepov za podporo trgom v okviru skupne kmetijske politike (SKP) odstotek opuščenih površin znotraj Evropske unije naraste za 8 %. Nezanemarljiv je tudi vpliv naravovarstvene in okoljevarstvene zakonodaje, ki lahko spodbuja kmetijsko pridelavo (npr. ohranjanje tradicionalne kmetijske krajine na območjih Natura 2000, košnja strmih travnikov) ali opuščanje (npr. vzpostavljanje naravne vegetacije za krepitev biodiverzitete) (MacDonald in sod., 2000; Diaz in sod., 2011).

Zaraščanje opuščenih kmetijskih površin ima lahko mnogo posledic. Skupna biotska raznovrstnost se sprva zaradi naselitve pionirskih vrst poveča, a se ob vzpostavitvi kompleksnejših združb (gozd) ponovno zmanjša in ustali. Izjema je vzpostavitev monokultur, kot so nekatere invazivne vrste pri spontanem zaraščanju ali iglavci pri tvorbi plantaž (EU, 2013). Mozaičnost in heterogenost kulturne krajine se s povečevanjem površine gozda manjša, kar vpliva na okolje, življenje ljudi, turizem, dostopnost za rekreacijo ipd. (Pogačnik in sod., 1995; Jankovič, 2003; Pogačnik in sod., 2011). Večje homogene enote enega razreda dejanske rabe lahko vodijo v poenostavljeno gospodarjenje (MacDonald in sod., 2000). Opuščena in zaraščena zemljišča predstavljajo vir nadaljnjega širjenja gozdov in divjadi, a hkrati prispevajo tudi k povečanju biodiverzitete (EC, 1980; Keenleyside in Tucker, 2010; Renwick in sod., 2013), stabilizaciji terena, zmanjšanju erozije tal (EU, 2013) in povečanju sposobnosti zadrževanja vode, kot je podaljšana retenzija vode v času obilnih padavin (Poyatos in sod., 2003; Garcia Ruiz in Lana Renault, 2011).

Preden se določi potrebne ukrepe za preprečevanje ali odpravljanje zaraščanja, je treba celostno analizirati vzroke opuščanja in zaraščanja kmetijskih zemljišč in na podlagi tega določiti ustrezne ukrepe za preprečevanje ali odpravljanje zaraščanja na nekem območju, če so tla primerna za kmetijsko obdelavo (Hočevar in sod., 2004; Pogačnik in sod., 2011). Če preprečevanje ali odpravljanje zaraščanja ni smiselno, se določi alternativno sedanji dejanski rabi zemljišča, ali se jih prepusti sukcesiji (Cunder, 1998; MacDonald in sod., 2000).

Zaraščajoče se površine lahko vzpostavimo nazaj v kmetijske s pomočjo rekultivacije (Pogačnik in sod., 1995). Rekultivacija je odvisna od oddaljenosti, dostopnosti in oblike parcele ter pedogeoklimatskih dejavnikov (Corbelle Rico in sod., 2012). Poznamo več načinov rekultivacije, kot so požig zaraščene površine, ročno ali mehanično odstranjevanje lesne biomase in rekultiviranje s pomočjo živali (Vidrih in sod., 1996; Vodlan, 2006). Če preprečevanje in odpravljanje zaraščanja ni možno ali smiselno, je možno alternativno ekstenzivno kmetovanje v obliki manj intenzivne pridelave zelišč, dišavnic (npr. pridelava sivke na Krasu), različnih vrst jagodičja (npr. pridelava brinovih jagod na Krasu) (Cunder, 1998) ali drevesno-pašne dejanske rabe (Pogačnik in sod., 1995; Vidrih, 2010). V oddaljenih, težje dostopnih območjih lahko pogozdovanje služi za pridobivanje lesa, lov, rekreacijo ali kot naravovarstveno območje (Cunder, 1998; Cojzer, 2011; Corbelle Rico in sod., 2012). Pri oblikovanju kmetijske politike, ki bi preprečevala opuščanje kmetovanja, je potreben teritorialen in prostorski pristop (Renwick in sod., 2013). Zakonodaja se običajno razvija v smeri spodbujanja lokalnega gospodarstva (npr. združevanje kmetov, razvoj izdelkov z dodano vrednostjo), poenostavljanja spremembe lastništva, pravice do koriščenja zemljišča, milejše davčne politike, neposrednih plačil in pomoči pri odkupu pridelkov.

V prispevku nameravamo s pomočjo javno dostopnih prostorskih podatkov ugotoviti prostorsko razporeditev kmetijskih zemljišč v zaraščanju in kmetijskih zemljišč, poraslih z gozdnim drevjem po slovenskih občinah in statističnih regijah. Ugotoviti smo želeli tudi, kakšen je vpliv izbranih naravnih dejavnikov (naklon, nadmorska višina, ekspozicija, poplavnost, delež gozda) ter statusa upravljanja območja (NATURA 2000, parki in rezervati, območje z omejeni dejavniki, vodovarstveno območje, območje življenjskega prostora rjavega medveda) na razporeditev izbranih razredov dejanskih rab. V analizo smo vključili podatke o dejanski rabi zemljišč, kot je bila zaznana v času nastajanj tega prispevka.

2 MATERIALI IN METODE

2.1 Prostorski sloji in opis območja raziskovanja

Za potrebe prostorske analize smo v državnih ustanovah pridobili ustrezne prosto dostopne georeferencirane digitalne prostorske sloje (Preglednica 1, Slika 1).

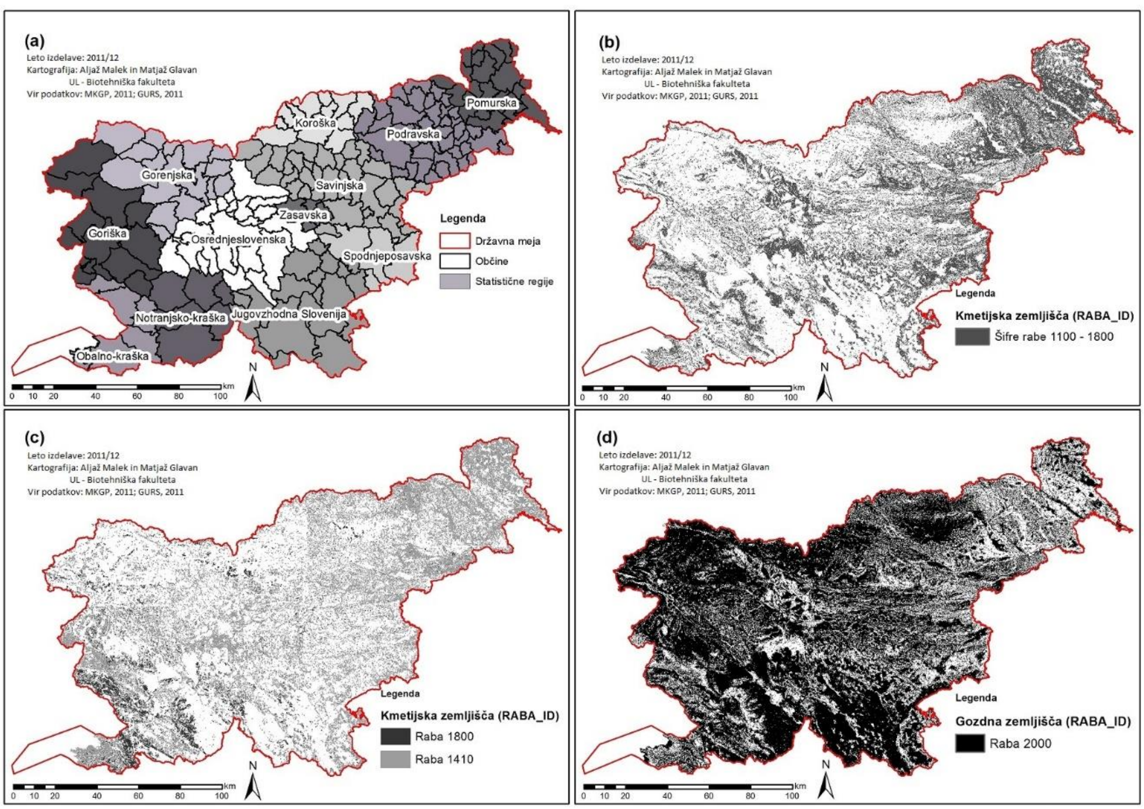
Preglednica 1: Nabor in vir georeferenciranih digitalnih podatkovnih slojev, uporabljenih v prostorskih analizah kmetijskih zemljišč v zaraščanju v Sloveniji
Table 1: Dataset and source of georeferenced data used in spatial analysis of the abandonment of agricultural land in Slovenia

Podatkovni prostorski sloj	Vir podatka
Dejanska raba zemljišč (RABA)	Ministrstvo za kmetijstvo, gozdarstvo in prehrano (MKGP, 2011)
Območja z omejenimi dejavniki (OMD)	
Državna meja	Geodetska uprava Republike Slovenije (GURS, 2011)
Meje občin	
Digitalni model višin 25mx25m (DMV)	
Ekološko pomembna območja (EPO)	Agencija Republike Slovenije za okolje (ARSO, 2011)
Območja Nature 2000 (NATURA)	
Zavarovana območja narave (ZO)	
Življenjski prostor rjavega medveda (ŽPRM)	
Poplavna območja (PO)	
Vodovarstvena območja (VVO)	
Meje statističnih regij	Statistični urad Republike Slovenije (SURS, 2011)

Po definiciji iz določanja razredov dejanske rabe je kmetijsko zemljišče v zaraščanju (v nadaljevanju: raba 1410) zemljišče, ki se zarašča zaradi opustitve kmetovanja ali preredke obdelave zemljišč, kar je lahko posledica neugodnih naravnih razmer za kmetovanje (naklon, osončenost, nadmorska višina, lastnosti tal, itd.) ali socio-ekonomskih razlogov (starost in izobraženost lastnikov, velikost kmetije, itd.) (Slika 1c) (MKGP, 2012). Na njem se pojavlja invazivno in trnasto rastlinje ter drevesa in grmičevje različnih starosti, katerih pokrovnost je 20–75 %. Drevesa so majhna, mlada in posamično razporejena. Neobdelanih vinogradov in drugih trajnih nasadov ne uvrščamo v ta razred, dokler so na letalskih posnetkih (ortofoto) vidne vrste (MKGP, 2012). Kmetijsko zemljišče, poraslo z gozdnim drevjem (v nadaljevanju: raba 1800) je površina, porasla s travinjem, na kateri rastejo posamična gozdna drevesa oziroma grmi in se redno, vsaj enkrat letno, popase oziroma pokosi (Slika 1c). Rabo 1800 smo vključili v analizo, ker v primeru opuščanja kmetijske pridelave na teh zemljiščih predstavljajo izhodišče za zaraščanje. V primeru uveljavljanja neposrednih plačil so ta zemljišča uvrščena med kmetijska zemljišča. Pokrovnost travinja je vsaj 80 %, pokrovnost drevesnih krošenj oziroma grmov je manjša od 75 % (MKGP, 2012).

V Sloveniji je bilo v letu 2011 25.278 ha rabe 1410, kar predstavlja 1,25 % celotne površine države. Zemljišč rabe 1800 je bilo 9.496 ha, kar predstavlja 0,47 % celotne površine države (MKGP, 2011). Zemljišča rabe 1410 so razporejena po celotni Sloveniji z glavnino v osi od jugozahoda proti severovzhodu, medtem ko je zemljišč rabe 1800 največ v jugozahodnem delu države.

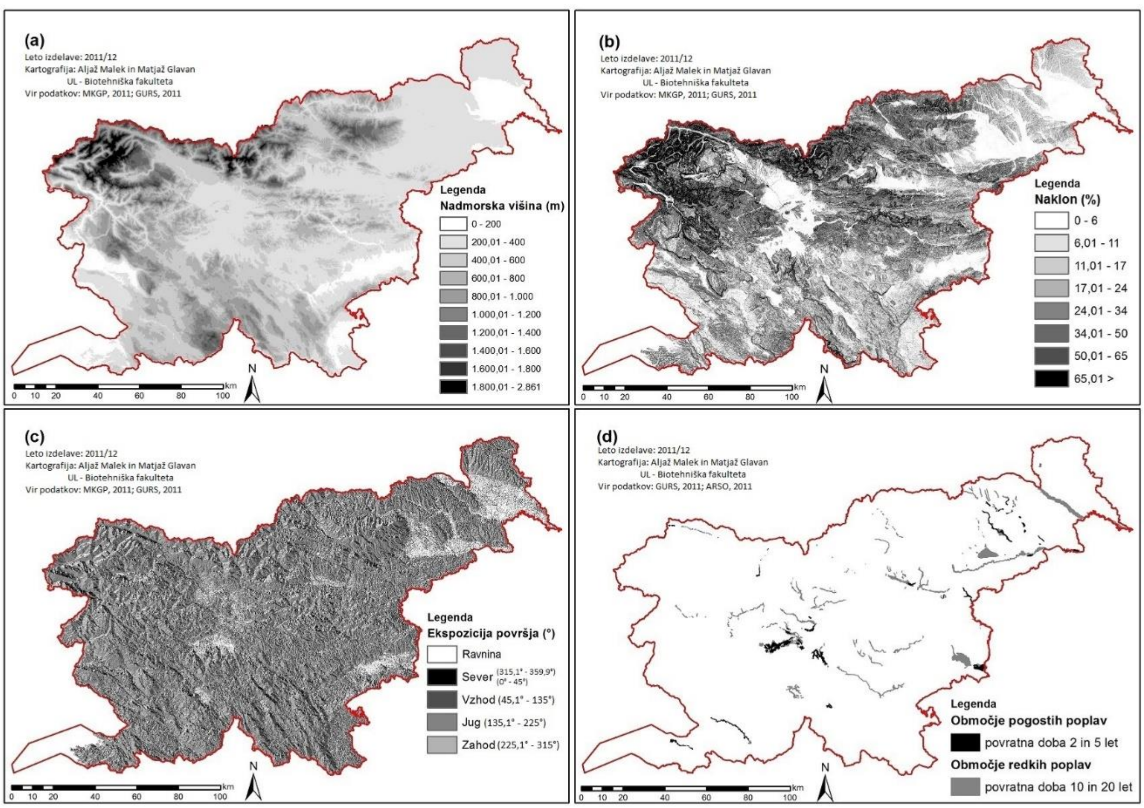
V razred dejanske rabe gozd (v nadaljevanju: raba 2000) spadajo zemljišča, porasla z gozdnim drevjem v obliki sestoja ali drugim gozdnim rastjem, ki zagotavlja katero koli funkcijo gozda. Med gozd se uvršča tudi zemljišča, poraščena z ruševjem (če je pokrovnost večja kot 75 %), površine, kjer je bila izvedena pomladitvena sečnja in so zato začasno brez drevja, poseke na trasah daljnovodov, cevovodov in smučarskih vlečnic, sanacije pogorišč, površine z mladimi drevesi, plantaže gozdnega drevja in gozdne drevesnice, ki so del gozda in jih na podlagi letalskih posnetkov (ortofoto) ne moremo posebej izločiti (MKGP, 2012). V razred gozd ne spadajo površine, poraščene z drevjem, kot so npr.: mestni parki in vrtovi (te se uvrščajo pod razred rabe 3000 – pozidano in sorodno zemljišče), posamične skupine dreves, ki imajo manjšo površino kot 2.500 m^2 in rekreacijska območja. Jase v gozdu se izločijo kot kmetijska zemljišča, če so večje od 5.000 m^2. Izločajo se tudi manjše površine, če so ta zemljišča v register kmetijskih gospodarstev vpisana kot GERK. Iz gozda so izločene ceste, ki so širše od 2 m in vodijo do naselij ali posameznih hiš (MKGP, 2012). Po evidenci MKGP je bilo v Sloveniji v času raziskave 1.210.010 ha gozdov, kar predstavlja 59,89 % površine države.



Slika 1: Prostorska razporeditev: (a) občin in statističnih regij, (b) združenih razredov kmetijske dejanske rabe od 1100 do 1800, (c) kmetijskih zemljišč v zaraščanju (1410) in kmetijskih zemljišč poraslih z gozdnim drevjem (1800) – meje poligonov so odebeljene za boljšo vidljivost, (d) gozda (razred rabe 2000) v Sloveniji

Figure 1: Spatial distribution of: (a) municipalities and statistical regions, (b) joined agricultural land use classes from1100 to 1800, (c) abandoned agricultural land use class (1410) and agricultural land use with forest trees class (1800) – polygon borders are made bold for better visibility, (d) forest (land use class 2000) in Slovenia

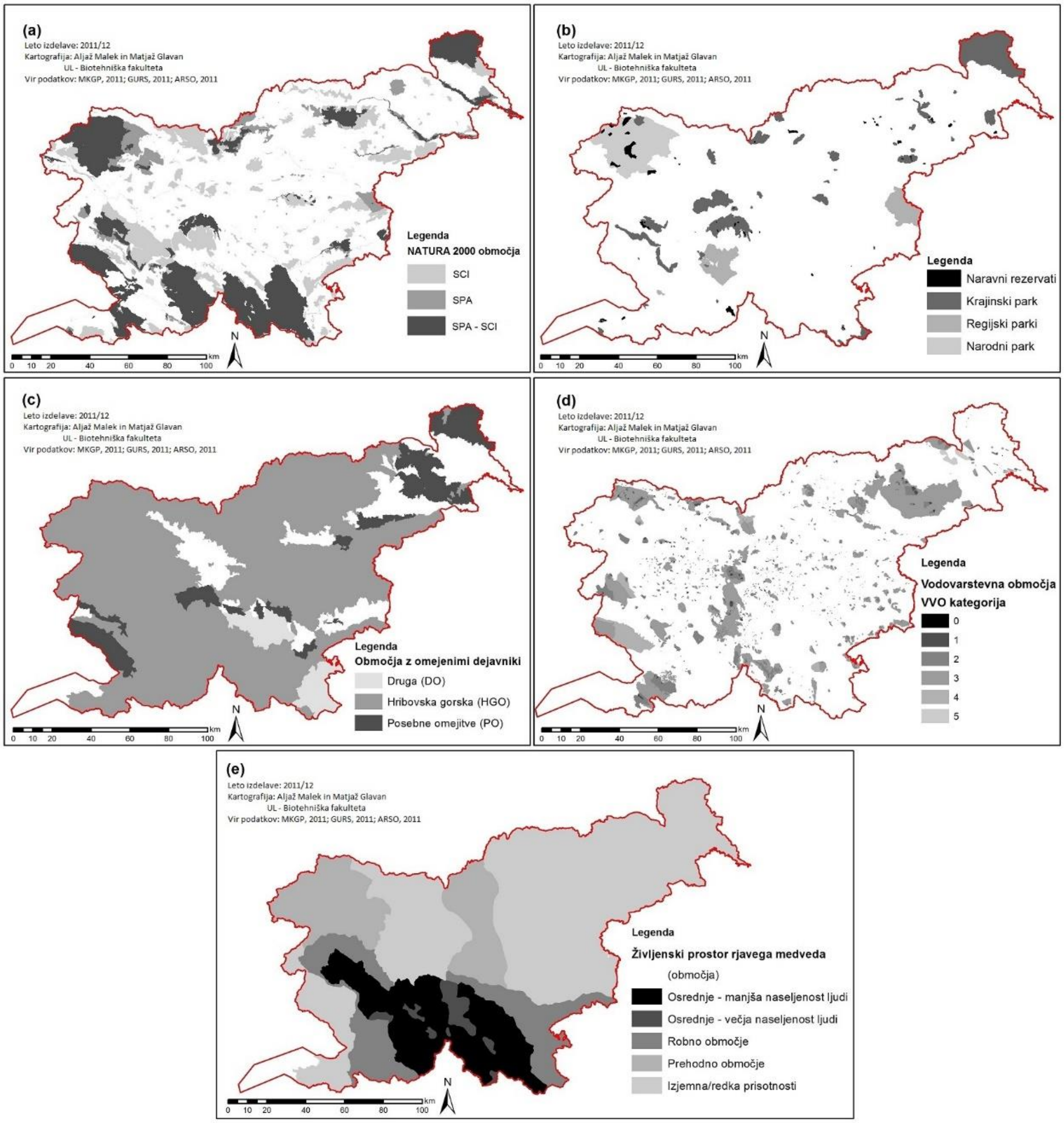
Digitalni model višin (DMV) smo za potrebe analize razvrstili v 10 razredov nadmorskih višin oz. v 200 metrske višinske pasove (Slika 2a). Največji odstotek od celotne površine države predstavljajo višinski pas od 200,01 do 400,00 m (33,07 % površine države). Najmanjši odstotek od celotne površine države predstavljajo višinski pas od 1600,01 do 1800,00 m (0,88 % površine države). Razrede naklona smo razdelili po merilih za bonitiranje zemljišč na 8 razredov, kot jih določa Pravilnik o določanju in vodenju bonitete zemljišč (RS, 2008) (Slika 2b). Največji odstotek od celotne površine države predstavljajo območja z naklonom od 0 do 6 % (21,12 %). Najmanjši odstotek predstavljajo območja z naklonom, večjim od 65 % (7,29 %). Površino Slovenije smo razdelili na pet razredov ekspozicije površja (Slika 2c). V analizi smo uporabili prostorske podatke poplavnih območij (Slika 2d) redkih poplav z dogodki s povratno dobo deset (Q10) do dvajset (Q20) let in pogostih poplav z dogodki s povratno dobo dve (Q2) do pet (Q5) let. Območij redkih poplav je v Sloveniji 29.344 ha (1,45 % Slovenije), območij pogostih poplav je 6.422 ha (0,32 % Slovenije).



Slika 2: Prostorska razporeditev razredov (a) nadmorske višine, (b) naklon, (c) ekspozicija površja in (d) pogostost poplavljanja v Sloveniji

Figure 2: Spatial distribution of (a) altitude above sea level classes (m), (b) slope classes (%), (c) surface exposition (°), and (d) flood areas in Slovenia

V analizo smo vključili območja petih izbranih upravljavskih statusov (Slika 3). Izbor je temeljil na: (1) območja so določena v zakonodaji; (2) območja so umeščena v prostor z jasnimi mejami; (3) režim upravljanja območij vpliva na kmetijsko dejavnost in odločanje lastnikov o vrsti dejanske rabe zemljišč. Ta območja so: (a) NATURA 2000; (b) zavarovana območja; (c) območja z omejenimi dejavniki za kmetijsko dejavnost; (d) vodovarstvena območja in (e) območje življenjskega prostora rjavega medveda. Območja NATURA 2000 so bila potrjena z Uredbo o posebnih varstvenih območjih (RS, 2004a) in jih delimo na območja, določena na podlagi direktive o habitatih – SCI (79/409/EGS) in območja, določena na podlagi direktive o pticah – SPA (92/43/EGS) (Slika 3a). Prostorski podatkovni sloj zavarovanih območij narave prikazuje območja, ki so zavarovana po različnih predpisih o ohranjanju narave (Slika 3b). Zavarovana območja se po Zakonu o ohranjanju narave delijo na ožja zavarovana območja (naravni spomenik, strogi naravni rezervat in naravni rezervat) in širša zavarovana območja (narodni, regijski in krajinski park) (RS, 2004b).



Slika 3: Prostorska razporeditev: (a) območij NATURA 2000, (b) območij nacionalnih, regijskih ali krajinskih parkov, (c) območij z omejenimi dejavniki za kmetijsko dejavnost, (d) vodovarstvenih območij in (e) območij življenjskega prostora rjavega medveda v Sloveniji

Figure 3: Spatial distribution of (a) NATURA 2000 areas, (b) national, regional or landscape parks areas, (c) naturally less favoured areas for agricultural activities, (d) water protection areas, and (e) areas of life environment of brown bear in Sloveni

Leta 2011 je bilo 286 območij Natura 2000 s skupno površino 720.286 ha, kar je predstavljalo 35,53 % površine države (Slika 3a). Prostorski podatkovni sloj zavarovanih območij narave obsega 252.929 ha (12,48 % Slovenije) in prikazuje območja, ki so zavarovana po različnih predpisih o ohranjanju narave (Slika 3b). Območja z omejenimi dejavniki za kmetijsko dejavnost (OMD) obsegajo 1.751.251 ha (86,38 %) in se v prostorskem sloju delijo na hribovska gorska območja v obsegu 1.467.573 ha (72,39 %), druga območja v obsegu 81.196 ha (4,00 %) in območja s posebnimi omejitvami v obsegu 202.482 ha (9,99 %) (RS, 2015) (Slika 3c). Vodovarstvena območja (VVO) v Sloveniji obsegajo 350.202 ha (17,27 %) in se glede na režim varovanja za zaščito vodnih virov delijo na državni nivo v obsegu 129.261 ha (6,38 %), občinski nivo v obsegu 216.160 ha (10,66 %) in vrelčni nivo v obsegu 4.781 ha (0,24 %) (Slika 3d). Območje življenjskega prostora rjavega medveda se deli na štiri dele (Slika 3e). Osrednje območje obsega 347.625 ha

(17,32 %), od tega predstavlja 305.972 ha (15,25 %) območje manjše naseljenosti ljudi in 41.653 ha (2,08 %) območje gostejše naseljenosti ljudi. Robno območje obsega 255.662 ha (12,74 %), prehodno 308.523 ha (15,37 %), območje izjemne (redke) prisotnosti medveda pa obsega 1.095.003 ha (54,56 %) (Slika 3e).

Pravilnik o razvrstitvi kmetijskih gospodarstev v območja z omejenimi možnostmi za kmetijsko dejavnost (OMD) določa tri vrste območij (RS, 2015) (Slika 3c). V hribovska gorska območja (HGO) uvrščamo območja, kjer je pridelava na zemljiščih omejena zaradi nadmorske višine in/ali naklona zemljišč na manjši nadmorski višini. Za druga območja z omejenimi dejavniki (DO) so značilne tako neugodne naravne razmere (geologija, tla, konfiguracija terena, parcelna struktura) kot tudi socio-ekonomska in demografska struktura (odseljevanje, negativni prirast, brezposelnost, velikost kmetij). V območja s posebnimi omejitvami (PO) prištevamo območja, kjer se kmetovanje nadaljuje, da bi zagotovili ohranjanje ali izboljšanje okolja, ohranjanje življenjskega prostora na podeželju in varovanje turističnega potenciala območja.

VVO območja se glede na režim varovanja za zaščito vodnih virov delijo na državni, občinski in vrelčni nivo (Slika 3e). Nivoji se delijo še na kategorije varstvenih režimov (od 0 do 5). Najstrožji režim varovanja je v kategorijah 0, 1 in 2. V 3. in 4. kategoriji je režim blažji. Kategorija 5 zajema le vrelčni nivo vodovarstvenih območij. Vodovarstvena območja državnega nivoja se določajo glede na Pravilnik o kriterijih za določitev vodovarstvenih območij (RS, 2004c), vodovarstvena območja občinskega in vrelčnega nivoja se določajo z odloki in medobčinskimi uradnimi vestniki s pripadajočimi strokovnimi podlagami. Kategorija varstvenega režima vpliva predvsem na način gnojenja kmetijskih zemljišč in uporabe fitofarmacevtskih sredstev (strožji režim – manjša uporaba gnojil in sredstev za varstvo rastlin), kar se odraža v obsegu pridelka in prihodkov.

Območje življenjskega prostora rjavega medveda se deli na pet delov: (a) osrednje območje – manjša naseljenost ljudi; (b) osrednje območje – območje gostejše naseljenosti ljudi; (c) robno območje; (d) prehodno in (e) območje izjemne (redke) prisotnosti medveda. Območja oz. cone opredeljuje Strategija upravljanja z rjavim medvedom v Sloveniji iz leta 2002, ki je vodilo za načrtovanje akcijskega načrta, za upravljanje z rjavim medvedom (MOP, 2002, 2007).

2.2 Metodologija prostorske analize

Prostorska analiza je bila opravljena z geografskim informacijskim sistemom Esri ArcGis 10.0. Iz podatkovnega sloja RABA smo za nadaljnjo uporabo pripravili sloje rabe 1410, rabe 1800 in rabe 2000. Urejanju površin novih slojev v atributnih tabelah je sledil njihov uvoz v MS Office Excel, kjer je sledila statistična analiza. Za ugotavljanje odvisnosti pojavljanja rabe 1410 in rabe 1800 od izbranih naravnih danosti (nadmorska višina in naklon) sta bila uporabljena regresijski model in Pearsonov koeficient korelacije (povezanost odstotka rabe 1410 in rabe 1800 z rabo 2000 na območju posameznih občin). Pri preučevanju razširjenosti rabe 1410 in rabe 1800 so bili uporabljeni odstotki določene rabe glede na celotno površino kmetijskih zemljišč v izbranem razredu nadmorske višine oz. naklona. Za ugotavljanje povezave med rabo 1410 in rabo 1800 ter rabo 2000 so bili uporabljeni odstotki rabe od površin občin.

3 REZULTATI IN DISKUSIJA

3.1 Občine in statistične regije

Zemljišča z rabo 1410 so razporejena po celi Sloveniji z glavnino v osi od jugozahoda proti severovzhodu. Glede na delež površine regije je raba 1410 najbolj razširjena v obalno-kraški statistični regiji in pomurski statistični regiji. Glavnina kmetijskih zemljišč rabe 1800 je v jugozahodnem delu države (obalno-kraška, goriška in notranjsko-kraška statistična regija) (Preglednica 2).

Preglednica 2: Površina (ha) in odstotek (%) zemljišč rabe 1410 (kmetijsko zemljišče v zaraščanju) in 1800 (kmetijsko zemljišče poraslo z gozdnim drevjem) glede na celotno površino in površino kmetijskih zemljišč (KZ) po statističnih regijah Slovenije

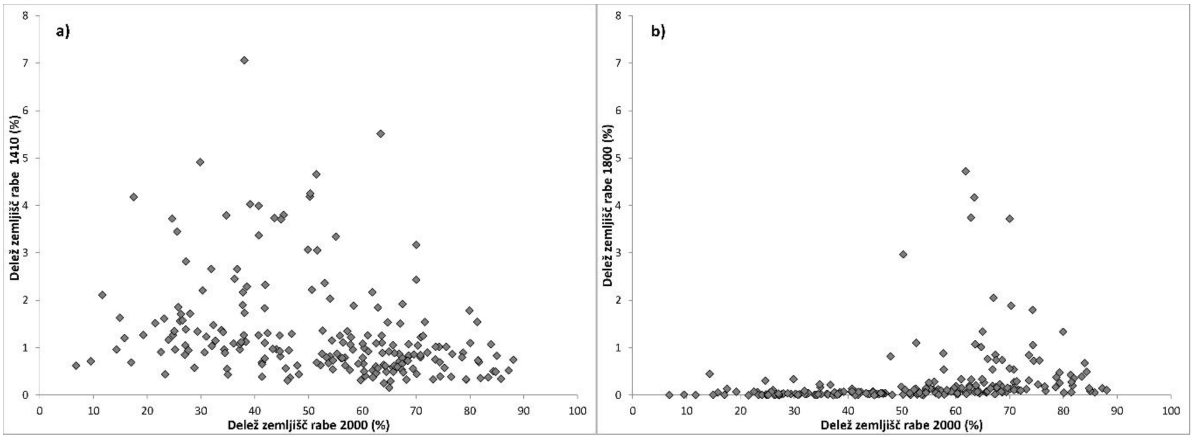
Table 2: Area (ha) and share (%) of abandoned agricultural land class (1410) and agricultural land use with forest trees class (1800) in comparison to total and agricultural land area (KZ) in statistical regions in Slovenia

Statistična regija	**Površina**							
	območje	**KZ**	**Raba 1410**			**Raba 1800**		
	ha	**ha**	**ha**	**% regije**	**% KZ**	**ha**	**% regije**	**% KZ**
Gorenjska	213.660	43.312	1.360	0,64	3,14	704	0,33	1,63
Goriška	232.550	52.000	3.613	1,55	6,95	1.734	0,75	3,33
Jugovzhodna Slovenija	267.509	69.891	2.842	1,06	4,07	679	0,25	0,97
Koroška	104.080	24.351	737	0,71	3,03	205	0,20	0,84
Notranjsko-kraška	145.634	37.685	1.275	0,88	3,38	1.789	1,23	4,75
Obalno-kraška	104.445	36.083	3.072	2,94	8,51	3.270	3,13	9,06
Osrednjeslovenska	255.496	80.372	2.570	1,01	3,20	513	0,20	0,64
Podravska	216.967	107.897	3.550	1,64	3,29	162	0,07	0,15
Pomurska	133.753	81.425	3.126	2,34	3,84	34	0,03	0,04
Savinjska	238.398	86.021	1.800	0,76	2,09	294	0,12	0,34
Spodnjeposavska	88.514	39.145	1.112	1,26	2,84	54	0,06	0,14
Zasavska	26.375	7.602	222	0,84	2,91	57	0,22	0,75
Skupaj	**2.027.380**	**665.782**	**25.278**	**1,25**	**3,80**	**9.496**	**0,47**	**1,43**

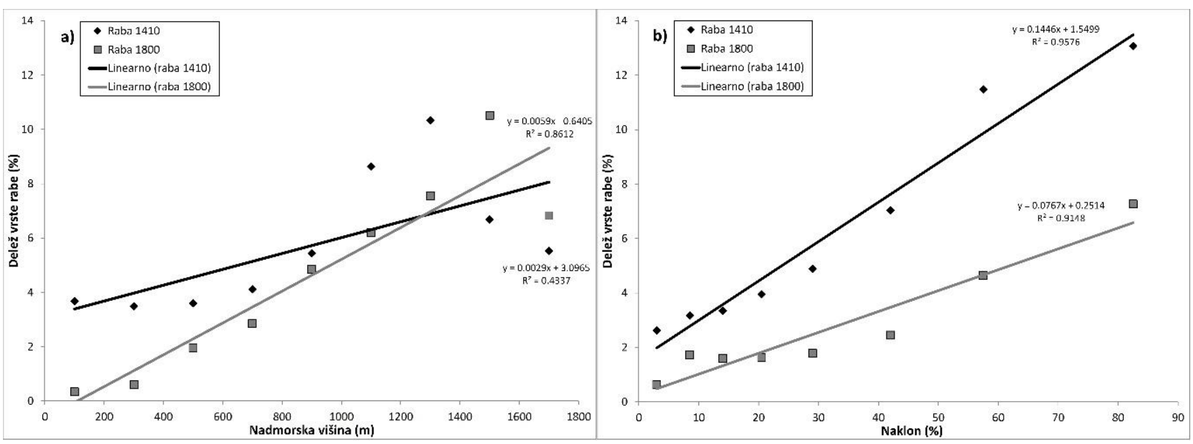
Največ rabe 1410 je v delih regij (Kras, Brkini, Barje, Haloze, Slovenske gorice, Goričko), kjer ob sočasnih spremembah razmer na trgu dela in na kmetijskem trgu prihaja do neugodnih socio-ekonomskih razmer. Tudi v analizi ukrepov upravljanja progama Natura 2000 za sektor kmetijstva so kot enega pomembnih razlogov za zaraščanje in zmanjševanje biotske pestrosti navedli socio-ekonomske vidike (starost, bolezen, ni naslednika) (Žvikart in sod., 2013). Ob tem se kmetijstvo ni zmožno dovolj hitro prilagoditi npr. z lastniško strukturo ali velikostjo parcel. Pogosto je omejujoč faktor naravna danost (naklon, tla), zato je v takih razmerah edina možnost ekstenzifikacija pridelave. Obalno-kraška statistična regija predstavlja izredno priložnost za krepitev drevesno-pašnega sistema na že obstoječih kraških gmajnah in za njegovo uvedbo na širšem območju, po vzoru savan in dehes. O možnosti ekstenzivnega koriščenja manj zanimivih kmetijskih zemljišč ter njegovih prednostih ob omejenih naravnih danostih za intenzivno kmetijsko pridelavo piše več avtorjev (Knap, 2008; Eler in sod., 2008; Vidrih, 2010). V regijah in občinah, kjer je zaraščanja manj, bi bilo na podlagi sosednje vrste dejanske rabe in lastnosti zemljišč treba razmisliti, v katero smer razvijati nadaljnjo dejansko rabo teh zemljišč. Ena od možnosti je sečnja lesne biomase iz zaraščenih območij in predelava v sekance ali pelete (Vodlan, 2006; Humar, 2008).

3.2 Naravni dejavniki

Statistična analiza je pokazala, da imajo glede na površino največji odstotek rabe 2000 občine Osilnica (3.191 ha, 88,11 %), Črna na Koroškem (13.605 ha, 87,23 %), Dolenjske Toplice (9.459 ha, 85,83 %), Kostel (4.769 ha, 85,03 %) in Lovrenc na Pohorju (7.160 ha, 84,80 %). Zemljišča rabe 1410 in 1800 v teh občinah predstavljajo manj kot 1 % površine občine. S pomočjo Pearsonovega koeficienta korelacije smo ugotavljali povezanost deležev rabe 1400 in rabe 1800 z rabo 2000 (Slika 4). Med rabama 1410 in 2000 (Pearsonov koeficient korelacije: - 0,3) in med rabama 1800 in 2000 (Pearsonov koeficient korelacije: 0,26) ni opaznih izrazitih vzorcev (Slika 4). Občine na JZ države z večjim odstotkom gozda imajo večji odstotek rabe 1800. Z izvajanjem kmetijske dejavnosti (paša, občasna sečnja in mulčenje) se slednja počasneje ali sploh ne zaraščajo, že obstoječa starejša drevesa rastejo naprej, medtem ko se podrast stalno odstranjuje. Vendar vzdrževanja rabe 1800 ni možno izvajati strojno, zato so pašne živali nujno potrebne. Vzreja pašnih živali zahteva stalno fizično prisotnost pastirja ali dobro varovano ograjo, ki preprečuje dostop divjim zverem, ki na območje rabe 1800 dostopajo iz sosednjih gozdov (Černe in sod., 2010). Prav škodni dogodki kot posledica napada divjih živali (volkovi, medvedi) so glavni razlog opuščanja pašništva na območju obalno-kraške in notranjsko-kraške regije (Pogačnik in sod., 2006).



Slika 4: Razsevni grafikon predstavlja povezanost razredov dejanske rabe (a) kmetijska zemljišča v zaraščanju (1410) in (b) kmetijska zemljišča porasla z gozdnim drevjem (1800) z odstotkom gozdne rabe (2000) po občinah
Figure 4: Scattered chart presenting connections between classes of actual land use (a) abandoned agricultural land (1410) and (b) agricultural land with forest trees class (1800) and forest (2000) by municipality



Slika 5: Primerjava deležev razredov dejanske rabe kmetijsko zemljišče v zaraščanju (1410) in kmetijsko zemljišče poraslo z gozdnim drevjem (1800) v odvisnosti od (a) nadmorske višine in (b) naklon po regresijskem modelu
Figure 5: Comparison between shares of actual land use classes of abandoned agricultural land (1410) and agricultural land with forest trees (1800) in dependence from (a) altitude above sea level and (b) slope by regression model

Ugotavljali smo, kako se z nadmorsko višino in naklonom spreminja delež posameznih razredov rabe 1410 in rabe 1800. Uporabili smo regresijski model (Slika 5). Opazovan je bil odstotek rabe 1410 in 1800 od vseh in samo od kmetijskih zemljišč. V primeru upoštevanja celotne površine območja posameznega višinskega pasu se z večanjem nadmorske višine in naklona odstotek kmetijskih površin in s tem tudi rabe 1410 in 1800 zmanjšuje (Preglednica 3). V povprečju se odstotek zemljišč rabe 1410 in 1800, glede na odstotek kmetijskih zemljišč, na vsakih 100 m nadmorske višine poveča za 0,29 % in 0,59 %. V povprečju se odstotek zemljišč rabe 1410 in 1800, s povečanjem naklona za 10 %, poveča za 1,5 % in 0,8 %. Rezultati naše analize kažejo, da imata nadmorska višina in naklona vpliv na zaraščanje in se ujema z ugotovitvami drugih raziskav (Poyatos in sod., 2003; Hočevar in sod., 2004; Gellrich in Zimmermann, 2007, Ketiš in sod., 2014). Rezultat analize vpliva nadmorske višine in naklona nakazuje na povečanje površin rabe 1410 in 1800 v primeru opuščanja kmetijske obdelave v obliki paše ali košnje strmih travnikov v hribovitih predelih Slovenije. Še posebej izrazit je pojav v hribovsko-gorskem okolju, kjer opažajo upad populacije gozdnih kur zaradi zaraščanja gozdih jas, gorskih pašnikov in gozdnih robov, ki zaradi oddaljenosti niso več zanimivi za obdelavo (Kus Veenvliet, 2012). Kmetijska politika se zaveda vpliva opuščanja kmetijskih zemljišč, zato v okviru Programa razvoja podeželja Republika Slovenija

(2014–2020) namenja neposredna plačila za košnjo habitatnih travnikov na naklonih, večjih od 50 %.

Iz podatkov (Preglednica 3) je razvidno, da so severne in zahodne osojne lege manj ugodne za kmetovanje, kar privede do opuščanja in vodi v zaraščanje. Delno na večji odstotek zaraščanja vpliva tudi relief in smer poteka gorskih grebenov v Sloveniji, ki marsikje potekajo od severozahoda protu jugovzhodu in so na severnih in zahodnih legah bolj strmi ter s tem manj primerni za kmetijstvo. Zemljišča zahodnih in južnih leg na manjših nadmorskih višinah do 400 metrov imajo dober potencial za nadaljnjo, tudi bolj intenzivno kmetijsko pridelavo, kot so vinogradništvo in sadjarstvo ter oljkarstvo v Slovenski Istri in Goriških Brdih. Rabe 1800 se večinoma pojavljajo v jugozahodni Sloveniji, na območjih apnenčastih, dolomitnih in flišnih kamnin. Brez prisotnosti človekovega delovanja bi jih prekrival gozd s ekonomsko manj zanimivimi termofilnimi vrstami, ki so prilagojene tudi sušnim razmeram. Kmetovalci so jih zato izkoristili kot površine s kombiniranim gozdno-pašnim sistemov. Če teh površin ne vzdržujemo z redno pašo, se lahko hitro zarastejo z grmovno zarastjo in postanejo neprehodne. Večina teh površin je z obstoječo pašno rabo dosega svoj maksimalen izkoristek (Vidrih, 2005).

Površin rabe 1400 je glede na odstotek razreda in odstotek kmetijskih zemljišč več na območjih pogostih kot redkih poplav (Preglednica 3). Analiza je pokazala večji vpliv poplavnih območij na rabo 1410 kot na rabo 1800. Manjši vpliv na rabo 1800 izhaja iz dejstva, da je večina te rabe na specifičnih lokacijah, kjer se zaradi matične podlage (apnenec) in topografije (naklon) poplave ne pojavljajo. To potrjuje tudi majhen obseg (48 ha) teh zemljišč na poplavnih območjih. Poplavljanje posredno vpliva na zaraščanje kmetijskih zemljišč, saj lahko redne poplave otežujejo proces kmetovanja in zmanjšujejo pridelek. To lahko na mokrotnih travnikih in mokriščih (Cerkniško polje, Ljubljansko barje), privede do zmanjšanja rastlinske in živalske pestrosti, ki se najbolj očitno odrazi v številu ptic vlažnih travnikov (Žgavec in sod., 2013; Bordjan in Bordjan, 2014).

Preglednica 3: Površine (ha) in odstotek (%) rab kmetijsko zemljišče v zaraščanju (1410) in kmetijsko zemljišče poraslo z gozdnim drevjem (1800) v različnih razredih (a) nadmorskih višin (m), (b) naklon (%), (c) ekspozicije površja (°) in (d) pogostosti poplav glede na površino kmetijskih zemljišč (KZ)

Table 3: Area (ha) and share (%) of abandoned agricultural land use class (1410) and agricultural land use with forest trees class (1800) in different classes of (a) altitude above sea level classes (m), (b) slope classes (%), (c) solar radiation exposition (°), and (d) flood areas in comparison to total area of agricultural land use (KZ)

	Površina							
	območje	**KZ**	**raba 1410**			**raba 1800**		
	ha	**ha**	**ha**	**% razreda**	**% KZ**	**ha**	**% razreda**	**% KZ**
(a) Razredi nadmorske višine (m)								
0 - 200	180.280	103.525	3.803	2,11	3,67	347	0,19	0,33
200,01 - 400	670.932	319.051	11.127	1,66	3,49	1.923	0,29	0,60
400,01 - 600	472.631	134.435	4.841	1,02	3,60	2.620	0,55	1,95
600,01 - 800	308.479	62.029	2.554	0,83	4,12	1.767	0,57	2,85
800,01 - 1000	168.112	24.142	1.312	0,78	5,43	1.169	0,70	4,84
1000,01 - 1200	95.741	7.621	659	0,69	8,64	472	0,49	6,20
1200,01 - 1400	61.972	4.298	444	0,72	10,34	325	0,52	7,56
1400,01 - 1600	31.449	5.047	337	1,07	6,69	531	1,69	10,52
1600,01 - 1800	17.768	3.235	179	1,01	5,53	221	1,24	6,83
1800,01 >	19.927	2.302	59	0,30	2,58	120	0,60	5,22
Skupaj	**2.027.293**	**665.685**	**25.316**	**1,25**	**3,80**	**9.495**	**0,47**	**1,43**
(b) Naklon (%)								
0 - 6	428.089	265.174	6.947	1,62	2,62	1.665	0,39	0,63
6,01 - 11	191.912	83.824	2.660	1,39	3,17	1.448	0,75	1,73
11,01 - 17	228.292	89.362	2.989	1,31	3,35	1.432	0,63	1,60
17,01 - 24	247.958	82.288	3.254	1,31	3,95	1.333	0,54	1,62
24,01 - 34	300.696	77.089	3.770	1,25	4,89	1.374	0,46	1,78
34,01 - 50	315.478	49.780	3.501	1,11	7,03	1.218	0,39	2,45
50,01 - 65	167.137	11.315	1.299	0,78	11,48	526	0,31	4,65
65,01 >	147.731	6.852	896	0,61	13,07	498	0,34	7,27
Skupaj	**2.027.293**	**665.685**	**25.316**	**1,25**	**3,80**	**9.495**	**0,47**	**1,43**
(c) Razredi ekspozicije površja (°)								
Sever	436.290	96.624	4.116	0,94	4,26	1.382	0,32	1,43
Vzhod	483.991	153.663	5.800	1,20	3,77	1.925	0,40	1,25
Jug	532.092	198.361	7.918	1,49	3,99	3.657	0,69	1,84
Zahod	445.154	126.822	5.445	1,22	4,29	2.368	0,53	1,87
Ravnina	129.766	90.214	2.037	1,57	2,26	163	0,13	0,18
Skupaj	**2.027.293**	**665.685**	**25.316**	**1,25**	**3,80**	**9.495**	**0,47**	**1,43**
(d) Razredi pogostosti poplav								
Redke poplave	29.344	20.496	803	2,74	3,92	44	0,15	0,21
Pogoste poplave	6.422	4.984	209	3,25	4,19	4	0,06	0,07
Skupaj	**35.766**	**25.480**	**1.012**	**2,83**	**3,97**	**48**	**0,13**	**0,19**

Opomba: Zaradi uporabe rastrskih slojev v izračunu je skupna površina Slovenije nekoliko manjša.

3.3 Dejavniki upravljanja območij

Poudariti je treba, da je vpliv upravljavskega statusa območja na zaraščanje težje ovrednotiti, saj se različni statusi prostorsko prekrivajo. Iz analize je razvidno, da sta odstotka površin rabe 1410 in rabe 1800 glede na površino območja različno zastopana znotraj in zunaj območij. Glede na odstotek kmetijskih zemljišč na območjih je površina rab 1410 in 1800 večja znotraj kot zunaj obravnavanih območij (Preglednica 4). Upravljavski status območja lahko vpliva tako na zaraščanje kot na preprečevanje slednjega. Nekatera območja Natura 2000 so opredeljena kot kmetijske krajine. Različne raziskave potrjujejo, da opuščanje kmetijske dejavnosti negativno vpliva na populacije vrst, ki so od nje odvisne (MacDonald in sod., 2000; Kraj in Koren, 2011; Miličić in sod., 2011; Žgavec in sod., 2013; Živkart in sod., 2013; Bordjan in Bordjan, 2014). Vpliv upravljavskega statusa območja ni omejen le na območje izvajanja, temveč tudi na njegovo neposredno okolico. Diaz in sodelavci (2011) ugotavljajo, da je zaraščanja več v bližini narodnih parkov, kar je posledica vzpostavljanja prvotne vegetacije.

Območja Nature 2000 in različne oblike parkov lahko z režimi upravljanja ali kmetijsko-okoljsko podnebnih plačil vplivajo na intenzivnost obdelave (npr. čas ali število košenj) in način kmetovanja (konvencionalno, integrirano, ekološko) (Žgavec in sod., 2013; Živkart in sod., 2013). Zemljišča so lahko zaradi omejitev in okoljskih ukrepov manj produktivna in stroški vloženega dela in materiala večji, kar lahko privede do opuščanja kmetijske dejavnosti in posledično do zaraščanja (Glavan in Pintar, 2013). Območja Nature 2000 in parkov lahko ob primernem upravljanju pripomorejo k varovanju zaščitenega tipa krajine in tako vplivajo na preprečevanje zaraščanja kmetijskih zemljišč, a je za to potrebna kmetijska obdelava in pridelava (Knap, 2008; Gutman, 2011; Pogačnik, 2011; Miličić, 2011; Kus Veenvliet, 2012; Žgavec in sod., 2013). Na varovanih območjih, ki ležijo na izjemno dobrih tleh in legah, je opaziti trend intenziviranja obdelave in odpravljanja grmovne in drevesne zarasti, kar ima lahko kvarne posledice za biodiverziteto (Žgavec in sod., 2013; Živkart in sod., 2013; Glavan in sod., 2015). Le vključevanje in izobraževanje kmetov ter sprememba odnosa nacionalne okoljske politike do kmetov bosta omogočila varovanje habitatov kulturne krajine.

Preglednica 4: Površine (ha) in delež (%) razredov dejanske rabe (a) kmetijsko zemljišče v zaraščanju (1410) in (b) kmetijsko zemljišče poraslo z gozdnim drevjem (1800) na območjih z in brez izbranega upravljavskega statusa ter glede na površino območja in kmetijskih zemljišč (KZ)
Table 4: Area (ha) and share (%) of actual land use classes (a) abandoned agricultural land (1410) and (b) agricultural land with forest trees (1800) in the areas with and without selected management status in comparison to total area and area of agricultural land (KZ)

Območje	Površina							
	območje	KZ	Z upravljavskim statusom			Brez upravljavskega statusa		
	ha	ha	ha	% območja	% KZ	ha	% območja	% KZ
(a) Raba 1410								
Natura 2000	720.286	158.011	9.478	1,32	6,00	15.800	1,21	3,11
Območja z omejenimi dejavniki	1.751.251	506.082	21.387	1,22	4,23	3.891	1,41	2,44
Življenjski prostor medveda*	2.006.813	665.588	24.884	1,24	3,74	-	-	-
Narodni park	83.808	8.913	731	0,88	8,25	24.543	1,26	3,74
Regijski parki	43.442	16.113	509	1,17	3,16	24.770	1,25	3,81
Krajinski parki	117.004	47.708	2.534	2,17	5,31	22.744	1,19	3,68
Naravni rezervati	8.675	1.132	95	1,1	8,42	25.183	1,25	3,79
Vodovarstvena območja	350.202	106.954	4.776	1,36	4,47	20.502	1,22	3,67
(b) Raba 1800								
Natura 2000	720.286	158.011	6.065	0,84	3,84	3.430	0,26	0,68
Območja z omejenimi dejavniki	1.751.251	506.082	9.380	0,54	1,85	116	0,04	0,07
Življenjski prostor medveda*	2.006.813	665.588	9.403	0,47	1,41	-	-	-
Narodni park	83.808	8.913	428	0,51	4,81	9.067	0,47	1,38
Regijski parki	43.442	16.113	143	0,33	0,89	9.352	0,47	1,44
Krajinski parki	117.004	47.708	236	0,20	0,49	9.260	0,48	1,50
Naravni rezervati	8.675	1.132	22	0,25	1,92	9.474	0,47	1,43
Vodovarstvena območja	350.202	106.954	3.462	0,99	3,24	6.033	0,36	1,08

* Življenjski prostor rjavega medveda je določen na celotnem ozemlju Republike Slovenije.

Prisotnost rjavega medveda in njegovo povzročanje gospodarske škode lahko otežujeta rejo živali na prostem, predvsem rejo drobnice. Rjavi medved je zelo domač v zaraščajočih in opuščenih kmetijskih površinah, ki se marsikje zaradi intenzivnega zaraščanja širijo do urbanih naselij in kmetijskih površin v okolici (Jarni, 2011). Zato je akcijski načrt za upravljanje z rjavim medvedom predvidel intenzivno čiščenje zaraščajočih površin v okolici naselij (MOP, 2007). Prav gotovo bo morala kmetijska politika slediti zavezam, zapisanim v zakonu o kmetijskih zemljiščih, in nameniti finančna sredstva za odpravljanje zaraščanja.

Največji delež zemljišč rabe 1410 je glede na celotno površino OMD na območjih PO in glede na površino kmetijskih zemljišč na območjih DO (Preglednica 5). Odstotek zemljišč rabe 1800 je glede na površino območja največji na območjih s posebnimi omejitvami in glede na površino kmetijskih zemljišč na hribovskih gorskih območjih (Preglednica 5). Največje površine in velik odstotek zaraščanja na HGO so posledica tega, da ta območja obsegajo skoraj 75% države in zemljišča na večjih nadmorskih višinah ter večjih naklonih. Slednja naravna dejavnika imata izrazit vpliv na odločanje lastnikov zemljišč o obdelavi (Slika 5). Tipi OMD so glede na odstotke med seboj dokaj izenačeni. Neposredna plačila za OMD so bila uvedena z namenom blaženja vplivov neugodnih razmer za kmetovanje (nadmorska višina, naklon, kraška skalovitost in razgibanost terena ter poplavnih območij), s čimer se preprečuje opuščanje kmetovanja. S sistemom točkovanja, ki ga je pripravil Kmetijski inštitut Slovenije, je to prostorsko in analitsko en od najnatančneje dodelanih ukrepov skupne kmetijske politike v Sloveniji. Pri določanju zunanjih mej območij se lahko poleg naravnih danosti upošteva še demografske razmere (odseljevanje, negativni prirast, brezposelnost, velikost kmetij). Kljub temu, da se ob predstavitvi ukrepa OMD pogosto navaja ugodne vplive na preprečevanje zaraščanja, so v strokovni literaturi analize vpliva ukrepa na nivoju kmetij redke. Obstoječi študiji, ki obravnavata kmetijsko-okoljske ukrepe v okviru Programa razvoja podeželja (PRP) navajata, da na intenziteto obdelave in sestavo travne ruše močno vplivajo socio-ekonomski dejavniki, kot sta starost in izobrazba kmeta ter premajhna finančna vrednost neposrednih plačil, ki ne spodbujajo k vključitvi v ukrepe (Žgavec in sod., 2013; Ketiš in sod., 2016). Lastniki oz. obdelovalci zemljišč se za vključitev v OMD-ukrepe odločajo prostovoljno in za vsako parcelo pred uveljavljanjem ukrepa pretehtajo ekonomsko upravičenost obdelave. Razlogi za opuščanje ali nadaljevanje obdelave ne vključujejo le neposrednih plačil, temveč tudi pridelek na parceli, stroške, nastale z obdelavo, čas, potreben za vzdrževanje, dostopnost, možnost strojne obdelave. Če le en od teh dejavnikov ne izkazuje pozitivnega izida, lahko to vodi v opustitev obdelave in zaraščanje parcele.

Preglednica 5: Površine (ha) in odstotek (%) zemljišč dejanske rabe kmetijsko zemljišče v zaraščanju (1410) in kmetijsko zemljišče poraslo z gozdnim drevjem (1800) znotraj območij (a) Natura 2000, (b) varovanih narodnih, regijskih in krajinskih parkov ter naravnih rezervatov, (c) življenjskega prostora rjavega medveda, (d) z omejenimi dejavniki in (e) vodovarstvenih območij glede na površino območja in kmetijskih zemljišč (KZ)

Table 5: Area (ha) and share (%) of actual land use abandoned agricultural land (1410) and agricultural land with forest trees (1800) within the areas of (a) Natura 2000, (b) national, regional or landscape parks, (c) life environment of brown bear, (d) naturally less favoured areas for agricultural activities and (e) water protection areas in comparison to total area and area of agricultural land (KZ)

Območja	Površina							
	območja	KZ	raba 1400			raba 1800		
	ha	ha	ha	% območja	% KZ	ha	% območja	% KZ
(a) Natura 2000								
Območje, pomembno za skupnost (SCI)	257.082	51.265	2.788	1,08	5,44	1.000	0,39	1,95
Posebno območje varstva (SPA)	82.178	25.440	883	1,07	3,47	318	0,39	1,25
Kombinacija SCI - SPA	381.026	81.306	5.807	1,52	7,14	4.747	1,25	5,84
Skupaj	**720.286**	**158.011**	**9.478**	**1,32**	**6**	**6.065**	**0,84**	**3,84**
(b) Varovana območja narodnih, regijskih in krajinskih parkov ter naravnih rezervatov								
Narodni park	83.808	8.913	736	0,88	8,25	428	0,51	4,81
Regijski parki	43.442	16.113	509	1,17	3,16	143	0,33	0,89
Krajinski parki	117.004	47.708	2.534	2,17	5,31	236	0,20	0,49
Naravni rezervati	8.675	1.132	95	1,10	8,42	23	0,25	1,92
Skupaj	**252.929**	**73.866**	**3.874**	**1,53**	**5,24**	**830**	**0,33**	**1,12**
(c) Življenjski prostor rjavega medveda								
Območje izjemne/redke prisotnosti	1.095.003	465.454	15.759	1,44	3,39	3.891	0,36	0,84
Osrednje območje	347.625	65.635	3.030	0,87	4,62	2.439	0,70	3,72
- Območje gostejše naseljenosti ljudi	41.653	18.931	550	1,32	2,90	129	0,31	0,68
- Območje manjše naseljenosti ljudi	305.972	46.704	2.481	0,81	5,31	2.310	0,76	4,95
Prehodno območje	308.523	55.082	2.431	0,79	4,41	1.304	0,42	2,37
Robno območje	255.662	79.418	3.664	1,43	4,61	1.770	0,69	2,23
Skupaj	**2.006.813**	**665.588**	**24.884**	**1,24**	**3,74**	**9.403**	**0,47**	**1,41**
(d) Območja z omejenimi dejavniki (OMD)								
DO - druga območja	81.196	24.387	1.144	1,41	4,69	114	0,14	0,47
HGO - hribovska gorska območja	1.467.573	375.745	16.305	1,11	4,34	7.599	0,52	2,02
PO - območja s posebnimi omejitvami	202.482	105.950	3.938	1,94	3,72	1.668	0,82	1,57
Skupaj	**1.751.251**	**506.082**	**21.387**	**1,22**	**4,23**	**9.380**	**0,54**	**1,85**
(e) Vodovarstvena območja (VVO) – državni, občinski, vrelčni nivo								
0	97	20	0	0,14	0,71	0	0,01	0,05
1	7.778	2.591	126	11,65	22,57	39	0,85	2,17
2	67.512	17.637	995	5,19	15,48	618	1,02	3,95
3	238.749	75.481	2.964	1,24	3,93	1.942	0,81	2,57
4	31.303	8.281	610	1,95	7,37	861	2,75	10,40
5	4.781	2.944	80	1,68	2,72	2	0,04	0,06
Skupaj	**350.202**	**106.954**	**4.776**	**1,36**	**4,47**	**3.462**	**0,99**	**3,24**

Rezultati za VVO potrjujejo opozorila kmetov, da se bodo območja najstrožjih vodovarstvenih režimov začela zaraščati, saj so pogoji za izvajanje običajne ekonomsko upravičene kmetijske dejavnosti zelo strogi in kmete odvračajo od obdelovanja teh zemljišč (Preglednica 5). Tako je omejena uporaba mineralnih gnojil v ravninskih območjih ter izvajanje paše na kraških in visokogorskih pašnikih. Z vidika varovanja vodnih virov je tak trend zaželen, a po drugi strani kvari krajinsko podobo območij in socio-ekonomski položaj kmeta. Povečanje zarasti in prehajanje v gozdno vegetacijo ima tudi negativen vpliv, saj se poveča evapotranspiracija, kar zmanjšuje količino vode, ki napaja vodni vir (Ulaga in sod., 2008). Predlagamo, da se v bodoče pred uvajanjem režima predhodno preveriti kratko-, srednje- in dolgoročne vplive na kmetijsko dejavnost, vrsto dejanske rabe in možnost pojava grmovne in drevesne zarasti.

4 SKLEPI

S to raziskavo smo prvič po sprejetju prenovljenega zakona o kmetijskih zemljiščih (RS, 2011), ki v sedmem členu lastnikom, zakupnikom in drugim uporabnikom nalaga preprečevanje in odpravljanje zaraščanja, opravili prostorsko in statistično analizo z namenom identifikacije območij, ki so najbolj izpostavljena zaraščanju.

Raziskava je pokazala, da je zaraščanje proces, ki se pojavlja po celotni Sloveniji z glavnino v osi od jugozahoda proti severovzhodu. Analiza povezave zaraščanja s prisotnostjo gozda na območju občine je pokazala, da ni izrazitih vzorcev, ki bi potrjevali domnevo, da večji odstotek gozda pomeni večji odstotek zaraščanja. Povezave med preučevanimi razredi dejanske rabe bi bilo treba podrobno preučiti na ravni posameznega območja zaraščanja, kjer bi opravili analizo učinka sosedstva rabe 2000 na razširjenost rabe 1410 in analizo opuščanja kmetijske obdelave na zemljiščih rabe 1800 (npr. vpliv napadov zveri na pašno živino).

Površine rabe 1410 in rabe 1800 so povezane z naklonom in nadmorsko višino, saj se z večanjem vrednosti obeh spremenljivk odstotek obeh rab glede na celotno površino kmetijskih zemljišč v občini poveča. Analiza je pokazala, da je raba 1410 na poplavnih območjih bolj razširjena od rabe 1800. Manjši vpliv na rabo 1800 izhaja iz dejstva, da je razširjena na specifičnih lokacijah, kjer se zaradi prepustnih apnenčastih kamnin in topografije (naklon) poplave ne pojavljajo. To potrjuje tudi majhen obseg rabe 1800 (48 ha) na poplavnih območjih. Vpliv posameznega upravljavskega statusa območja na zaraščanje je težje ovrednotiti, ker se jih ponekod izvaja več vzporedno. Analiza je pokazala, da je zaraščanje večje na območjih z upravljavskim statusom. Še posebno izstopa dejstvo, da bolj kot je striktno okoljsko varovanje (kombinacija Nature 2000 območij SCI – SPA, narodni park, naravni rezervat, osrednje območje življenjskega prostora rjavega medveda, 1. in 2. kategorija VVO), večji je odstotek rabe 1410 od vseh kmetijskih zemljišč.
V prostoru bodo z nadaljnjim razvojem kmetijske tehnologije in pridelovalnih procesov vedno obstajala zemljišča, ki bodo podvržena procesom zaraščanja. Dinamiko zaraščanja ali krčitve zarasti narekuje ekonomski interes lastnikov in zakupnikov zemljišč. Raziskava je pokazala, da se zaraščajo tudi kmetijska zemljišča v ravnini, saj do naklona 6 % obsegajo skoraj 7.000 ha rabe 1410 in 1.700 ha rabe 1800. Skupno te površine presegajo obseg vseh delujočih (6.077 ha) in delno delujočih (647 ha) namakalnih sistemov Sloveniji.

Na območjih, kjer je zaraščanje najbolj razširjeno, bi bilo treba pospešeno izvajati zakon o kmetijskih zemljiščih, ki določa odpravljanje zaraščanja na območjih z večjo boniteto zemljišč. Opraviti bi bilo treba analizo vzrokov za opuščanje kmetijske obdelave na posameznih območjih in predlagati ukrepe za preprečevanje (komasacije, izboljšanje dostopnosti, finančna spodbuda ali kazni) in odpravljanje zaraščanja (agromelioracije za izboljšanje fizikalnih, kemijskih in bioloških lastnosti tal, rekultivacija s pomočjo drobnice) ter nadaljnjo obdelavo zemljišč, ki bi ustrezala naravno pridelovalnim razmeram območja (reja avtohtonih pasem, izdelki z dodano vrednostjo, npr. sivka s Krasa), načrtno pogozdovanje (biomasa, plantaže gozdnega drevja). Podatki o dejanski rabi zemljišč kažejo, da se proces zaraščanja nadaljuje, zato bi morali odločevalci nadaljevati aktivnosti, ki spodbujajo preprečevanje in odpravljanje zaraščanja (ARSO, 2016). Vendarle je pomembno, da vlogo pri tem prevzamejo tudi lokalne skupnosti, ki bolje poznajo razmere na terenu in možnosti izkoriščanja prostora.

Dober ukrep za zmanjševanje zaraščanja je, v januarju 2017 sprejeta, uredba o izvajanju ukrepa odpravljanja zaraščanja na kmetijskih zemljiščih, katere izvajanje se financira s sredstvi, zbranimi iz naslova odškodnine zaradi spremembe namembnosti zemljišč (RS, 2017). Uredbo je pripravilo Ministrstvo za kmetijstvo, gozdarstvo in prehrano z namenom financiranja izvedbe agromelioracij na kmetijskih zemljišči v zaraščanju, s čimer bi jih ponovno usposobili za kmetijsko pridelavo. A uredba ureja le odpravljanje zaraščanja in ne deluje preventivno. Zato bo v prihodnje treba določiti tudi ukrepe za preprečevanje zaraščanja, kot so preprečevanja plazenja, odvodnjavanje talne vode, izgradnja teras, odpravljanje skalovitosti in izravnave terena, izgradnja poljskih in gozdnih poti, vzdrževanje osuševalnih sistemov in komasacije preko povečevanja površine kmetijskih parcel. Preventivni ukrepi preprečujejo zaraščanje, kar je dolgoročno finančno in energijsko učinkoviteje. Pomembno je, da s preventivnimi ukrepi omogočimo, da je zemljišče ves čas v pridelovalni funkciji in da z vlaganji izboljšujemo dostopnost in pridelovalni potencial zemljišč.

5 ZAHVALA

Delo je bilo financirano strani Javne agencija za raziskovalno dejavnost Republike Slovenije (ARRS) in Ministrstva za kmetijstvo, gozdarstvo in prehrano (MKGP) v okviru Ciljnega raziskovalnega programa. Naslov projekta je Strokovne podlage za določitev območij primernih za odpravljanje zaraščanja (V4-1149).

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Effect of CO_2 elevation and UV-A radiation on growth responses of Zinnia, Petunia, Coxcomb, and Marigold

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ABSTRACT

In order to evaluate the effect of CO_2 elevation and UV radiation on growth responses of zinnia, petunia, coxcomb, and marigold, a study was conducted in 2015 at Arsanjan Islamic Azad University, Iran. The experimental design was factorial arranged in completely randomized design with three replications. Treatments were included four ornamental species (zinnia, petunia, coxcomb, and marigold), CO_2 concentration at two levels (350 and 700 ppm), and UV radiation at two levels (with and without UV radiation). Results showed that elevating of CO_2 concentration from 350 ppm to 700 ppm increased morphological and physiological characters of C_3 plants, especially marigold. Meanwhile, increasing CO_2 concentration from 350 ppm to 700 ppm, decreased effects of UV damage on plants' morphological and physiological characters. The highest leaf number, shoot dry mass, plant height and water use efficiency of C_4 plant (coxcomb flower) were observed at 350 ppm of CO_2 concentration without UV radiation while, the highest leaf number, shoot dry mass and leaf pigments of C_3 plants (zinnia, petunia, and marigold flower) were obtained at 700 ppm of CO_2 concentration without UV radiation. The results showed that the activity of catalase and peroxidase enzymes under UV radiation was increased in all of plants. Overall, it is concluded that, the recognition of plants resistant to UV radiation and high levels of CO_2 concentration in the future may be better for environmental production and distribution as ornamental plants in town landscapes, where ecophysiological traits should be considered.

Key words: ornamental plants; climate change; morphological and physiological traits; UV radiation

IZVLEČEK

UČINEK POVEČANE KONCENTRACIJE CO_2 IN POVEČANEGA UV-A SEVANJA NA RASTNI ODZIV CINIJE, PETUNIJE, PETELINJEGA GREBENA IN ŽAMETNICE

Z namenom ovrednotenja učinka povečanega CO_2 in UV sevanja na rastni odziv cinije, petunije, petelinjega grebena in žametnice je bil v letu 2015 izveden poskus na Arsanjan Islamic Azad University, Iran. Poskus je bil popoln naključni faktorski poskus s tremi ponovitvami. Obravnavanja so obsegala štiri vrste okrasnih rastlin (cinija, petunija, petelinov greben in žametnica), dve koncentraciji CO_2 (350 in 700 ppm), in dve jakosti UV sevanja (brez in z UV sevanjem). Rezultati so pokazali, da je povečana koncentracija CO_2 iz 350 ppm na 700 ppm povečala vrednosti morfoloških in fizioloških znakov C_3 rastlin, še posebej žametnice. Povečanje koncentracije CO_2 iz 350 ppm na 700 ppm je zmanjšalo učinke poškodb po UV v morfoloških in fizioloških znakih. Največje število listov, največja masa suhe snovi in največja učinkovitost izrabe vode so bili pri C_4 rastlinah (petelinji greben) zabeleženi pri 350 ppm CO_2 brez UV sevanja, pri C_3 rastlinah (cinija, petunija in žametnica) je bila največja vrednost znakov kot so število listov, suha masa poganjkov in vsebnost listnih pigmetov ugotovljena pri 700 ppm CO_2 brez UV sevanja. Aktivnosti katalaze in peroksidaze sta se v razmerah UV sevanja povečali pri vseh rastlinah. V splošnem lahko zaključimo, da je pri izbiri okrasnih rastlin za učinkovitejše ozelenjevanje urbanih površin potrebno upoštevati tudi njihove morfološko fiziološke lastnosti.

Ključne besede: okrasne rastline; morfološko fiziološke lastnosti; klimatske spremembe; UV sevanje

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1 INTRODUCTION

Nowadays, unstable symptoms on the Earth such as environmental pollution and species extinction caused by increased emissions of greenhouse gases, in combination with changes in solar radiations intensity appeared to be unavoidable (Xing, 2009; Ziska and Blumenthal, 2010). Morphological traits and physiological processes of plants are affected by different climate change aspects such as CO_2 elevation, high temperature, ultra violet (UV) radiation, and quantity and dispersal of rainfall (Fuhrer, 2003). It is reported that climate changes has affected flowering initiation, physiology, water relations, ions absorption, photosynthesis and respiration of plants (Mortensen, 1987).

CO_2 plays an important role in process of global warming and climate changes (Holden and Hoyer, 2005). The level of CO_2 in the atmosphere is rising at an unprecedented rate, has increased from 280 ppm at the beginning of the industrial revolution to 380 ppm today, and is expected to double pre-industrial levels sometime during this century (Hennessy et al., 2008; Karl et al., 2009). Generally, CO_2 elevation could increase net photosynthesis of potted plants, cut flowers, and vegetables (Croonenborghs et al., 2009). Significant increase of water use efficiency (WUE) and decrease of stomatal conductance were observed in plants treated by CO_2 elevation (Lincoln and Couvet, 1989; Prior et al., 2011). Kamali et al. (2011) reported that CO_2 elevation from 380 to 1050 ppm could increase shoot and root dry mass, height, number of leaves and leaf area of coxcomb (*Celosia argentea* L.). Shoor et al. (2010) showed that increasing CO_2 level to 700 ppm could accelerate marigold (*Tagetes patula* L.) flowering time.

Plants, as sessile organisms that require sunlight for growth and development, are inevitably exposed to UV wavelengths (200–400 nm), which represent almost 7 % of the electromagnetic radiation emitted from the sun. Plants responses to ozone layer destruction and high UV radiation include widespread range of bio-chemical, physiological, morphological, and anatomical changes (Zhang et al., 2003). High doses of UV radiation may damage macromolecules, including DNA and proteins, and induce the production of reactive oxygen species (ROS), affecting photosynthetic pigments, cell membrane integrity and viability (Horii et al., 2007). Rahimzadeh et al. (2011) reported that UV-A radiation decreased shoot dry mass, protein, and chlorophyll content of savory (*Satureja hortensis* L.). Kazemi Ghale et al. (2011) showed that in radish plants treated with UV radiation photosynthesis rate decreases due to leaf area reduction, leaf thickness increases, and observes bio-chemical changes of chlorophyll pigments. Golbazhagh et al. (2010) reported the reduction of sunflower growth such as shoot dry mass, root length, and leaf area caused by increasing exposure time of different doses of UV-A radiation. Sarikhani (2013) showed that the UV-A radiation reduced peppermint yield. Also, antioxidant and secondary metabolite activity increased when peppermint was treated by UV-A radiation.

By considering the climatic changes the objective of this research was to determine the influence of CO_2 elevation and UV-A radiation on morphological and physiological properties of zinnia, petunia, coxcomb, and marigold . Study of climate changes effect on zinnia, petunia, coxcomb, and marigold production, quality, and marketable properties are very important because these plants are widespread ornamental species.

2 MATERIALS AND METHODS

In order to evaluate the effect of CO_2 elevation and UV-A radiation on morphological and physiological parameters of zinnia (*Zinnia elegans* Jacq.), petunia (*Petunia x hybrida* ‘Grandiflorus’), coxcomb (*Celosia cristata* L.), and Mexican marigold (*Tagetes erecta* L.) a study was conducted in 2015 at Islamic Azad University, Arsanjan branch, Iran (53° 19' E, 29° 55' N and 1690 m). The Experimental design was factorial arranged in completely randomized design with three replications. Treatments included plant species (zinnia, petunia, coxcomb, and marigold), CO_2 concentration at two levels (ambient CO_2 (350 ppm) and elevated CO_2 (700 ppm)), and UV radiation at two levels (with and without UV-A radiation).

The experiment was conducted in two environmentally controlled growth chambers with four compartments to apply CO2 and UV-A treatments, with the mean air temperature of 25/14 °C (day/night) and relative humidity of 75 (day) and 60 % (night). At the beginning of the experiment, five seeds were planted in 5 cm deep of each of the 48 pots (18 cm height and 14 cm diameter) filled with a silty-loam soil with 1.16 % organic matter, 15 to 18 % sand, 50 to 56 % silt, 10 to 15 % clay and pH of 7.5. After seed germination, seedlings were thinned to one per pot at the four-leaf stage. Then, half of the pots were moved into the ambient CO_2 chamber and the other half, into the elevated CO_2 chamber. Pots were uniformly irrigated (EC = 0.78 ds m^{-1} of water) every 3 days. Half of the pots in the chambers was exposed by UV-A radiation (5 min per days) from fluorescent tubes (T9 Black light blue fluorescent- Schwan Company), used to produce UV-A radiation.They were installed at 50 height from each pot.

Measured traits were leaf number, shoot dry mass, plant height, water use efficiency, chlorophyll-a and carotenoid content, and activity of catalase and peroxidase enzymes. In order to the assessment leaf pigments content, 20 ml aceton (80 %) was mixed with 0.5 g of leaf fresh mass, then, the mixture was centrifuged (with 3000 rpm for 10 min). Then, leaf pigments were determined by spectrophotometer (UV2100 Plus model - USA) device (wavelength 470 and 663 nm) (Arnon, 1967).

In order to measure activity of catalase and peroxidase, 5 ml of potassium phosphate buffer (100 m mol and pH 7.5) was blend with 0.5 g of leaf fresh mass, then, the mixture was centrifuged (with 12000 rpm for 60 min). Afterwards, activity of catalase and peroxidase were determined using method described by Pereia et al. (2002) and Fielding and Hall (1978) , respectively. All data were submitted to an analysis of variance (ANOVA) and Duncan test was used to verify the significant differences among treatment means at the 5 % probability level (Little and Jackson, 1978).

3 RESULTS AND DISCUSSION

Effect of CO_2 and species interaction was significant on leaves' number, shoot dry mass, plant height, water use efficiency, chlorophyll a, activity of catalase and peroxidase (Table1). Results showed that the highest leaf number and shoot dry mass were observed in coxcomb flower + ambient CO_2 (Figure1 (a) and (b)). The highest WUE was obtained in coxcomb flower + ambient CO_2 (Figure1 (d)) and the highest catalase activity was achieved in coxcomb flower + elevated CO_2 (Figure1 (g)).

Results showed that the highest plant height was observed in *Zinnia* + elevated CO_2 (Figure1 (c)) also, the highest chlorophyll a was achieved in marigold + ambient CO_2 (Figure1 (e)) while, the highest peroxidase activity were achieved in petunia + ambient CO_2 (Figure1 (h)). Generally, the highest leaf number and shoot dry mass were observed in coxcomb in comparison to the C_3 plants due to quickly establishment and better use of soil nutrients by coxcomb seedlings (Hammer et al., 2005; Wortman et al., 2011). Shoor et al. (2010) reported that elevating CO_2 concentration to 700 ppm could be increased marigold height (approximately 50 %) as a result improved plant photosynthesis capacity and allocated more assimilates to vegetative growth. It seems that elevating CO_2 concentration improved WUE due to high CO_2 concentration into inter-cellular space and transpiration reduction by stomatal closer (Fuhrer, 2003). Furthermore, increasing chlorophylla and carotenoid pigments by elevated CO_2 had significant role in photosynthesis rate and photosystem II protection for improving radiation absorption and increasing photosynthesis capacity (Mavrogianopoulos et al., 1999; Joseph et al., 2008). Miri and Rastegar (2012) showed that elevated CO_2 could increase chlorophyll index (approximately 6 to 30 %) in soybean, lambsquarters, panicum, and pigweed.

In addition, shoot dry mass, WUE, chlorophyll a, catalase and peroxidase activity were significantly influenced by UV-A radiation and species interaction (Table 1). According to results, morphological and physiological parameters showed a reduction as affected by UV-A radiation. The highest leaf number, shoot dry mass, and WUE, and chlorophyll a were observed in coxcomb and marigold , respectively, without UV-A radiation (Figure 2 (a), (b), (d), and (e)). Meanwhile, the catalase activity in all plants increased by UV-A radiation (Figure 2 (g)). It seems that in plant subjected to UV-A radiation less transfer of photosynthetic assimilate occurred due to reduction of photosynthesis capacity (Balouchi et al., 2008). Reduction in growth and development and reduction of cell division also was observed by UV-A radiation (Smirnoff and Wheelev, 2000; Gao et al., 2003). But, plants response differently to UV-A radiation as affected by species and environmental factors such as plant water status, photosynthetically active radiation (PAR), and nutrients availability (Mark and Tevini, 1996; Balouchi et al., 2009).

In general, results showed that shoot dry mass, plant height, WUE, chlorophyll a and carotenoid pigments, and catalase activity were significantly influenced by CO_2 concentration, UV-A radiation, and plant species (Table 1). The highest leaf number and shoot dry mass were obtained in coxcomb + ambient CO_2 + without UV-A radiation (Figure 3 (a) and (b)). Also, the highest WUE was observed in coxcomb + at elevated CO_2 + without UV-A radiation (Figure 3 (d)). Meanwhile, the highest plant height was achieved in *Zinnia* + elevated CO_2 + without UV-A radiation (Figure3 (c)). According to our research, results showed that UV-A radiation could decrease growth parameters of plant species due to its impact on photosynthesis capacity, but, elevating CO_2 could lead to improve photosynthesis capacity and high assimilate transfer to vegetative growth (Ziska and McClung, 2008; Croonenborghs et al., 2008; He et al., 2013).

Table 1: The summary of the source of variation and the mean square of shoot dry mass (g per plant), leaf number (per plant), water use efficiency (g l^{-1}), chlorophyll a and carotenoid (mg g^{-1}), catalase and peroxidase activity (u mol g^{-1} min^{-1})

Source of variation	df	Shoot dry mass	Leaf number	Plant height	Water use efficiency	Chlorophyll a	Carotenoid	Catalase activity	Peroxidase activity
Plant species	3	0.521^{**}	154.750^{**}	1689.607^{**}	3.81^{**}	46.192^{**}	21.175^{**}	3.644^{**}	1207.464^{**}
CO2 level	1	0.000^{ns}	4.083^{ns}	552.028^{**}	1.31^{**}	1.602^{ns}	1.522^{ns}	1.367^{**}	54.957^{**}
Plant×CO2	3	0.013^{**}	26.750^{**}	30.184^{*}	1.10^{**}	3.323^{*}	0.102^{ns}	2.719^{**}	41.788^{**}
UV radiation	1	0.003^{*}	24.083^{*}	0.445^{ns}	1.97^{ns}	6.237^{*}	0.0471^{ns}	4.025^{**}	145.718^{**}
Plant×UV	3	0.003^{*}	0.528^{ns}	8.196^{ns}	7.68^{**}	9.105^{**}	0.622^{ns}	0.533^{**}	39.719^{**}
CO2×UV	1	0.001^{ns}	0.083^{ns}	80.808^{**}	1.11^{ns}	4.466^{*}	0.218^{ns}	0.035^{ns}	11.903^{ns}
Plant×CO2×UV	3	0.009^{**}	9.417^{ns}	95.731^{**}	1.35^{**}	6.633^{**}	1.828^{*}	4.572^{**}	7.987^{ns}
Error	32	0.001	4.042	10.386	1.72	0.945	0.460	0.055	4.438
CV (%)		12.70	14.10	18.00	13.60	11.50	13.07	18.20	16.90

Note: * and ** significant at the 0.05 and 0.01 level, respectively; ns, not significant

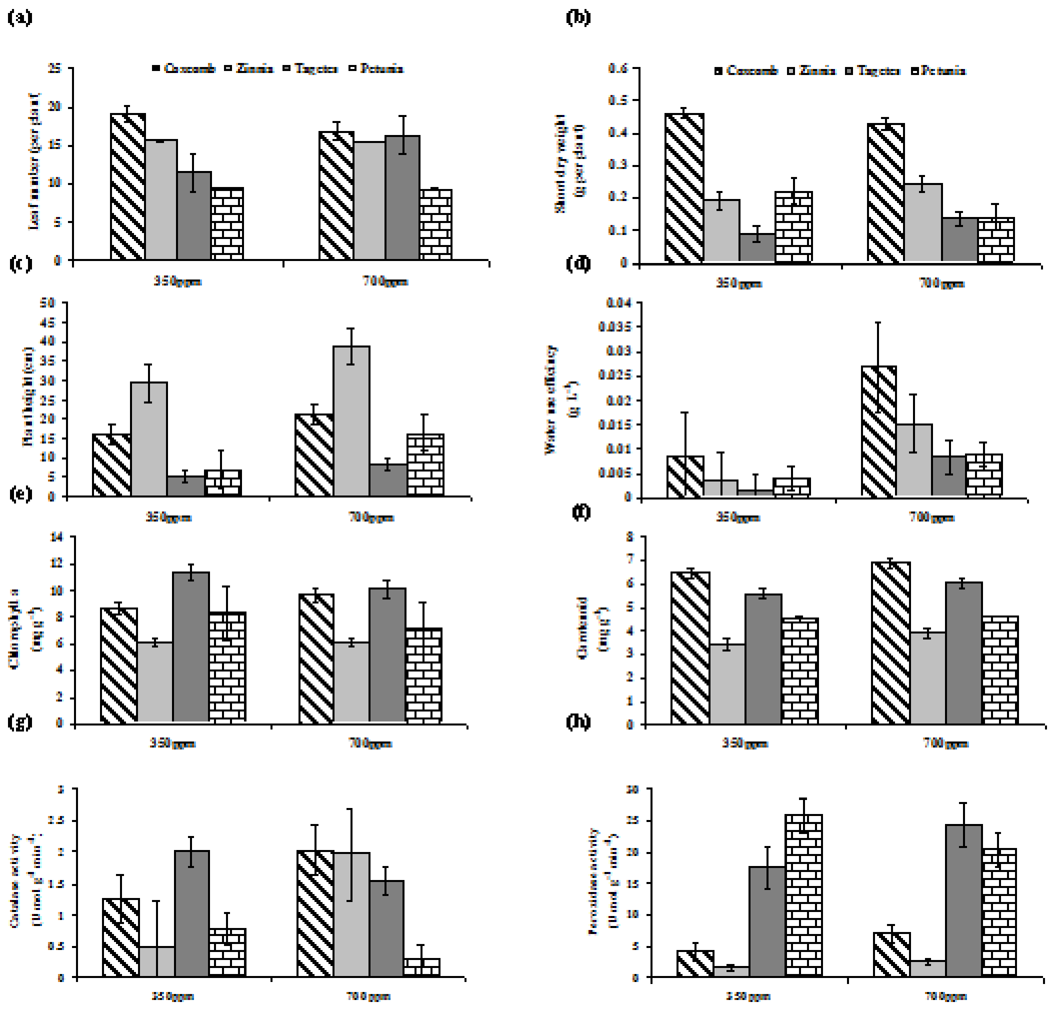

Figure 1: The effect of flower species and CO_2 concentration interaction on leaf number (a), shoot dry mass (b), plant height (c), water use efficiency (d), chlorophyll a content (e), carotenoid content (f), catalase activity (g), and peroxidase activity (h). (According to standard error, the means with same overlap not significant)

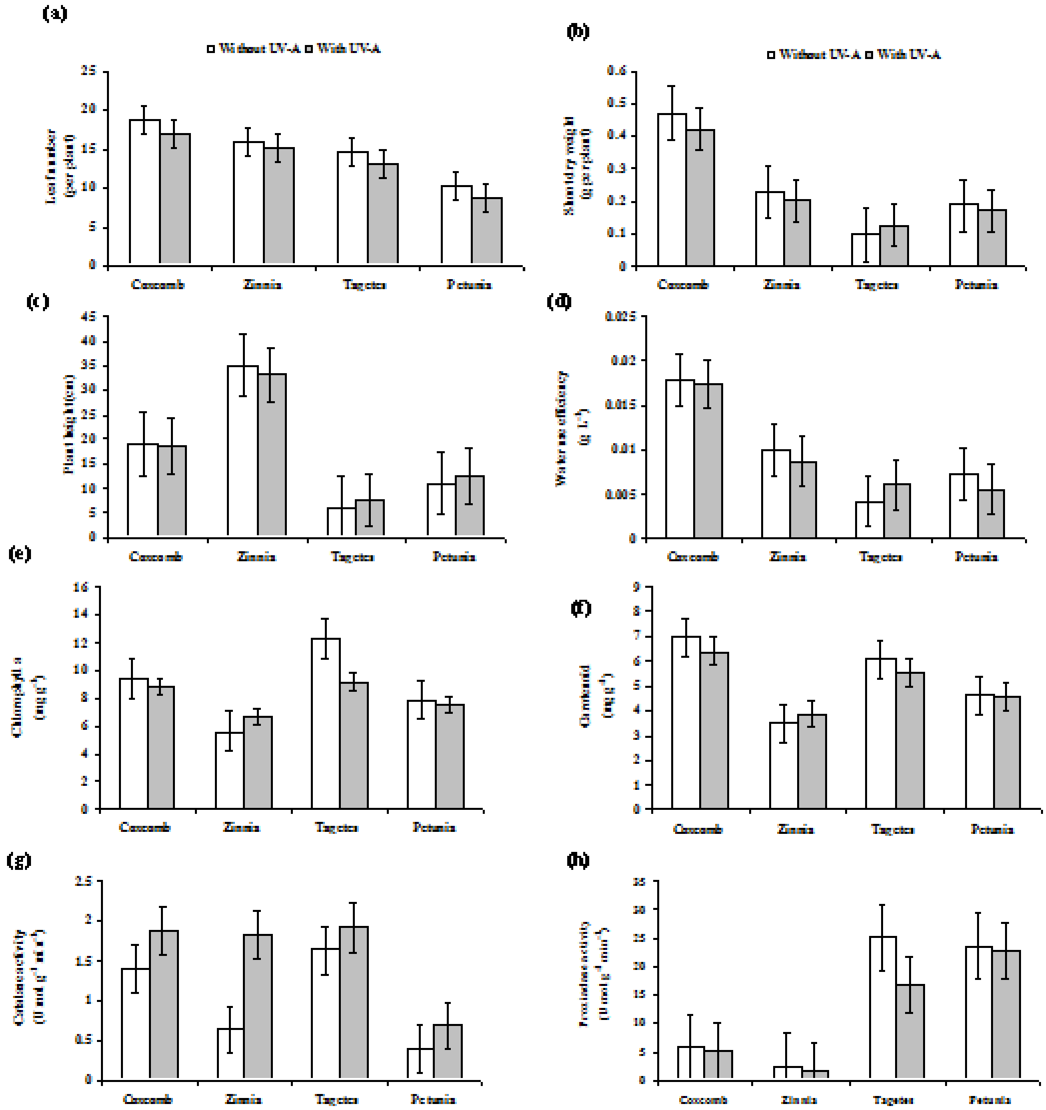

Figure 2: The effect of plant species and UV-A radiation interaction on leaf number (a), shoot dry mass (b), plant height (c), water use efficiency (d), chlorophyll a content (e), carotenoid content (f), catalase activity (g), and peroxidase activity (h). (According to standard error, the means with same overlap not significant)

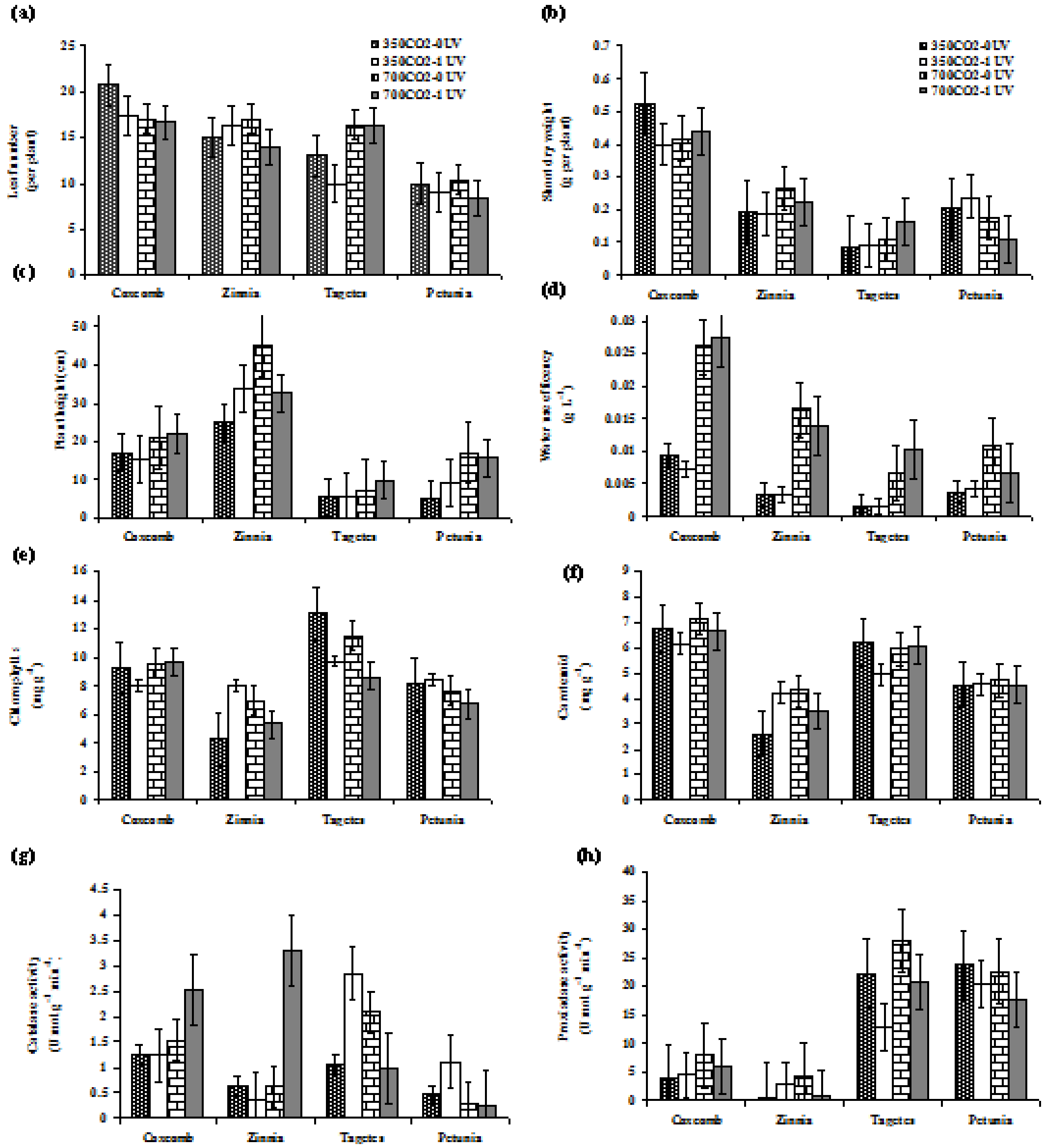

Figure 3: The effect of plant species, CO_2 concentration, and UV-A radiation interaction on leaf number (a), shoot dry mass (b), plant height (c), water use efficiency (d), chlorophyll a content (e), carotenoid content (f), catalase activity (g), and peroxidase activity (h). (According to standard error, the means with same overlap not significant).

It seems that elevating CO_2 concentration could increase growth of C_3 plants (zinnia, petunia, and marigold) in comparison to the C_4 ones (coxcomb) due to a better use of environmental sources for growth and development. However, UV-A radiation reduced plant growth parameters, but, increasing CO_2 concentration could reduce destructive effects of UV-A radiation on analysed plants species. It is concluded that, the highest growth parameters of zinnia, petunia, and marigold were achieved under elevated CO_2 without UV-A radiation, but, the highest growth parameters of coxcomb were obtained in ambient CO_2 without UV-A radiation.

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Salinity induced changes in water relations, oxidative damage and morpho-physiological adaptations of pistachio genotypes in soilless culture

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ABSTRACT

Selecting salt tolerant rootstocks is a sustainable approach for developing fruit trees in salinity prone areas. 60-day-old seedlings of *Pistacia vera* 'Akbari' and 'Ghazvini', and *P. vera* 'Ghazvini' × *P. atlantica* (G×A) were subjected to 0, 50, 100 and 150 mM NaCl in half strength Hoagland's nutrient solution. After 45 days, the growth, water relations, and oxidative damage parameters were investigated. Salt stress reduced plant biomass, height, crown diameter and leaf number, but increased specific leaf area (SLA) of the seedlings. Under salt stress, the growth of 'Akbari' seedlings was higher than the other genotypes. Accumulation of malondialdehyde (MDA) and proline was observed in the leaves of salt affected seedlings. 'Ghazvini' seedlings had the highest MDA concentration and the lowest cell membrane stability in their leaves. Degredation of photosynthetic pigments under salt stress was lower in the leaves of 'Akbari' seedlings than that in other genotypes. Increase in leaf succulence was observed in 'Akbari' and G×A seedlings in response to salt stress. Relative water content and concentration of anthocyanins in the leaves of pistachio genotypes remained unchanged under salt stress. The results revealed that monitoring leaf abscission, SLA, leaf succulence, MDA concentration, and photosynthetic pigments provide suitable contrast for screening salt tolerance in pistachio. Furthuremore, 'Akbari' was found to be the most salt tolerant genotype.

Key words: interspecific hybrid; leaf pigments; morphophysiological adaptation; salt stress; oxidative stress; *Pistacia vera*

IZVLEČEK

S SLANOSTJO VZPODBUJENE SPREMEMBE V VODNEM REŽIMU, OKSIDATIVNE POŠKODBE IN MORFOLOŠKO-FIZIOLOŠKE PRILAGODITVE GENOTIPOV PISTACIJE V BREZTALNEM GOJENJU

Izbor na slanost tolerantnih podlag je primeren pristop pri razvoju sadnih dreves na območjih podvrženih zasoljevanju. 60-dni stare sejanke pistacije (*Pistacia vera* 'Akbari' in 'Ghazvini', ter *P. vera* 'Ghazvini' × *P. atlantica* (G×A)) so bile izpostavljene 0, 50, 100 in 150 mM NaCl v polovični Hoaglandovi hranilni raztopini. Parametri rasti, vodnih razmer in oksidativnih poškodb so bili preučeni po 45 dneh. Solni stres je zmanjšal biomaso rastlin, višino, premer krošnje in število listov, a povečal specifično listno površino (SLA) sejank. Pod solnim stresom je bila rast sejank 'Akbari' večja kot drugih genotipov. Akumulacija malondialdehida (MDA) in prolina je bila opažena v listih od soli prizadetih sejank. Sejanke 'Ghazvini' so imele največjo koncentracijo MDA in najmanjšo stabilnost celičnih membran listov. Razgradnja fotosinteznih pigmentov v listih sejank 'Akbari'je bila v solnem stresu manjša kot pri drugih genotipih. Povečanje sukulence listov kot odziv na solni stres je bilo opaženo pri sejankah 'Akbari' and G×A. Relativna vsebnost vode in vsebnost antocianinov v listih sta pri vseh genotipov pistacije ostali nespremenjeni v solnem stresu. Izsledki so odkrili, da daje spremljanje odpadanja listov, SLA, listne sukulence, koncentracije MDA in fotosinteznih pigmentov primernen nabor znakov za odkrivanje tolerance na sol pri pistaciji. 'Akbari' je bil prepoznan kot na sol najbolj tolranten genotip.

Ključne besede: medvrstni križanci; listni pigmenti; morfološko-fiziološke adaptacije; solni stres; oksidativni stres; *Pistacia vera*

1 INTRODUCTION

Salinity is one of the most serious constraints to agricultural crop production in arid and semi-arid areas. More than 1.5 Mha of irrigated lands are taken out of production each year due to high salt accumulation in the soil (Munns and Tester, 2008). Salinity reduces the growth and development of plant by affecting water availability to plant, absorption of mineral nutrients, and ion homeostasis (Parida and Dos, 2005). Furthermore,

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burst of regeneration of reactive oxygen species (ROS) under this situation damages proteins, lipids and the genetic material (Gill and Tuteja, 2010). Morphological, physiological, and biochemical adaptations to salt stress, such as stomatal closure, osmotic adjustment, ion exclusion and compartmentation, and increase in the antioxidative activity have been widely discussed in the literature (Hishida et al., 2014).

Pistachio (*Pistacia vera* L.) is originally native to arid and semi-arid regions of the middle east and Persia. Although the plant is relatively salt tolerant (Ferguson et al., 2002), intensified salt built up in the soil of these regions due to using poor quality water for irrigation in association with frequent drought periods has reduced its production over recent decades (Karimi and Rahemi, 2012). Selecting salt tolerant rootstocks is an effective approach for sustainable development of pistachio in such salt prone areas. In this regard, understanding the effects of salinity on pistachio is of a crucial importance for establishing a successful rootstock breeding program.

Although there are many reports on evaluating salt tolerance of pistachio genotypes and related species, some controversies in results of these researches can be found. The issue is mainly due to physico-chemical differences in growing media, and using convenient criteria for evaluating salt tolerance of pistachio which are mainly developed for herbaceous annual crop species. However, performing such experiments in soilless culture may reduce experimental errors by increasing uniformity of growing medium. Absence of complex interactions between soil, the stressor, and plant in soilless condition enhances repeatability of results and provide a clear understanding of the plant responses to salinity. Therefore, in this study the effects of salt stress were evaluated on the growth, morpho-physiological adaptations, and biochemical characteristics of pistachio seedlings in soilless culture in order to screen salt tolerance. Moreover, effectiveness of different criteria for screening salt tolerance in pistachio seedlings was investigated.

2 MATERIAL AND METHODS

2.1 Plant material and experimental conditions

This study was conducted at the Department of Horticultural Science of University of Tehran in 2014-15. Seeds of pistachio ‘Ghazvini’ and ‘Akbari’ were obtained from Pistachio Research Station of Damghan, Iran. Seeds of interspecific hybrid *P. vera* ‘Ghazvini’ × *P. atlantica* Desf. (G×A) were obtained by controlled hybridization (Morovati, 2013). The seeds were soaked for 24 hrs and then treated with 0.2 % captain fungicide for 4 hrs. The seeds were transferred to clean containers with moistened filter paper and allowed to germinate for 5 days at room temperature. Three germinated seeds were sown in 4.5 liter plastic pots containing 3.0 kg of coco-peat and perlite mixture (2:1 volume ratio) in a greenhouse with average day/night temperatures of 30/24 °C and air humidity of 10/22 %. The pots were irrigated with tap water for about 30 days. At four leaved stage, the seedlings started to receive half-strength Hoagland’s nutrient solution (Hogland and Arnon, 1950) for a 60-day period and then subjected to four NaCl concentrations (0, 50, 100 and 150 mM) in the nutrient solution for 45 days. The seedlings were irrigated with the nutrient solutions every 48 hrs. To avoid salt build-up in the pots, the plants were irrigated enough to ensure drainage of 30 % of the solutions.

2.2 Growth parameters

After 45 days of exposure to the salt stress plant height, leaf number and crown diameter of the seedlings were measured. Then, the plants were harvested and their fresh mass and dry mass were determined. Specific leaf area (SLA) was measured by determining dry mass of fifteen leaf discs (0.90 cm diameter) from the 4-5th fully expanded leaves from shoot top according to Eq. 1:

$$SLA = {\text{Leaf area}}/{\text{Leaf dry mass}} \qquad \text{Eq. 1}$$

2.3 Water relations

Leaf water potential (Ψ_{Leaf}) was measured at midday (11:00-12:00) using a portable pressure chamber device (Soil Moisture Equipment Corp., USA). Ψ_{Leaf} was measured immediately after excising the 4-5th fully expanded leaves from the top of the stem. Leaf relative water content (RWC) was measured by punching fifteen discs (0.90 cm diameter) from the developed leaves. The discs were weighted (FM), floated on distillated water for 24 hrs (at 4 C in dark) to obtain turgid mass (TM), and finally their dry mass was recorded 72 hrs after placing at 70 °C (DM). RWC was calculated according to Eq. 2:

$$RWC = \frac{FM - DM}{TM - DM} \times 100 \qquad \text{Eq. 2}$$

Leaf water content was expressed as the percentage equivalent of the ratio of the mass of water (FM - DM) to the leaf dry matter (M_d) (Eq. 3).

$$\text{Leaf water content} = \frac{FM - DM}{Md} \times 100 \qquad \text{Eq. 3}$$

Area basis leaf water content (succulence index) was expressed as the ratio of the mass of water to the area of the leaf sample (LA_S). In this order, fifteen foliar discs (0.90 cm diameter) from the developed leaves of shoot top were used (Eq. 4).

$$\text{Succulence} = \frac{FM - DM}{LAS} \qquad \text{Eq. 4}$$

2.4 Leaf proline concentration

Leaf proline concentration was measured according to the method described by Bates et al. (1973). Leaf tissue (0.1 g) was extracted in 10 ml of 3 % sulphosalicylic acid and then, 2 ml of ninehydrin reagent and 2 ml acetic acid were added to 2 ml of the extract. Then, the samples were heated in boiling water for 60 min. Four milliliters of toluene was added to each sample and vortexed for 15-20 seconds. The absorbance of toluene phase was measured at 532 nm using a spectrophotometer (Perkin Elmer, Lambada 25, USA). The concentration of proline was determined according to an external standard curve.

2.5 Oxidative damage parameters

Cell membrane stability index (CMS) was determined by measurement of electrolyte leakage from leaf samples. Fifteen leaf discs (0.90 cm diameter) were excised from fully expanded young leaves. The leaf discs were washed three times in deionized water and incubated in 15 ml deionized water (40°C) for 30 min. The initial conductance (C*i*) of the incubation solution was measured using an electrical conductance meter. Leaf tissue in the incubation solution was killed by placing the samples in boiling water for 10 min. The conductance of the solution (C*max*) was determined at room temperature. CMS was calculated by using the following formula (Eq. 5):

$$\text{CMS} = 1 - \left({Ci}/{C_{max}}\right) \times 100 \qquad \text{Eq. 5}$$

Lipid peroxidation was assessed by measurement of malondialdehyde (MDA) concentration in fully expanded young leaves, according to the method described by Heath and Parker, (1986). Leaf tissue (200 mg) was homogenized in 10 ml of 0.1 % trichloroacetic acid (TCA) and centrifuged at 1000 g for 5 min. Then 4 ml TCA acid 20 % and thiobarbituric acid 0.15 % were added to 1 ml of the supernatant. The absorbance was measured at 532 nm by spectrophotometry (PerkinElmer, Lambda 25, USA).

2.6 Leaf pigments

In addition to evaluation of leaf greenness by using a SPAD 502 chlorophyll meter (Minolta Co., Japan), concentrations of chlorophylls and carotenoids were measured in the 4-5th developed leaves according to the method described by Lichtenthaler (1987). Fresh tissue (15×0.64 cm^2 leaf discs) was extracted in 80 % acetone and after centrifuging at 4800 rpm for 20 min, the absorption of supernatant was read at 470, 647 and 664 nm using a spectrophotometer (PerkinElmer, Lambda 25, USA). The concentrations of chlorophyll a (Chl a) and b (Chl b), and carotenoids were calculated according to the following formulas (Eq. 6-8):

$$\text{Chl a} = 12.25A_{664} - 2.79A_{647} \qquad \text{Eq. 6}$$

$$\text{Chl b} = 21.51A_{647} - 5.10A_{664} \qquad \text{Eq. 7}$$

$$\text{Carotenoids} = (1000A_{470} - 1.8C_a - 85.02C_b)/198 \qquad \text{Eq. 8}$$

For measuring anthocyanins, 500 mg fresh leaf tissue was extracted in 10 ml of methanol acidified with 1 % HCl at 4 ^{o}C for 24 hrs. The absorbance of the extract was determined at 550 nm using a spectrophotometer (PerkinElmer, Lambda 25, USA). Anthocyanin concentration was calculated using an extinction coefficient of 33000 mol^{-1} cm^{-1} (Wagner, 1979).

2.7 Statistical analyses

The experiment was conducted as factorial (3 genotypes × 4 NaCl concentrations) based on a completely randomized design with three replications. Three pots were considered in each replication and mean of these pots were considered as a replication for each treatment. In sum, 108 pots were used in the experiment. The data were subjected to ANOVA and the means were compared using Duncan's multiple range test (DMRT) at $P \leq 0.05$. The statistical analyses were performed using the SPSS software (v. 21.0).

3 RESULTS

The effects of salt stress on growth of pistachio seedlings are represented in Table 1. Shoot height, crown diameter, leaf number, and dry mass of the seedlings were significantly reduced under 100 and 150 mM NaCl treatments. Shoot height and leaf number of 'Akbari' seedlings were significantly higher than the other genotypes. Trunk diameter of 'Akbari' seedlings was significantly higher than the other genotypes and the lowest value was found in G×A. Fresh mass of the seedlings significantly decreased by increasing NaCl concentration in the nutrient solution. Among the genotypes, seedlings of 'Akbari' had the highest fresh and dry mass at the end of the experiment; no significant differences were observed between fresh and dry mass of 'Ghazvini' and G×A seedlings. SLA significantly increased under 100 and 150 mM NaCl treatments. The highest SLA was found in G×A and 'Akbari' seedlings had the lowest SLA.

Table 1: The effects of salt stress and plant genotype on growth of pistachio seedlings

	Plant height (cm)	Crown diameter (mm)	Leaf number	Fresh mass (g)	Dry mass (g)	SLA (cm^2 g^{-1})
Genotype						
Akbari	40.3^{a†}	5.79^{a}	22.9^{a}	81.0^{a}	36.1^{a}	0.010^{b}
Ghazvini	35.1^{b}	4.78^{b}	19.2^{b}	56.6^{b}	24.7^{b}	0.011^{ab}
G×A	32.9^{b}	4.44^{c}	18.5^{b}	47.8^{b}	20.7^{b}	0.012^{a}
NaCl (mM)						
0	39.9^{a}	5.43^{a}	24.6^{a}	77.2^{a}	35.0^{a}	0.010^{b}
50	35.8^{ab}	5.12^{ab}	21.7^{ab}	65.5^{b}	28.4^{ab}	0.010^{b}
100	33.8^{b}	4.87^{bc}	18.8^{bc}	53.1^{c}	23.6^{b}	0.012^{a}
150	34.2^{b}	4.63^{c}	16.2^{c}	48.5^{c}	21.5^{c}	0.012^{a}
ANOVA						
Genotype	**	**	*	**	**	*
NaCl Level	*	**	**	**	**	**
NaCl×Genotype	ns	ns	ns	ns	ns	ns

Difference among the treatments was analyzed by 3 genotypes × 4 salt stress ANOVA; ns, *, ** indicate non-significant, and significant differences at 0.05 and 0.01, respectively. The data are means of 3 replicates; Mean separation was performed according to DMRT ($P \leq 0.05$) and similar letters indicate no significant difference between mean values.

Relative water content (RWC) and water potential (Ψ_{Leaf}) of the leaves were not affected by the NaCl treatments. However, Ψ_{Leaf} was significantly higher in the leaves of 'Akbari' seedlings (-0.68 MPa) and the lowest value (-0.892 MPa) was found in 'Ghazvini' leaves (Table 2). Leaf water content and succulence were significantly affected by the interactive effects of salt stress and plant genotype ($P \leq 0.01$). Water content significantly increased in the leaves of 'Akbari' and G×A under 100 and 150 mM NaCl stress up to 21 and 41 percent, respectively. However, no significant changes were observed in leaf water content of 'Ghazvini' under salt stress (Figure 1). Unlike the other genotypes, area basis leaf water content was significantly reduced in the leaves of 'Ghazvini' under 150 mM NaCl by 16.2 percent (Figure 1).

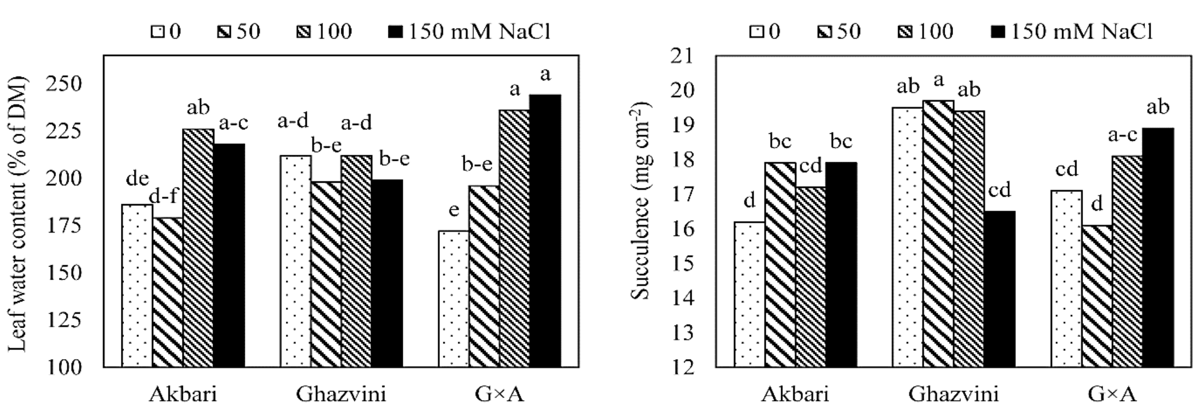

Figure 1: The interactive effects of salt stress and genotype on water content and succulence of the leaves of pistachio seedlings. Mean separation according to DMRT at $P \leq 0.05$ (n = 3)

Significant increase in leaf proline concentration was observed under severe salt stress. Among the genotypes, 'Ghazvini' and 'Akbari' had more leaf proline content than G×A (Table 2). Cell membrane stability index (CMS) remained unchanged under salt stress, too (Table 2). However, CMS in the leaves of 'Ghazvini' was significantly lower than the other genotypes. Significant accumulation of malondialdehyde (MDA) concentration was found in the leaves of the seedlings under salt stress and the highest increase in MDA concentration (45.9 %) was observed under 150 mM NaCl treatment. G×A had the highest leaf MDA concentration (15.5 mmol g^{-1}), and the lowest concentration (10.56 mmol g^{-1}) was found in 'Akbari' (Table 2).

Table 2: The effects of salt stress and plant genotype on relative water content (RWC), water potential (Ψ_{Leaf}), proline, cell membrane stability index (CMS), and concentration of malondialdehyde (MDA) in the leaves of pistachio seedlings

	RWC (%)	Ψ_{Leaf} (MPa)	Proline (µmol g $^{-1}$)	CMS (%)	MDA (mmol g $^{-1}$)
Genotype					
Akbari	83.3	-0.680^{a†}	267.8^{ab}	90.7^{a}	10.65^{b}
Ghazvini	84.0	-0.892^{b}	296.7^{a}	83.5^{b}	12.26^{ab}
G×A	85.3	-0.744^{ab}	218.1^{b}	92.1^{a}	15.50^{a}
NaCl (mM)					
0	84.2	-0.851	231.2^{b}	88.8	10.72^{b}
50	84.0	-0.683	258.2^{b}	90.7	11.94^{ab}
100	85.3	-0.755	271.0^{ab}	88.2	13.25^{ab}
150	83.6	-0.851	283.3^{a}	87.0	15.65^{a}
ANOVA					
Genotype	ns	**	*	**	*
NaCl Level	ns	ns	*	ns	*
NaCl×Genotype	ns	ns	ns	ns	ns

Difference among the treatments was analyzed by 3 genotypes × 4 salt stress ANOVA; ns, *, ** indicate non-significant, and significant differences at 0.05 and 0.01, respectively. The data are means of 3 replicates; Mean separation was performed according to DMRT ($P \leq 0.05$) and similar letters indicate no significant difference between mean values.

Table 3 represents the effects of salt stress and plant genotype on concentration of leaf pigments. Chlorophyll a:b ratio (Chl a:b) significantly increased in the leaves of pistachio seedlings in response to salt stress. Chl a:b in the leaves of 'Akbari' was significantly lower than the other genotypes. Leaf greenness, which was measured by SPAD, significantly reduced under salt stress. However, no significant difference was observed in leaf color of the genotypes. Salt stress significantly reduced total chlorophyll concentration in the leaves. 'Akbari' had the highest leaf chlorophyll concentration than the other genotypes. Concentration of anthocyanins and carotenoids in the leaves remained unchanged under salt stress, however, concentration of carotenoids in the leaves of 'Akbari' was higher than 'Ghazvini' and G×A.

Table 3: The effects of salt stress and plant genotype on concentration of pigments in the leaves of pistachio seedlings

	SPAD	Chl a:b	Total Chls (mg cm^{-2})	Carotenoids (mg cm^{-2})	Anthocyanins (mmol g^{-1})
Genotype					
Akbari	55.0	0.395^{b†}	29.2^{a}	2.23^{a}	280.9
Ghazvini	55.9	0.420^{a}	25.4^{b}	2.03^{b}	282.1
G×A	56.1	0.423^{a}	23.6^{b}	1.90^{b}	282.3
NaCl (mM)					
0	57.8^{a}	0.396^{b}	27.9^{a}	2.13	289.5
50	55.4^{b}	0.422^{a}	23.8^{b}	1.90	279.3
100	54.6^{b}	0.421^{a}	26.8^{ab}	2.08	283.3
150	54.9^{b}	0.416^{a}	25.2^{b}	2.06	275.3
ANOVA					
Genotype	ns	**	**	**	ns
NaCl Level	*	**	*	ns	ns
NaCl×Genotype	ns	ns	ns	ns	ns

Difference among the treatments was analyzed by 3 genotypes × 4 salt stress ANOVA; ns, *, ** indicate non-significant, and significant differences at 0.05 and 0.01, respectively. The data are means of 3 replicates; mean separation was performed according to DMRT ($P \leq 0.05$) and similar letters indicate no significant difference between mean values.

Chlorophylls:carotenoids ratio (Chl:Crt) was significantly affected by the interactive effect of salt stress and plant genotype ($P \leq 0.01$). Chl:Crt ratio remained unchanged in the leaves of salt stressed 'Akbari' and 'Ghazvini' seedlings. A significant decrease in Chl:Crt ratio was found in the leaves of G×A under salt stress (Figure 2).

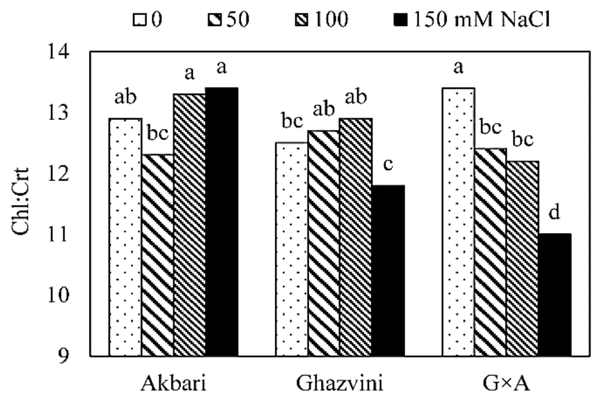

Figure 2: The interactive effects of salt stress and plant genotype on chlorophylls:carotenoids ratio (Chl:Crt) in the leaves of pistachio seedlings. Mean separation according to DMRT at $P \leq 0.05$ (n = 3)

4 DISCUSSION

4.1 Plant growth

The growth and biomass of pistachio genotypes reduced with increasing NaCl concentration in the nutrient solution. This was in accordance with the earlier reports on studying pistachio responses to salt stress (Hokmabadi et al., 2005; Tavallali et al., 2008; Karimi et al., 2009). Limitation of plant growth under salt stress can be attributed to osmotic stress, leaf injuries, and reduced photosynthesis active area (Wahome et al., 2001). Biomass of G×A and 'Ghazvini' seedlings was significantly lower than that in 'Akbari' seedlings. Munns (2002) stated that salt stress reduces plant growth by causing premature senescence of leaves and reducing supply of assimilates for growth. Leaf abscission is a major cause of loss of biomass and growth inhibition of pistachio under salt stress (Karimi et al., 2009). Necrosis and abscission of leaves of the plants started from the older leaves at the base of stem (Rahemi et al., 2017). Leaf abscission is primarily dependent upon accumulation of Na^+ and Cl^- ions in the leaves (Brumós et al., 2009). Accordingly, rate of leaf abscission under salt stress could indirectly determine plant sensitivity to ionic stress of salinity. In this regard, monitoring leaf abscission indicated that G×A and 'Ghazvini' seedlings were more susceptible to salt stress than 'Akbari' seedlings.

Specific leaf area (SLA) is known to be negatively correlated with relative growth rate (Osone et al., 2008) and water use efficiency of plant (Rao et al., 1995). The higher SLA of G×A and 'Ghazvini' seedlings under salt stress indicated the lower growth rate and higher salt sensitivity of these plants than those in 'Akbari' seedlings (Ball, 2002). Moreover, the increase in SLA of pistachio leaves in response to salt stress indicated that the leaves became thinner due to mesophyll destruction and limitation of photoassimilate accumulation and starch biosynthesis (Sefton et al., 2002). Plants with higher SLA have fewer cells per unit area with lower concentration of chlorophylls in their leaves, which reduce plant photosynthetic capacity and WUE. Such plants have poor performance under salinity (Omamt et al., 2006) and osmotic stress (Karimi et al., 2012). The increase in SLA of pistachio seedlings under salt stress was in accordance with Behboudian et al. (1986) who reported that NaCl destructs leaf mesophyll and reduces CO_2 assimilation and photosynthetic efficiency of pistachio.

Stem diameter changes are usually monitored for investigating tree growth and water transport. Decrease in trunk diameter under salt stress was previously reported in *Carya illinoinensis* (Wangenh.) K.Koch (Miyamoto et al., 1986), *Persea americana* Mill. (Bernstein et al., 2001), *Pistacia* spp. (Ferguson et al. 2002), and *Olea europea* L. (Aragüés et al., 2005). Yaron et al. (1969) stated that decrease of stem diameter with increasing salt stress was primarily due to leaf abscission. Ashraf and Sarwar (2002) also found that decreased stem diameter under salt stress was due to the reduction in turgor potential and cell division. In sum, the significant decrease in crown diameter of pistachio seedlings confirmed the limitation of photoassimilates accumulation in stem and limitation of root function in water transport under salt stress (Escalona et al., 2002). Moreover, the larger crown diameter of 'Akbari' seedlings emphasized on higher growth and performance of this genotype than the others under salt stress.

4.2 Water relations

The growth inhibition under salt stress is partially related to water deficiency or osmotic stress, which induces changes in the cell metabolic activity (Jaleel et al., 2007) and reduces cell division and expansion (Taiz and Zeiger, 2006). In the present study, salt stress did not affect RWC of leaves of the pistachio seedlings. Hokmabadi et al. (2005) also reported the same result in pistachio genotypes under salt stress. The significant increase in proline concentration under salt stress revealed that maintenance of RWC in pistachio leaves was owed to accumulation of osmolytes and osmotic adjustment. Accumulation of osmolytes, such as proline, is of curtail importance for water conservation in tissues and preventing cytoplasm dehydration under osmotic stress (Hoekstra et al., 2001). Previous researchers also reported that proline concentration increased in pistachio leaves in response to salt stress (Hokmabadi et al., 2005; Karimi et al., 2009; Karimi et al., 2017). Since the highest leaf proline concentration was extracted from leaves of 'Ghazvini' seedlings with the lowest water content in the leaves, it can be assumed that higher leaf proline concentration reflects osmotic pressure of salt stress on the plants. But, some researchers believe that higher accumulation of proline in the leaves is related to salt tolerance of this species (Hokmabadi et al., 2005; Szabados and Savoure, 2010). This seems not to be a universal fact, since our results indicated that other physiological responses of plant should be considered in drawing conclusions in this respect. 'Akbari' and G×A seedlings were able to preserve leaf RWC with lower proline concentration in their leaves. These observations suggest that mechanisms other than osmoregulation help these plants preserving leaf water content under salt stress.

Stomatal closure is the earliest response of plants for water conserving under water deficit conditions (Flexas and Medrano, 2002; Pérez-López et al., 2009). Studying stomatal characteristics of some pistachio cultivars and species has indicated that 'Ghazvini' has large stomata (Arzani et al., 2013). Therefore, inefficiency of stomatal regulation in its leaves is expected (Bosabalidis and Kofidis, 2002). In this regard, Saadatmand et al. (2007) revealed that the salt tolerance of pistachio 'Ghazvini' is dependent on water availability to this plant. They compared salt tolerance of 'Ghazvini' seedlings with another Persian rootstock, 'Sarakhs', under different irrigation regimes. Their results indicated that 'Ghazvini' was more salt tolerant than 'Sarakhs' at the lowest irrigation intervals; but, with increasing irrigation intervals, 'Ghazvini' turned to the salt sensitive genotype. In contrast with 'Ghazvini', increases in leaf succulence and leaf water content were observed in 'Akbari' and G×A seedlings in responses to salt stress. Increase in leaf succulence is resulted from increase in leaf cell number and volume. This is a common response of many glycophytes to salinity (Kim and Park, 2010) which reduces ion toxicity pressure on plant by diluting the ions in the leaves (Larcher and de Assis Prado, 2000; Ottow et al. 2005). Moreover, succulence is important for regulating leaf temperature and water balance under salinity induced osmotic stress (Fitter and Hay, 2002). Thus, the increased leaf succulenece in 'Akbari' and G×A seedlings considered to be a morphophysiological adaptation to salt stress which reduces the internal salt concentration and ionic pressure (Jennings, 1968). Lack of such adaptation in 'Ghazvini' probably is linked with the higher sensitivity of the plant to salt stress.

4.3 Oxidative damage

In addition to ion toxicity, oxidative stress is another cause of leaf injury and necrosis under salt stress (Munns, 2002). Accumulation of toxic ions in cell and experiencing water stress disrupt photosynthesis and photorespiration, alter the homeostasis of cells, and increase the production of reactive oxygen species (ROS). Accumulation of ROS cause oxidative damage to cell membrane and other subcellular structures (Miller et al., 2010). Leaf MDA concentration and CMS are widely determined to evaluate the effects of oxidative damage on plasma membrane integrity under environmental stress (Foyer et al., 1997). MDA is the most abundant individual aldehyde resulting from lipid peroxidation (Dmitriev and Titov, 2010); thus, its accumulation in the leaves of pistachio genotypes under 150 mM NaCl stress confirmed oxidative damage to the leaf cells. However, salt stress did not affect CMS in the leaves. These observations suggested that accumulation of MDA in the leaves is an earlier response than CMS loss and it can provide a higher contrast in assessment of oxidative damage under salt stress. It should be noted that ion toxicity and leaf necrosis are mainly started from the older leaves on pistachio shoot; therefore, the young and fresh leaves which remain on the plant for evaluating CMS may not reflect the actual pressure of salt stress on the plant. In sum, according to higher leaf MDA content, G×A and 'Ghazvini' seedlings were found to be more prone to oxidative damage and ion toxicity than 'Akbari' seedlings.

Significant decrease of photosynthetic pigments in the leaves also indicated oxidative damage to cell under salt stress. Since chloroplasts are of the primary sites of ROS production under abiotic stress, the early symptoms of oxidative injuries become evident on chloroplast structure and degradation of photosynthetic pigments (Maiale et al., 2004). The significant reductions in total chlorophylls, Chl:Crt ratio and the greenness of the leaves of pistachio genotypes under salt stress were in parallel with MDA accumulation in the leaves. The increase in chlorophyll a:b ratio under salt stress indicated that destruction of chlorophyll b was higher than chlorophyll a (Karimi et al., 2009). This observation indicated structural damages to photosystem II reaction center (Mehta et al., 2010). The lower Chl:Crt ratio in the leaves of 'Ghazvini' and G×A seedlings than that in 'Akbari' seedlings, indicated that the degradation of chlorophylls was higher than carotenoids in the sensitive genotypes. Loss of photosynthetic pigments and structural damage to chloroplasts in salt sensitive pistachio genotypes have been correlated with limitation of photosynthesis rate and the plant growth and productivity under salt stress (Behboudian et al., 1986; Ferguson et al., 2002; Karimi et al., 2009). The higher oxidative stress tolerance of 'Akbari' seedlings than the other genotypes was partially owed to its lower SLA. Low SLA minimizes light capture, while enhances water use efficiency via reducing water loss potential (Field et al., 1983). Higher self-shading within the leaf tissue and higher water conservation capability of the leaves with lower SLA are of crucial importance for reducing the production of ROS under salinity or drought stress situations.

Some leaf pigments and cell antioxidant defense also involve in reducing ROS-derived injury under salt stress (Sevengor et al., 2011). Increase in biosynthesis of sun screen pigments including carotenoids and anthocyanins help plants to cope with excess light energy and reduce ROS production in the leaves (Sherwin and Farrant, 1998). Carotenoids preserve chloroplast structure and prevent degradation of chlorophylls under abiotic stress (Nishida et al., 2007; Karimi et al., 2013; Jain and Gould, 2015). These pigments by regulation of light harvesting by the chlorophyll antenna system of photosystem II, detoxification of ROS and protecting membrane lipids against peroxidation involve in plant defense system against abiotic stresses (Havaux, 2014). Therefore, the higher concentration of carotenoids in the leaves of 'Akbari' may explain lower chlorophyll b destruction in its leaves in comparison with that in other genotypes. Anthocyanin have shown to accumulate in plant vacuoles in response to salinity (Piao et al., 2001; Keutgen and Pawelzik, 2007) and drought stress (Basu et al., 2010; Sperdouli and Moustakas, 2012). Increase in biosynthesis of these pigments is thought to mask chlorophyll and act as filters preventing excess light absorption by the photosynthesis aparathus (Hopkins, 1999). In addition to their photoprotective function (Mittler, 2002), anthocyanins also involve in osmoregulation and ROS scavenging in plant defense network (Jain and Gould, 2015). However, in this study, salt stress did not affect concentration of anthocyanins in the leaves of pistachio seedlings. Chalker-Scott (2002) stated that the toxic effects of Na^+ and Cl^- ions may prevent anthocyanin biosynthesis and accumulation in plant leaves. Furthermore, since biosynthesis of these metabolites is greatly under control of environmental factors, more studies are needed to clarify their role in defense system of this species against salt stress.

5 CONCLUSION

In sum, progressive leaf abscission and loss of shoot biomass, limitation of stem growth, and higher SLA of 'Ghazvini' and G×A seedlings under salt stress revealed that these genotypes are more prone to salt stress than 'Akbari' seedlings. The water relations data confirmed that 'Ghazvini' seedlings experienced water stress under severe salt stress. The highest proline accumulation in the leaves of 'Ghazvini' was in parallel with water deficit development in its leaves. Therefore, leaf proline concentration is a good indicator of osmotic stress pressure on pistachio plants. Increase in leaf succulence and leaf water content indicated morpho-physiological adaptations in the leaves of 'Akbari' and G×A, which reduced ion toxicity in their leaves. However, higher MDA concentration and chlorophyll degradation in the leaves of G×A seedlings revealed that this genotype is prone to oxidative stress. The higher concentration of carotenoids in the leaves of 'Akbari' protected the photosynthetic apparatus and improve oxidative tolerance of this genotype. Moreover, the higher oxidative stress tolerance of 'Akbari' seedlings was partially related to their lower leaf SLA, which resulted in higher self-shading and lower water loss potential in their leaves..

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Response of onion to different nitrogen levels and method of transplanting in moderately salt affected soil

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ABSTRACT

Rapid increase in population and consumption, urged upon the agronomists to develop a comprehensive site specific agro technology to boost up production per unit area and quality of daily dietary onion crop in salt affected soils by improving some basic components of the prevailing onion production technology in Pakistan. In this perspective a detailed and systematic series of field studies were undertaken for three consecutive years (2013 - 2015) at Soil Salinity Research Institute, Pindi Bhattian, Pakistan to evaluate different nitrogen levels and the cost-effective planting technique for onion production under salt affected conditions. The experiment was laid out in split plot arrangement using randomized complete block design having three replications. Two planting methods, ridge and bed planting and 4 nitrogen levels, 1- recommended dose (RD) of N (90 kg ha^{-1}), 2 -75 % N of RD (67.5 kg ha^{-1}), 3 -125 % N of RD (112.5 kg ha^{-1}), 4 - 150 % N of RD (135 kg ha^{-1}) were used. Planting methods were kept in main plots and nitrogen levels in sub plots keeping sub-plot size of 4 m x 6 m. Measurements included were: plant height, number of leaves/plants/m^{-2}, bulb diameter, bulb mass, total bulb yield, number of flowers/umbel, seed mass/plant and 1000-seed mass. Results showed that maximum onion yield and yield attributes were recorded with nitrogen application at rate of 150 & 125 of RD in ridge planting. However nitrogen application at rate of 125 of RD in ridge planting recorded higher economic returns over all the other treatments and is recommended as most cost effective technique for onion production under salt affected soil as compare to other treatments.

Key words: onion; nitrogen fertilization; planting technique; salinity; cost benefit

IZVLEČEK

ODZIV ČEBULE NA RAZLIČNA GNOJENJA Z DUŠIKOM IN TEHNIKE SADNJE V RAZMERAH ZMERNO SLANIH TAL

Hitra rast prebivalstva in z njim povezana poraba hrane zahtevata od agronomov, da razvijajo in izboljšujejo primerne, rastiščem prilagojene tehnike pridelave čebule glede na enoto površine in kakovost dnevne porabe v razmerah slanih tal v Pakistanu. V treh zaporednih rastnih sezonah (2013 - 2015) so bili opravljeni podrobni in sistematični poljski poskusi na Soil Salinity Research Institute, Pindi Bhattian, Pakistan z namenom ovrednotenja učinkov različnega gnojenja z dušikom in različnih tehnik sadnje čebule v razmerah slanih tal. Poskus je bil zasnovan na osnovi deljenk kot popoln naključni bločni poskus s tremi ponovitvami. Uporabljena sta bila dva načina sadnje, na grebene in v brazde ter štiri načini gnojenja z dušikom glede na priporočeno dozo (RD, 90): 1 - priporočena doza (RD) (90 kg ha^{-1}), 2 - 75 % N od RD (67.5 kg ha^{-1}), 3 - 125 % N od RD (112.5 kg ha^{-1}) in 4 - 150 % N od RD (135 kg ha^{-1}). Obravnavanja z načini sadnje so bila na glavnih ploskvah, obravnavanja gnojenja z N pa na podploskvah velikost 4 m x 6 m. Meritve so obsegale višino rastlin, število listov/rastlino/m^2, premer čebule, maso čebul, celokupni pridelek čebul, število cvetov na kobul, maso semena na rastlino in maso 1000 semen. Rezultati so pokazali največji pridelek čebule in njegovih delov pri obravnavanjih z dušikom 150 in 125 % od priporočene doze (RD) in pri sadnji na grebene. Pri tem je obravnavanje z dušikom 125 % od priporočene doze in sadnja na grebene dalo največji ekonomski učinek v primerjavi z drugimi in ga priporočamo kot stroškovno najbolj učinkovit način pridelave čebule v razmerah zasoljenih tal.

Ključne besede: čebula; dušikova gnojila; načini sadnje; slanost; analiza stroškov in koristi

1 INTORODUCTION

Among vegetables, *Allium* species have worldwide consumption and emerged as popular dietary items in many countries because of its high nutritive value and specific flavor properties (Griffith et al., 2002). In Pakistan onion crop is grown approximately on 0.153 million hectares with a reported production of 2.015

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million tons whereas average yield is 13.2 t. ha-1 (GOP, 2015). Across the world, average onion consumption is approximately 6.5 kg per capita per annum while in Pakistan, it is 10 kg per annum (Kabir, 2007). Keeping in view the rapid increase in population and consumption level, Pakistan has to produce about 15 million tons of onion per annum. So as to remain self-sufficient in onion, not only the area of production but also productivity per unit area needs to be increased extensively. Rapid increase in population is exerting more pressure on its demand. Hence the farmers can in turn obtain good remuneration by producing this important crop by utilization of marginal lands owing to its increasing demand in local market and exportation commodity. Due to arid to semi-arid climate, Pakistan has 6.67 m ha of salt affected land which is approximately 1/3rd of the total cultivated area (Khan,1998) and therefore great scope exists to increase production of this cash value crop through strengthening research and development activities in salt affected soils.

Onion is classified as relatively salt sensitive crop with threshold level of 1.2 dSm-1 electrical conductivity (EC) (Mass and Hoofman, 1977). Consequently, for better yield and quality of onion in salt affected soil, there is need to improve cultivation methods, like planting method, planting time, proper planting geometry, optimum fertilization and other management practices for farmers to grow onion crops in salt affected soils at a commercial scale.

Nitrogen performs a critical role during photosynthesis and is also an indispensable part of protein. It is vital for many physiological and biochemical reactions of plant metabolism (Balasubramaniyan and Palaniappan, 2001; Epstein and Bloom, 2006). Nitrogen management efficiency assumes greater importance in salt affected soils and have to follow an optimal nitrogen fertilization strategy for sustainable onion production (Songzhong, 2009). Because nitrogen, constitute approximately 80 % of the total mineral nutrients absorbed by plants (Marschner, 1995). Under most agro ecological conditions, nitrogen has been reported as one of the most yield limiting nutrients for crop production and its effective use is imperative for the economic sustainability of cropping systems (Fageria and Baligar, 2005). Considering the benefits of fertilization, some researchers have reported that addition of nitrogen and phosphorus alleviate the harmful effect of salt stress and osmotic stress on plants (Grattan and Grieve, 1999; Kaya and Higgs, 2002; Kaya and Higgs, 2003; Abu-Romman and Suwwan, 2012; Abu-Romman et al., 2013). Onion productivity could be increased substantially through use of improved cultivars and optimum use of fertilizers (Shaheen, 2007). Nitrogen application in onion significantly influences the flavor, development and quality of bulb (Randle, 2000). Several researcher have reported higher levels of N considerably improved the production of onion over low level of nitrogen (Khan et al., 2002; Ghaffoor et al., 2003; Al Abdulsalam and Hamaiel, 2004; Moursy et al., 2007; Yaso et al., 2007; Nemat et al., 2011; Dhital et al., 2015).

Suggested agronomic practices for saline soils comprise irrigation at night to avoid evaporation loss (Rhoades, 1999), better cultivation approaches like sowing on raised beds, pre-sowing seed treatments to improve germination even in salt stress environment (Sayre, 2007; Egamberdiev, 2007; Bakker et al., 2010). Improved planting technique not only help to maintain optimize plant population by avoiding excessive crowding but also allow plants to exploit land, light, and other input resources uniformly and efficiently. Saline soil, owing to its high bulk density and low porosity, confines the plant roots in the upper surface layer and reduces the soil volume which could be explored by the plants for water and nutrients acquisition. In ridges planting plants establish well developed root system due to loose fertile layer of soil with more surface area (Bucher, 2007; Ao et al., 2010; Khan et al., 2012). Bakht et al. (2011) reported that planting methods had significant effect on the yield and yield components of different maize varieties, maximum plant height, number of plants ha^{-1}, more grain and biological yield was recorded in ridge planting. Khan et al. (2000) reported maximum yield of canola in ridge planting method as irrigation next to seed resulted movement of salts away from seed producing less saline environment in ridges. Malik et al. (2001) also reported the maximum yield of sunflower in ridge planting pattern than other conventional planting methods. Keeping above facts, series of experiments were taken with view to understand a cohesive evaluation of onion fertilizer requirements and planting method while meeting specific extension needs of growers in salt affected areas.

2 MATERIALS AND METHODS

Field trial was set and carried out for three consecutive winter season from 2013 to 2015 at

Soil Salinity Research Institute Pindi Bhattian. The pH of saturated soil paste was 8.67, electrical conductivity of soil extract (EC_e) = 4.10 (dS m-1) and sodium absorption ratio (SAR) = 34.41 (mmol $l^{-1})^{1/2}$. The experiment was laid out in split plot arrangement using randomized complete block design having three replications. Two planting methods (ridge planting and raised bed planting) and 4 nitrogen levels 1- recommended dose (RD) of N (90 kg ha^{-1}), 2 - 75 % N of RD (67.5 kg ha^{-1}), 3 - 125 % N of RD (112.5 kg ha^{-1}), 4 - 150 % N of RD (135 kg ha^{-1}) were used in the study. Planting methods were kept in main plots and nitrogen levels in sub plots keeping sub-plot size of 4 m x 6 m. Bulbs of onion (*Allium cepa* L.) cultivar namely 'Phulkara' were planted in 3rd week of December for three consecutive years. Fertilizer dose of P, K (90-60 kg ha^{-1}) and nitrogen according to treatment plan was used at the time of land preparation in the form of single super phosphate, sulphate of potash and urea respectively. All the standard agronomic management practices were adopted. The data regarding different agronomic parameters like plant height, number of leaves/plants, bulb diameter, bulb mass, bulb yield, number of flowers/umbel, seed yield/plant and 1000-seed mass were recorded and subjected to analysis of variance following the method of Steel et al. (1997) to sort out significant differences among treatments at 5 % probability level using STATISTIX 8.1 package software. In order to assess the economic feasibility of different nitrogen levels and planting methods used, an economic analysis was evaluated (Shah et al., 2013).

3 RESULTS AND DISCUSSION

3.1 Plant height (cm)

Pooled data of three years regarding plant height evidenced linear response with increasing nitrogen levels, as well as for N by planting methods interaction (Table 1). Treatment using nitrogen at rate of 150 % of RD recorded the highest plant height (56.16 cm) which was followed by 125 % N of RD with plant height of (55.33 cm) however difference between both treatments was not statistically significant ($P \leq 0.05$). The lowest plant height (36.00 cm) was estimated with nitrogen dose at rate of 75 % of RD. Planting method, however, did not influenced the plant height, ridge planting produced greater plant height (49.16 cm) than bed planting (46.41 cm) but both treatments were statistically non-significant. Data regarding interactive effect of nitrogen levels and planting method showed that nitrogen at rate of 150 % of RD in ridge planting documented the taller plant of (57.00 cm) which was statistically similar with N at rate of 150 % of RD with bed planting and nitrogen application at rate of 125 % of RD in ridge and bed planting respectively. Nitrogen is one of the most important growth-limiting macro nutrient in salt affected soils (Gupta and Abrol, 1990; Curtin and Naidu, 1998; Irshad et al., 2002) and its availability is also affected in these soils as NH_3 volatilized due to high pH (Gupta and Abrol, 1990) and NO_3 uptake is inhibited by Cl^{-1} which is usually present at toxic concentrations in salt affected soils (Grattan and Grieve, 1999). Salt stressed crop generally needs an additional 25 % more N than the same cropping sequence used on normal soils for improved plant growth and yield (Gupta and Abrol, 1990). In our study this remarkable increase in plant height with higher level of nitrogen than recommended may be explained by increased vegetative growth probably due to efficient exploitation of absorbed nutrients and improved nourishment through nitrogen application (Bhakher et al., 1997). Ours finding are in consistent with Aregawi (2006) who reported that N fertilization at 150 kg N ha^{-1} and 180 kg N ha^{-1}, respectively increased plant height more than in plots that received no N fertilization. Similarly Subedi (2001) reported the positive effect of different levels of N on plant height.

3.2 Number of leaves/plants

Concerning to number of leaves a pronounced effect was found for N. Results display in Table 1 showed that there was a notable increase ($P \leq 0.05$) in number of leaves/plants with increasing levels of nitrogen over 100 % N of RD than at lower level of N. With respect to planting techniques, ridge planting proved superior with number of leaves of 8.75 over bed planting with 7.91 number of leaves. Pooled data reflected that maximum number of leaves (10.16) was produced in 150 % N of RD followed by 125 % N of RD, however, the differences in level of N from 125 % to 150 % of RD could not show significant differences on this parameter and minimum number of leaves (6.16) was recorded in 75 % N of RD. Interaction between nitrogen levels and planting methods also showed pronounced effect on leaves number. Greater number of leaves (10.66) was recorded in ridge planting, where nitrogen was applied at rate of 150 % of RD however it was statistically non-significant with 125 % N of RD. Higher number of leaves in ridge planting against bed planting may be explained that ridge planting rendered favorable growth conditions and improved nutrient absorption capacity (Bakht et al., 2011). Similar results are also reported by Siddique and Bakht (2005) and Bakht et al. (2006) that maximum grain yield in maize was recorded in ridge planting. The positive effect of higher dose of nitrogen on leaf length may be attributed to its role in synthesis of proteins, enzymes, chlorophyll, and the occurrence of more protein leads to increase the leaves size and consequently carbohydrate synthesis is accelerated (Bungard et al., 1999). Our results are in accordance with findings of other authors (Neeraja et al., 2001; Diaz-Perez et al., 2003; Singh et al., 2004; Jilani 2004) that nitrogen fertilization significantly increases leaves length in onion.

3.3 Bulb diameter (cm)

A perusal of data regarding the bulb diameter it could be observed that nitrogen levels, planting methods and their interactive effect significantly ($P \leq 0.05$) increase bulb diameter (Table 1). Over all mean value of three years showed that the highest bulb diameter (5.80 cm) was recorded in 150 % N of RD but it was non-significant with 125 % N of RD and the minimum bulb diameter was found in 75 % N of RD obtaining value of 4.42 cm. In respect to the planting methods, onion planted on ridges produced the maximum bulb diameter of (5.29 cm) when compared with bed planting method (5.12 cm). Planting methods and nitrogen interaction was also found significant. Nitrogen at rate of 150 % of RD with ridge planting produced bulb diameter of 5.85 followed by125 % N of RD in ridge planting however statistically no difference was found among two fertilizer levels in ridge and bed planting. In salt affected field, reduction in root-zone salinity is one of effective approach to enhance crop emergence and stand establishment (Dong et al., 2008). Raised beds offered a large surface area than ridges and under saline conditions, salt accumulation on top of the beds increased due to upward movement of groundwater driven by evaporation (Deng et al., 2003; Qiao et al., 2006 and Bakker et al., 2010). Comparable findings are also stated by (Arif et al., 2001; Oswald et al., 2002; Rasheed et al., 2003) that in maize, ridge planting gave better results than other sowing methods. Results of present study coincide with the reports of many researchers that bulb length increase markedly in response to nitrogen application (Hussaini et al., 2000; Mandira and Khan, 2003; Lee et al., 2003; Yadav et al., 2003; Reddy et al., 2005; Nasreen et al., 2007).

3.4 Bulb mass (g)

As far as bulb mass is concerned pooled data of three consecutive seasons, showed pronounced effect of successive increasing levels of nitrogen on bulb mass and interaction of N x planting methods (Table 1). Treatment using nitrogen at rate of 150 % of RD for which the peak value of bulb mass (59.25 g) was recorded against N at rate of 125 % of RD with bulb mass of (58.78 g) however difference between these two treatments was not large enough to reach the level of significance. Results reflected that ridge planting produced significantly higher bulb mass of (53.86 g) than bed planting (50.94 g). Data regarding interactive effect of nitrogen levels and planting methods showed that nitrogen at rate of 150 % of RD with ridge planting produce maximum bulb mass (60.93 g) which was statistically alike with nitrogen at rate of 125 % of RD with ridge planting followed by nitrogen at rate of 150 & 125 % of RD with bed planting. Optimum fertilization in salt affected soil resulted in better crop yields by improving nutrient contents in plant tissue and soil solution (Adediran et al., 2004) and may produce 67 % of additional yield than normal field (Taiwo et al., 2001). So optimum level of nitrogen that precisely match crop requirements lessens the chances for N loss as the plant is established and rapidly taking it up (Andraski et al., 2000).The increase in bulb mass with application of N at rate of 125 & 150 % of RD could be ascribed to overall improvement in crop growth and vigor, due to more root proliferation, improved nutrients and water uptake, greater leaf number, more photosynthesis and accelerated rate of assimilation (Yadav et al., 2005). These results show responsiveness of the onion to nitrogen fertilization and support the earlier findings of many authors that the nitrogen contributes markedly to production of larger bulbs. (Baghour et al., 2001; Anwar et al., 2001; Ghaffoor et al., 2003; Lee et al., 2003; Mandira and Khan, 2003; Arian et al., 2004; Gautam and Pande, 2005). Moreover in ridge planting fertile soil is more loose with improved aeration and moisture supply and low resistance to new emerging roots for exploitation of more surface area for nutrient acquisition might be the reason of better growth in ridge sown maize crop (Chassot and Richner, 2002).

3.5 Bulb yield (t ha^{-1})

Bulb yield depicted in (Table 2) revealed significant difference among the planting methods and different nitrogen levels and interaction of nitrogen and planting methods. N at rate of 125 & 150 % of RD were most effective treatments in increasing bulb yield, 19.65 and 19.61 t ha^{-1} respectively however statistically both treatments were alike and the lowest value of bulb yield (15.60 t ha^{-1}) was noted where nitrogen was applied at rate of 75 % of RD. With respect to the planting methods, ridge planting has better effect on bulb yield (18.29 t ha^{-1}) as compared to bed planting (18.01 t ha^{-1}). Interaction of nitrogen levels and planting methods showed that the greater bulb yield (19.71 t ha^{-1}) was produced with nitrogen at rate of 150 % of RD with ridge planting and statistically was similar to nitrogen at rate of 125 % of RD in bed planting and the minimum bulb yield (15.38 t ha^{-1}) was achieved in 75 % N of RD with bed planting. Arid to semi-arid climate of Pakistan results in very low soil organic matter hence availability of nitrogen for optimum plant growth and development is very low and this situation becomes worse in salt affected soil due to excess of Cl^{-1} which antagonizes the uptake of nitrogen (Grattan and Grieve, 1999). The increased in bulb yield with increasing levels of nitrogen can be justified by the fact that nitrogen performs a critical role during photosynthesis and is also an indispensable part of protein. It is vital for many physiological and biochemical reactions of plant metabolism (Balasubramaniyan and Palaniappan, 2001; Epstein and Bloom, 2006) and ultimately yield is improved. The current results of this study are in strong agreement with findings of different authors who reported the stimulatory effect of nitrogen on onion yield (Ehsan et al., 2002 ; Cizauskas et al., 2003; Abdissa et al., 2011). Our results also confirmed the findings of Choudhary et al., (2008) that in salt affected field, salt accumulation increases on top of the beds, and bigger bulb yield in ridge planting might also be due to the more favorable soil conditions created by ridges. That result in better roots development allowing the plants to uptake more nutrients and moisture (Rasheed et al., 2004; Amin et al., 2006; Abdullah et al., 2007; Ghaffar et al., 2012). Similar results have been also reported by Liu and Yong (2008) and Belachew and Abera (2010).

3.6 Number of flower/umbel

As seems to be appearent from data, the Table 2 revealed that the additional response of nitrogen fertilization and planting method on number of flowers was statistically ($P \leq 0.05$) significant. 150 % N of RD produced maximum number of flowers (150.33) which was statistically alike with 125 % N of RD and consequently reduced number of flowers were evidenced in 75 % N of RD having flowers of (134.83) followed by N at rate of RD. In the case of planting methods the maximum number of flowers (146.17) was noted in ridge planting which differed significantly from bed planting with 142.83 of flowers/umbel. Interaction between nitrogen levels and planting methods showed that N at rate of 150 % of RD with ridge planting recorded the highest number of flowers (151.67) however statistically no significant difference was found among N at rate of 125 % of RD in ridge planting and N at rate of 150 % of RD in bed planting. Being shallow rooted crop, onion is highly responsive to nitrogen application at high rate which markedly improved the production. More surface area from bare top soils of bed results more evaporation rates which leads to more salt accumulation as compared to ridge. (Choudhary et al., 2008; Cardon et al., 2010). Our results coincide with findings of Liu & Young (2008) and Belachew and Abera (2010).

3.7 Seed yield/plant (g)

The results presented in Table 2 revealed that the effect of nitrogen fertilization and planting methods on seed yield/plant was statistically ($P \leq 0.05$) significant. 150 % N of RD produced the maximum seed yield (1.29 g) which was of slightly higher value than found in 125 % N of RD, so statistically both the treatments were similar and minimum seed yield was recorded in 75 % N of RD with seed yield of (0.73 g). In case of planting methods the maximum seed yield (1.11 g) was noted in ridge planting which differed significantly from bed planting with 1.03 g seed yield/plant. Interactive effect of nitrogen levels and planting methods showed that N at rate of 150 % of RD with ridge planting recorded the highest value of seed yield (1.32 g) however statistically no significant difference was found among N at rate of 150 % of RD in bed planting and N at rate of 125 % of RD in ridge and bed planting respectively. By analyzing results obtained from three consecutive seasons it could be concluded that N at rate of 125 % of RD is the dose which gave the best response in interaction with ridge planting. These findings are confirmed also by those, reported by Ali et al. (2007) that N dose up to 150 kg ha^{-1} in onion produced bigger number of flowers, better fruit set and seed yield significantly. Current findings corroborate the report of Bakht et al. (2011) who suggested that ridge planting rendered better growth conditions and improved nutrient absorption capacity.

3.8 1000-seeds mass (g)

The results presented in Table 2 depicted that the maximum 1000-seeds mass occurred by applying nitrogen level of 125 & 150 % of RD. Treatment where N was applied at rate of 150 % of RD produced the maximum seed yield (2.37 g) which was statistically alike with 125 % N of RD and the minimum 1000-seed mass of (1.98 g) was noted at the dose of 75 % N of RD. In case of planting methods the maximum 1000-seed mass (2.24 g) was noted in ridge planting which differed significantly from bed planting with 2.14 g seed mass. Nitrogen levels and planting methods interaction showed that N at rate of 150 % of RD with ridge planting recorded the highest value of 1000-seed mass (2.40 g) however statistically no significant difference was found among N at rate of 150 % of RD in bed planting and N at rate of 125 % of RD in ridge and bed planting respectively. These results are in agreement with previous findings which showed the positive responses of N application on onion crops (Neeraja et al., 2001; Diaz-Perez et al., 2003; Singh et al., 2004).

3.9 Economic analysis

Economic feasibility in financial terms of any innovation or technique has primary importance in deciding its wider adoption among farming community (Khan et al., 2012). Economic analysis was carried out at the end of study to evaluate the best and economical nitrogen level and planting technique to grow onion crop under salt affected conditions. Different planting technique and nitrogen levels resulted in different net income as indicated in the Table 3. Data regarding economic analysis for different treatments revealed that the highest net income (Rs. 300800 ha^{-1}) with cost benefit ratio of (4.34) was earned with nitrogen application at rate of 125 % of RD in ridge planting as compare to other treatments which may be due to more economic yield.

4 CONCLUSION

In salt affected field among the agronomic practices, planting methods are of substantial importance as proper adjustment of plants in such field not only ensures optimum plant population but also allows the plants to exploit the soil and other external resources more expeditiously and judicially towards growth and higher yield production. Findings of the present studies suggested the level of N at rate of 125 % of RD as a way to unify with ridge planting method that would promote the highest yield of onion crop under salt affected soils.

Table 1: Different parameters of onion as affected by different nitrogen rates and planting methods under saline conditions

Treatments	Ridge Planting	Bed Planting	Mean
Plant height (cm)			
Recommended dose (RD)	45.66 b	41.66 c	43.66 b
75 % N of RD	37.66 d	34.33 d	36.00 c
125 % N of RD	56.33 a	54.33 a	55.33 a
150 % N of RD	57.00 a	55.33 a	56.16 a
Mean	49.16 a	46.41 a	
Number of leaves/plant			
Recommended dose (RD)	7.66 d	6.66 e	7.16 b
75 % N of RD	6.33 e	6.00 e	6.16 c
125 % N of RD	10.33 ab	9.33 c	9.83 a
150 % N of RD	10.66 a	9.66 bc	10.16 a
Mean	8.75 a	7.91 b	
Bulb diameter (cm)			
Recommended dose (RD)	5.02 b	4.66 c	4.84 b
75 % N of RD	4.48 cd	4.36 d	4.42 c
125 % N of RD	5.81 a	5.73 a	5.77 a
150 % N of RD	5.85 a	5.75 a	5.80 a
Mean	5.29 a	5.12 b	
Bulb mass (g)			
Recommended dose (RD)	49.33 c	47.10 c	48.21 b
75 % N of RD	44.73 d	42.00 e	43.36 c
125 % N of RD	60.46 a	57.10 b	58.78 a
150 % N of RD	60.93 a	57.56 b	59.25 a
Mean	53.86 a	50.94 b	

Means sharing the same small letters are statistically similar by LSD at $P \leq 0.05$

Table 2: Different parameters of onion as affected by different nitrogen rates and planting methods under saline conditions

Treatments	**Ridge Planting**	**Bed Planting**	**Mean**
Bulb yield (t ha^{-1})			
Recommended dose (RD)	17.93 b	17.54 c	17.73 b
75 % N of RD	15.83 d	15.38 e	15.60 c
125 % N of RD	19.68 a	19.54 a	19.61 a
150 % N of RD	19.71 a	19.60 a	19.65 a
Mean	18.29 a	18.01 b	
Number of flowers/umbel			
Recommended dose (RD)	145.00 c	141.67 d	143.33 b
75 % N of RD	137.33 e	132.33 f	134.83 c
125 % N of RD	150.67 ab	148.33 b	149.50 a
150 % N of RD	151.67 a	149.00 ab	150.33 a
Mean	146.17 a	142.83 b	
Number of seeds/plant			
Recommended dose (RD)	1.06 b	0.92 c	0.99 b
75 % N of RD	0.76 d	0.71 d	0.73 c
125 % N of RD	1.29 a	1.25 a	1.27 a
150 % N of RD	1.32 a	1.27 a	1.29 a
Mean	1.11 a	1.03 b	
1000-seeds mass (g)			
Recommended dose (RD)	2.12 b	1.98 cd	2.05 b
75 % N of RD	2.05 bc	1.92 d	1.98 b
125 % N of RD	2.39 a	2.32 a	2.35 a
150 % N of RD	2.40 a	2.34 a	2.37 a
Mean	2.24 a	2.14 b	

Means sharing the same small letters are statistically similar by LSD at $P \leq 0.05$

Table 3: Effect of different nitrogen rates and planting methods on net income and benefit: cost ratio (BCR) of onion crop

Nitrogen levels	**Planting methods**							
	Ridge Planting				Bed Planting			
	Cost of production (Rs.)	Gross income (Rs.)	Net income (Rs.)	Benefit: Cost	Cost of production (Rs.)	Gross income (Rs.)	Net income (Rs.)	Benefit: Cost
Recommended dose (RD)	85000	358600	273600	4.218824	85000	350800	265800	4.12
75 % N of RD	75000	316600	241600	4.221333	75000	307600	232600	4.10
125 % N of RD	90000	393600	303600	4.373333	90000	390800	300800	4.34
150 % N of RD	92000	394200	302200	4.284783	92000	392000	300000	4.26

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Willingness to pay for excreta pellet fertilizer: Empirical evidence from Ghana

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ABSTRACT

This study examined farmers' willingness to pay for excreta pellet fertilizer in Ghana. Primary data was obtained from 461 farmers in 10 districts in the Western and Greater Accra regions of Ghana through randomized questionnaire administration. The contingent valuation method was used in eliciting the farmers' willingness to pay decisions (WTP) and maximum amount they were willing to pay. The Tobit regression model results revealed that being a household head, unit cost of current fertilizer used, and farm size positively influenced the willingness to pay amount whereas previous use of organic fertilizer influenced the willingness to pay amount negatively. These results have implications for marketing of excreta pellet as fertilizer in Ghana.

Key words: excreta pellet fertilizer; farmers; willingness to pay; Tobit regression; Ghana

IZVLEČEK

PRIPRAVLJENOST ZA NAKUP GNOJILNIH PELET IZ FEKALIJ: EMPIRIČNI PRIMER IZ GANE

Raziskava analizira pripravljenost kmetov za nakup gnojilnih pelet iz fekalij v Gani. Osnovni podatki so bili pridobljeni od 461 kmetov z naključnim vprašalnikom iz 10 okrožij v zahodnem in širšem območju Akre. Za ovrednotenje pripravljenosti za nakup pelet in njihovo najvišjo ceno je bila uporabljena kontingenčna metoda. Rezultati regresijskega Tobit modela so pokazali, da so parametri kot so gospodar, cena ob anketi uporabljenih gnojil in velikost kmetije pozitivno vplivali na pripravljenost za nakup pelet medtem, ko je poprejšnja uporaba organskih gnojil nanj vplivala negativno.

Ključne besede: gnojilne pelete iz fekalij; pripravljenost za nakup; Tobit regresijski model; Gana

1 INTRODUCTION

Food prices have been on the increase and this is attributed mainly to increases in prices of inputs, such as land, machinery, feed and chemical fertilizers. Conventional chemical fertilizers, although having the potential of polluting surface and ground water, have become more expensive. With agriculture becoming more dependent on conventional fertilizers due to loss of soil fertility and scarce land, stakeholders have sorted to find alternatives that are ecofriendly, cheaper and more productive (Mariwah & Drangert, 2011).

Organic fertilizers have become the main alternative due to their significant agricultural, health and environmental benefits and the premium price received when organic produce are sold. Most often, the use of organic fertilizers lead to improved microbial activity and soil structure and are slow release fertilizers. Organic fertilizers are produced from plant, animal and human waste. Human waste consists of urine and faecal matter. Millions of tons of faecal matter is generated each day worldwide and are collected as faecal sludge, which could be a major resource for the production of organic fertilizers. In urban and peri-urban Areas in sub Saharan Africa (SSA), organic waste is normally not seen as a resource and hence it is collected and dumped in the sea and landfill sites (Hofny-Collins, 2006).

Municipal authorities have over the years regarded waste recycling as a low yielding and high risk business venture, hence in West Africa, these authorities focused

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on waste collection and disposal (Danso et al, 2006). Human excreta are common urban wastes and could be used as fertilizers especially for urban and peri-urban agriculture (Cofie, 2009). In some countries like China, Japan and Sweden, excreta have served as fertilizers traditionally. However, consumers of vegetables cultivated with raw excreta in Vietnam risk diseases caused mainly by parasites such as *Ascaris* spp. and *Trichuris* spp. (Phuc et al., 2006). These concerns worsened with the growth in urbanization and industrialization, or modernization, and a majority of farmers worldwide now depend mainly on chemical fertilizers as their main soil input.

With worsening faecal waste management issues, expensive and environmentally detrimental disposal methods coupled with the clarion call for sustainability in the use of natural resources, there is a shift to more adaptive and mitigating approaches to waste disposal. Now cities are looking for ways of sustainable waste management, hence a return to the human nutrient cycle. In the effort to reduce pathogen levels and make human excreta more presentable as fertilizers, various products have been developed. These include treated compost sludge, dried sludge and excreta pellets. Excreta pellets were developed not just as a way of decreasing pathogen levels but also to reduce water levels thereby solving the problem of bulkiness.

The use of dried pellets leads to a decrease in volume of 50 % - 80 % of the fertilizer required on the field. Applications of such products (faecal sludge) have remained on a low or pilot scale and its performance in the field is virtually unknown to farmers. Farmers in Europe would use fertilizers derived from waste but were not willing to pay a higher price, whilst those in Thailand and Japan were relatively more willing to pay a higher price (Harris et al, 2001). Most projects involving the recycling of waste have failed due to the over reliance on the technological aspect with little or no attention on social and marketing aspects (Zurbrügg et al., 2005). Some religious and cultural beliefs also serve as constraints to marketing of a product such as excreta pellets fertilizers. It is imperative therefore to study and recognize the decision making process of end users as this will help in identifying attitudes, barriers, reactions to incentive and finally their motivation to accept a new product. The general opinion that excreta pellets are a good source of much needed plant nutrients is good. However, the factors of successful introduction of such product needs to be better understood. The objectives of the study were twofold. First, to determine the amount that farmers were willing to pay (per 50 kg bag). Second, to determine the factors influencing the amount that farmers were willing to pay.

1.1 Production of excreta pellets

The production of pelleted excreta begins with dewatered faecal sludge (DFS). Faecal sludge is received from vehicle (tanker) operators, who discharge the sludge on a sand filter for drying. After at least 15 days of sun drying and sand filtering, dewatered faecal sludge is produced. The DFS is in the form of lumps as shown in Figure It is usually packed in polypropylene bags and transported to the composting site.

Figure 1: A bag of packed dewatered faecal sludge; source: Nikiema et al., 2013

The first step of the composting process is to manually break the DFS lumps into smaller particles, then add the required amount of water to reach a moisture content of about 65 % and to finally form the heap (0.87 m in height and 2.8 m in diameter). The moisture content is increased and a heap formed to aid in anaerobic respiration by microbes. Temperature is monitored daily while turning the heap with a spade or shovel, and moistening (to maintain moisture content of 65 %) for about 60 days. The heap is then levelled by spreading it on a platform to be sun dried, hence reducing its moisture content to less than 10 %. The dried composted DFS is grinded to produce a powder. The total organic carbon, nitrogen, phosphorus and potassium concentrations in the compost are then analyzed.

The pre-gelatinization process involves combining the required amounts of water with dry starch to form a paste. This paste, composted DFS and ammonium sulfate which is added for nutrient enrichment is then mixed up and put into the pelletizer to produce the pellets (Figure 2).The figure shows freshly produced pellets, which will undergo sun drying before being packed into sacks of 50kg each. Microbiological characteristics of the dry pellets produced are then analyzed; this is done by checking if *E. coli* T. Escherich, 1885 concentrations do not exceed the threshold given in the WHO guidelines (103 CFU/g of pellet) average concentrations of total coliforms being around 824 ± 511. The CFU refers to the colony-forming units.

Figure 2: Freshly produced excreta pellets; source: Nikiema et al., 2013

2 MATERIALS AND METHODS

2.1 Data

The study used primary data from 461 respondents in 10 districts in Western and Greater Accra regions of Ghana. Questionnaires were administered to farmers in a face to face interview[1]. The questionnaire was divided into four parts with the first part focusing on the socio-economic characteristics of the respondent. The next part was on the history of fertilizer purchases which included the number of bags used in the previous year, cost per unit, preferred form, amongst others. The third part addressed perception of previous fertilizer purchases and faecal based fertilizer. Respondents had the opportunity to indicate their agreement using a five point Likert scale on issues ranging from labelling, packaging, certification, and religious and cultural beliefs. The final part focused on the 'maximum amount they were willing to pay'. The procedure by which the data was collected from the respondents is presented as follows. A sample of the excreta pellet was shown to the respondent then the production process was explained with emphasis on the main ingredient being human excreta[2]. Afterwards, the agronomical and environmental benefits were explained to the respondent. The respondent was then asked if he/she would use such a product with a positive answer leading to the following question: "are you willing to pay"? A response in the affirmative then led to the respondent rolling a dice to randomly choose one of the three starting bids. Each respondent was asked if he/she was willing to pay the first bid. If he/she said 'yes' to the first bid, a second higher bid was given and willingness to pay was asked. A 'yes' or 'no' answer to the second bid led to the last follow-up question which was the maximum he/she was willing to pay. If a respondent said 'no' to the initial bid, a second lower bid was provided. If the respondent said 'no' or 'yes' to the second lower bid, he/she was asked to mention the maximum that he/she was willing to pay[3].

[1] The respondents include food crop farmers such as maize, vegetables, fruits and rice, non-food crop farmers like cocoa and flowers. Please note that the terms end users, farmers and respondents are used interchangeably in this paper.

[2] Please note that the terms 'excreta pellets' and 'faecal sludge-based fertilizer' are used interchangeably in this paper.

[3] The three opening bids were classified as low [GHS 15.00 ($ 6.33)], medium [GHS 18.00 ($ 7. 59)] and high [GHS 22.00 ($ 9.28)]. The opening bid for each respondent followed a random process. In this respect, a die was rolled for each respondent to enable the choice of the starting (opening) bid. This procedure was followed to eliminate starting point bias. Please note that 1 USD = GHS 2.37 when the data was collected in January, 2014.

2.2 Determinants of willingness to pay (wtp) amount for excreta pellet fertilizer

The Tobit model (1958) was employed to show the relationship between the observed maximum 'willingness to pay' amount (MWTP) and explanatory variables specified in equations (1), (2), and (3) as follows:

$$MWTP_i^* = \alpha + \beta X_i^{'} + \varepsilon_i \quad (1)$$

$$MWTP_i = MWTP_i^* \text{ if } MWTP_i^* > 0 \quad (2)$$

$$MWTP_i = 0 \text{ if } MWTP_i^* \leq 0 \quad (3)$$

where *MWTPi** is the end user's (farmer's) unobserved 'maximum willingness to pay' amount for the excreta pellets; *MWTPi* is an end user's (farmer's) actual 'maximum willingness to pay amount' for the excreta pellets; X_i' is vector of explanatory variables; β is vector of unknown true coefficients; α is the intercept; and ε_i is disturbance term, which is assumed to be normally independently distributed, i.e., NID (0, σ^2) and independent of x_i. Assuming that the variable is zero, then the dependent variable in the Tobit regression model is a *continuous variable* (i.e. the maximum amount quoted by end users to pay for 50 kg excreta pellets as fertilizer). The amount end users were willing to pay was hypothesized to be influenced by the following factors:

Age of the Respondent (AGE): This is a continuous variable indicating the age of the respondent in years. The age of respondent may have either negative or positive effect on WTP decision. Aged farmers may not see the need for long term sustainability of soil fertility and may be conservative to new ideas hence may show negative attitude towards the faecal sludge fertilizer. On the other hand, as they grow much older, they may become more experienced thereby willing to conserve the soil through the use of excreta pellets and hence positively influencing their WTP decision (Tessema & Holden 2006; Asgary et al., 2004; Dong et al., 2003).

Experience (EXP): Farming experience leads to increased willingness to improve the soil for better productivity (Tessema & Holden, 2006). Hence experience is expected to positively influence WTP decision.

Education Level of the Respondent (EDUCATION): This parameter refers to the number of years that the respondent has spent in a formal school. Following Paulos (2002) and Yitayal (2004), the study assumed that respondents who had high level of education better understood the problems of soil erosion or degradation and its consequences and therefore would be willing to invest in a product that increases fertility and conservation of the soil.

Household Head (HHD): This is a dummy variable which takes a value of 1 if the respondent is the head of the household; and 0 otherwise. It is hypothesized that being a household head is expected to influence willingness to pay positively.

Household Size (HSIZE): This is a continuous variable which refers to the number of family members in the household. This explanatory variable is included because it affects the labor supply at the household level. Also, households with more people to feed will be willing to pay for a new input that would increase yield (Agyekum et al., 2014).

Gender of the Respondent (Gender): dummy variable (1= male, 0 = otherwise) and is included in the model to find out the influence of gender on WTP. Males have a probability of getting better access to information than females. Therefore, a positive influence is hypothesized for male respondent. Female farmers tend to adopt new technologies at a lower rate than males due to seemingly limited access to information and resource (Doss & Morris, 2001).

Farm Size (Farmsize): It is a continuous variable expressed in terms of hectares of cultivated land and expected to have a positive effect on the willingness to pay. This is expected because farmers (end users) with larger land size tend to be commercially oriented and hence would invest in an input that will positively affect production (Oladele, 2008; Cofie, 2010).

Farm Income (FARMINC): This is a continuous variable. It is measured as the annual farm income from sale of produce. It is expected to have a positive relationship with WTP. As income increases it positively affects the WTP for waste as fertilizer. This can be linked to farmers having more money to afford such a product. Numerous empirical studies have reported positive relationships between income and adoption of agricultural technologies and input (Faye & Deininger, 2005; Holden & Shiferaw, 2002; Mbata, 2008).

Aware of Faecal Fertilizer (Aware): This is a dummy variable which assumes 1 for "yes I am aware of faecal fertilizers", and 0 otherwise. It is hypothesized that respondents who have prior knowledge of faecal sludge-based fertilizer would be more willing to use excreta pellets since they are less bulky and the unpleasant smell is removed. Also, farmers who were aware of other available agricultural inputs were more receptive to paying for these inputs (Asrat et al., 2004).

Quantity of Fertilizer Previously Used (Number of 50 kg bags): A continuous variable that measures the amount of fertilizer used in the previous year. A unit was pegged at 50 kg bag based on the fact that the majority of the respondents bought fertilizers packaged in 50 kg bags. It is hypothesized that an increase in the quantity used will increase the probability of end users WTP.

Unit Cost (unit cost): This is a continuous variable that measures the average cost of a unit (50 kg bag) of fertilizer used in the previous year. It is hypothesized that an increase in price of the cost of fertilizer would lead to an increase in the probability of WTP.

Previous Use of Organic Fertilizer (Used Organic): This is a dummy variable that assumes 1 for "yes I have ever used organic fertilizer" and 0 otherwise. It was hypothesized that previous use of organic fertilizer would positively influence the end users WTP decision.

Membership of Farmer Based Organization (member of FBO): This is a dummy variable which assumes 1 for "yes I am a member" and 0 for otherwise. It is hypothesized that being a member of a farmer-based organization (FBO) will positively affect the WTP amount.

Own Current Land (own land): This is a dummy variable which assumes 1 for "yes I own the land that am currently working on" and 0 for otherwise. Following Obuobie et al., (2006), a positive relation with maximum WTP amount is hypothesized. The previous study revealed that most farmers on hired land were not allowed by the land owners to use faecal sludge based fertilizer in the city of Tamale in the northern region of Ghana.

3 RESULTS AND DISCUSSION

3.1 Preliminary results

The study found that about 69 % of the farmers interviewed were aware of excreta pellets as fertilizer whilst 31 % of the total respondents were not aware. This indicates that the use of faecal sludge as fertilizer is not an alien concept to farmers. The farmers agreed with the assertion that fertilizer derived from human excreta should be certified by a relevant body such as the Environmental Protection Agency (EPA) before its introduction onto the market, and that certification by a relevant authority would lead to increased willingness to pay for excreta pellet fertilizer. Farmers also agreed that excreta pellets can be used as a replacement for conventional chemical fertilizers. Farmers in northern Ghana disclosed that human excreta when used in farming produce better and safer yields (i.e. enhance agricultural productivity) than conventional fertilizers, hence it should be used in place of the conventional fertilizers (Kranjac-Berisavljević, 2009). Farmers perceived that such a fertilizer has a positive effect on long term fertility of soils. With regard to health, farmers did not agree that using faecal-based fertilizer would expose them to several major health risks. However, they did indicate that use of raw or untreated human excreta on farmland is associated with some skin infections, diarrhea, foot rot and vomiting. The majority of respondents interviewed (91.1 %) claimed that they would not buy excreta pellets if it is sold at the same price as conventional fertilizer. However, they would buy excreta pellets if cheaper than conventional chemical fertilizers; in other words, they expect the price of the product to be lower than the price of conventional chemical fertilizers (Cofie et al, 2009).

Majority of respondents that indicated their WTP for excreta pellets, only 29 % stated they were not willing to pay. The reasons stated were: those excreta pellets have not been certified for use by farmers, especially under global GAP; and that some farmers also claimed that excreta pellets need to be tested on their produce before they would be willing to buy. One of the reasons for not willing to pay was that the product was not certified and not tested in their locality to ascertain its quality. Some were also of the view that since the product is made from human excreta which are waste coupled with disposal being a major problem to local authorities, it should not be sold. In general, over 70 % of respondents were willing to pay for excreta pellets as fertilizer and this level of approval is consistent with previous studies (Danso et al. 2006, and Agyekum et al. 2014).

Table 1 shows the distribution of the willingness to pay amounts by the respondents.[4] Nine respondents (representing 2.6 %) were willing to pay less than GHS 10.00 [$ 4.22] for a 50kg bag of the excreta pellets. Forty eight respondents (representing 14 %) were willing to pay between GHS 10.00 and GHS 19.99 [$ 4.22 – $ 8.43]. Also, one hundred and ninety seven respondents (representing 57.6 %) were willing to pay between GHS 20.00 and GHS 29.99 [$ 8.44 - $ 12.65].

[4] Please note that 342 respondents out of the total sample of 461 were willing to pay for the excreta pellets.

Seventy seven respondents (representing about 22.5 %) were willing to pay between GHS 30.00 and GHS 39.99 [$ 12.66 – $ 16.87], and eleven respondents (representing about 3.2 %) were willing to pay GHS 40.00 and GHS 50.00 [$ 16.88 - $ 21.10].

Table 1: Maximum willingness to pay amount

WTPA	Frequency	Percentage
<10	9	2.6
10-19.99	48	14.0
20-29.99	197	57.6
30-39.99	77	22.5
40-50	11	3.2
Total	342	100

3.2 Tobit regression model results of the factors influencing willingness to pay maximum amount

The minimum price per 50 kg bag quoted was GHS 2.00 [$ 0.84] and a maximum of GHS 50.00 [$ 21.10]. The mean price quoted was GHS 22.83 [$ 9.63]. This mean amount was quite higher than that quoted by respondents in Agyekum et al. (2014) for 50 kg of faecal compost. The Tobit regression model results of the factors that influenced willingness to pay amounts quoted by the farmers are presented in Table 2. Being a household head was significant and had a positive relation with the willingness to pay. A household head was willing to pay GHS 5.12 [$ 2.16] more for a bag of excreta pellets compared to an end user who is not the head of the household. This willingness to pay more for such an input may be due to the responsibilities associated with such a position that would make the farmer want to invest in soil conservation (Gebremedin, 2012). Farm size was also significant and positively influenced WTP amount, as farm size increased by a hectare, the WTP amount increased by GHS 0.48 [$ 0.20]. This could be due to high cost of use of inorganic fertilizer, as farm size increases end users would be more willing to use faecal sludge-based fertilizers. End users with larger farm size would also be willing to pay more for excreta pellets because as stated by Oladele (2008) they are commercially oriented and hence would like to invest in an input that will impact positively on production. The results also revealed a negative and significant relation between the amounts an end user was willing to pay and if he had ever used an organic fertilizer. Thus, if the end user has ever used organic fertilizer, he was willing to pay GHS 2.41 [$ 1.02] less for the 50 kg of the excreta pellets compared to a respondent who has never used organic fertilizer. This relation could be attributed to the cheaper prices of organic fertilizer available as some farmers acquired organic fertilizers for free and are only required to pay for the transportation cost. Hence, this category of farmers will not be willing to pay a high price for another organic product. This assertion is consistent with previous studies in which farmers with compost or manure experience were willing to pay less for faecal compost (Drechsel et al. 2004, Danso et al. 2006). Also, if organic fertilizer were available, use by farmers may be very low due to limited knowledge and management among farmers (Obour et al., 2015).

As expected, there is a positive relation between willingness to pay amount and the average unit cost of fertilizer currently being used by the end user. A GHS 1 increase in the unit cost of the current fertilizer being used will lead to paying GHS 0.06 [$ 0.03] more for excreta pellets (though negligible). This can be attributed to an end user looking to invest in cheaper alternatives.

Table 2: Tobit regression results of the factors influencing maximum willingness to pay amount

Variable	Coefficient	P>t
Gender	-2.160	0.147
Age	0.030	0.608
Household Head	5.119***	0.001
Household Size	0.050	0.813
Own Land	-0.146	0.903
Used Organic	-2.406**	0.030
No Of 50 kg	-0.006	0.271
Unit Cost	0.040**	0.022
Farm Size	0.480**	0.024
Income	0.000	0.376
Education	-0.058	0.660
FBO Member	1.731	0.147
Experience	-0.050	0.405
Aware Of Faecal Fertilizer	1.183	0.309
Constant	11.969	0.000
Number of obs		459
F(14, 445)		2.800
Prob > F		0.001
Pseudo R2		0.001
Log pseudolikelihood		-1770.3

*, ** and *** denotes 10 %, 5 % and 1 % significant level

4 CONCLUSIONS AND IMPLICATIONS

The results of this study revealed that the perceptions about fertilizer derived from human excreta are positive. Majority of respondents did not have religious, health and cultural hindrances to the use of excreta product. They described excreta pellet as a potentially good replacement for conventional fertilizer. Respondents also indicated that they would use a faecal sludge-based fertilizer provided it was certified by the Environmental Protection Agency since this certification and labeling would lead to trust in the product. Interestingly, almost all respondents indicated that they would not purchase excreta pellet if it is sold at the same price as conventional chemical fertilizer. However, these respondents mentioned that they would purchase the excreta pellet if it is sold cheaper than the conventional chemical fertilizers on the market. There is a potential for the marketing of excreta pellet as fertilizer in Ghana. Producers of different backgrounds were interested in using the product for both crop and non-crop agricultural enterprises as they expressed positive perception about the product. End users were willing to pay a minimum of GHS 2.00 [$ 0.84], a maximum of GHS 50.00 [$ 21.10] and a mean of GHS 22.83 [$ 9.63]. to obtain a 50 kg bag of excreta pellet fertilizer. The results of the Tobit regression model revealed that being a household head, unit cost of current fertilizer used, and farm size positively influenced the willingness to pay amount whereas previous use of organic fertilizer influenced the willingness to pay amount negatively. Thus, the marketing of excreta pellet as fertilizer has prospects in Ghana. End users are interested in reducing cost hence would welcome any input that is cheaper and effective. Based on these findings, this paper recommends that marketing of faecal sludge based fertilizer should target people in decision making positions such as household heads with large farm sizes and businesses in the manufacturing sector of Ghana should find innovative ways of reducing the cost of production of excreta pellet fertilizer so as to reduce the price when offered on the market. A cheaper product will be wholly accepted by end users. The Ghana Standards Authority should support manufacturing companies to satisfy certification requirements of the product before introduction onto the market. Demonstration plots should be identified at the district level so as to give first hand education on the use of the product. For florists, the product should be tested on their flowers to dispel the notion that it might be too strong for the plants and may cause wilting. Also a protocol or brochure should be added to the product (when sold), indicating how product should be applied to meet specific crop or plant nutrients requirements. Marketing of the product should target farmer-based organizations, people in social positions such as household heads and people with larger land holdings. Information about the efficacy of product will flow to other end users. Government institutions such as Environmental Protection Agency (EPA), Council for

Industrial and Scientific Research (CSIR) and the Ministry of Food and Agriculture (MoFA) should establish clear guidelines and standards on the use of faecal sludge-based fertilizers. When establishing quality guidelines, the institutional capacities for controlling and enforcing them should also be taken into account. More importantly, political will and legal tools should be adequate enough to enforce quality standards. This will go a long way to help maintain end users' confidence in the product for the long term since the raw material is inexhaustible. MoFA must intensify its education on the use of protective clothing especially gloves when applying the excreta pellet fertilizer.

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Unfair practices and illicit conduct in food supply chains in Slovenia

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ABSTRACT

The food supply chain in Slovenia is highly developed, but it involves unequally developed stakeholders with different bargaining power. Upon reviewing all stakeholders through participatory research, it is made clear that in the whole food supply chain, retail chains generally have the greatest and primary producers the smallest bargaining power. For this reason, in the process of regulating mutual relations in contractual commitments and mutual operations, unfair practices and illicit conduct often emerge, through which the parties with significant market power impose additional discounts, rebates and other contributions on the parties with smaller market power in order to improve their own financial management. Unfair practices and illicit conduct lead to the weakening of the entire food supply chain, so it is important to recognise such tendencies in the food chain. In this article, the autor would like to draw attention to the illicit conduct and unfair practices in Slovenia which are used by retail chains in their interpersonal relationships and their dealings with suppliers.

Key words: unfair practices; illicit conduct; food supply chain; significant market power; imposition of conditions

IZVLEČEK

NEPOŠTENE PRAKSE IN NEDOVOLJENA RAVNANJA V VERIGI PRESKRBE S HRANO V SLOVENIJI

Veriga preskrbe s hrano v Sloveniji je izredno razvita, vendar v njej sodelujejo različno razviti deležniki z različno pogajalsko močjo. Ob pregledu vseh deležnikov preko participativnega raziskovanja se izlušči, da imajo največjo pogajalsko moč v celotni verigi preskrbe s hrano trgovske verige in praviloma najmanjšo primarni pridelovalci. Zaradi tega so pri urejanju medsebojnih odnosov pogosto v pogodbenih zavezah in medsebojnem poslovanju prisotne nepoštene prakse in nedovoljena ravnanja, s katerimi stranke z znatno tržno močjo vsiljujejo dodatne popuste, rabate in druge prispevke strankam z manjšo tržno močjo z namenom izboljševanja svojega finančnega poslovanja. Zaradi nepoštenih praks in nedovoljenih ravnanj prihaja do slabitve celotne verige preskrbe s hrano, zato je pomembno, da jih v verigi preskrbe s hrano prepoznamo. V tem prispevku želim opozoriti na nepoštene prakse in nedovoljena ravnanja, ki so jih trgovske verige v Sloveniji vključile v medsebojne odnose pri poslovanju z dobavitelji.

Ključne besede: nepoštene prakse; nedovoljena ravnanja; veriga preskrbe s hrano; znatna tržna moč; vsiljevanje pogojev

1 INTRODUCTION

The food supply chain is a major employer in Europe and the Slovenian area. On the basis of the data in the report presented by the European Parliament (Jackiewicz, 2015), more than 47 million people in the EU are employed by the food sector. Production, processing, logistics and food sales in Slovenia employ all together about 87.000 people (ReSURSKŽ, 2011), and additional new jobs will be opened up, which is due to the increasing self-sufficiency in Slovenia. Because of the quality jobs in the operating agri-food chain, the needs of the state for various social transfers are being consequently reduced. Increased production and food processing have lead to the growing consumption of raw materials for agricultural production and thus the revenues to the state budget have increased. A well-functioning agri-food chain promotes economic growth equally well and increases the purchasing power of rural areas. Increasing demand for food of Slovenian origin generates other aspects of preserving the Slovenian countryside as well, enabling the development of other

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industries, such as tourism, sports, recreation, and more. In view of all these aspects, it is extremely important that the food supply chain is solid and sustainable. The strength of the food supply chain is undoubtedly influenced by the relations between its various partners as well. Because of the uneven market position of various partners involved in the food supply chain, smaller food producers in particular are affected by unfair practices which in the long run weaken the partners on whom these practices are imposed. In general, unfair practices can be defined as the practices that significantly deviate from sound business conduct, being contrary to good faith and honest conduct, and which are unilaterally imposed on the others by one partner or a group of partners. Illicit conduct can be classified as non-compliance with payment deadlines and the imposition of conditions (additional payments, discounts, promotions, unfair delivery conditions, counter trade by non-competitive conditions, transfer of business risk to trade suppliers, etc.) (ZKme1B, 2014). Such improper practices evolve there where is no balance in the financial and consequently in the bargaining power of business partners, impairing in this way the entire EU economy, because as a result of such actions, businesses (especially small and medium ones) are losing the ability to invest and foster innovations and they consequently do not decide to expand their business in the single market. Attention should be paid to the factor of fear, when the weaker partners decide not to initiate legal action in spite of the exisisting possibility to do so, and irrespective of the damage, they simply accept unfair practices because they are afraid that a stronger partner may otherwise terminate a business relationship.

Although the imbalances in bargaining power are a completely legitimate component of the functioning of the market, the abuse of a stronger position can distort the relationship between the companies, which often leads to unfair trading practices. This question concerning the transactions between enterprises has been increasingly coming to the force in recent years, and although it is difficult to evaluate all its dimensions, there are concrete statistics and market evidence revealing that unfair practices in the food chain are fairly widespread, especially in certain parts of the agri-food chain. It is being recognized by many Member States that such practices can cause a lot of damage, so they are taking action against them, while the other members are planning to do the same. The rules in this area as well as the extent of this problem vary greatly among the Member States. At the same time, market participants are trying to confront the problem by developing principles of good practice in vertical relationships and by designing self-regulatory frameworks for the implementation of these principles (Evropska komisija, 2014; Kocsis and Nedeczky, 2013). However, as unfair practices are widespread and they are becoming more and more problematic, the question is being raised in this article: to what extent can self-regulatory mechanisms actually help to restore market equilibrium? Voluntary mechanisms that encourage companies to refrain from unfair practices should supposedly mitigate this problem to some degree, but they certainly cannot solve it (Jackiewicz, 2015). For the effective prevention of unfair practices and illicit conduct, the identification of such unfair practices is of the utmost importance. For that reason, in the continuation of this article, its author will present the identified suspicions of unfair practices and illicit conduct that occur in the Slovenian food supply chain, with the emphasis on the retail chains recognized as the parties with significant market power.

2 MATERIAL AND METHODS

In Slovenia, the food supply chain is extremely dynamic and, due to the proverbial incoherence of individual actors, it is fairly different from similar chains in the neighbouring countries. So in Slovenia, there are the following types of food supply chains (Podgoršek, 2016):

1. agricultural holding — retail chain (typical for the sector of fresh vegetables, potatoes),
2. agricultural holding – cooperative – retail chain (some examples in the sector of vegetables and fruit),
3. agricultural holding — food processing company – retail chain (typical for the sector of meat and grain),
4. agricultural holding – cooperative – food processing company – retail chain (typical for the milk sector),
5. food processing company – retail chain (typically for the sector of drinks).

Due to the highly fragmented structure of different stakeholders, a decision has been taken by the author to analyze the relationships in the food supply chain at the point where all retail chains and their suppliers join in. To establish the suspicion of unfair practices and illicit conduct, the method of participatory active research has been chosen. This is one of the research tools connecting participants in this research with the purpose of finding a common definition and a solution to the problem. For this reason, one of the important objectives of this research is also its active contribution to the solution to certain social problems. It is oriented to promoting skills, community development, social justice, wider accessibility and the participation of different stakeholders (Podmenik and Bembič, 2015).

In accordance with the selected method, the author personally interviewed a variety of Slovenian suppliers of retail chains in Slovenia, maintaining in this way the anonymity of the participants in the research, as all the time throughout the present research, it was possible to recognize the participants' fear that sanctions might be imposed on them by retail chains in the case of their identity being revealed. For this reason, the obtained information was combined and merged in such a form that a clear source of information can no longer be identified. The research has been conducted in all the main groups of suppliers who have been divided into the following five groups:

- meat and meat products,
- milk and dairy products,
- fruits and vegetables (fresh and processed),
- the manufacture of grain mill and bakery-manufacture,
- the manufacture of other food products.

The research was conducted among different types of suppliers, such as farms, agricultural cooperatives and agro-processing companies.

3 RESULTS AND DISCUSSION

In the Slovenian area, unfair practices and illicit conduct have been actively dealt with for many years due to the increasing concern of both stakeholders and agricultural policy who recognized the seriousness of the existing anomalies. The first serious attempt to restrict the development of unfair practices was the signing of the *Code of good business practices* among stakeholders in the agri-food chain at the Agra Fair 2011. The Code signatories (the Slovenian Chamber of Commerce, the Slovenian Chamber of Commerce and Industry, The Chamber of Agriculture and Forestry, the Slovenian Chamber of Craft and Small Business and the Cooperative Association of Slovenia) then agreed to develop positive relationships and promote the joint development of the food supply chain. The Code also envisaged the introduction of the net purchase prices in retail chains, planning to implement the agreement by early 2013 (Kodeks, 2011). Due to non-compliance with the commitments contained in the Code, the *Law on amending the Law on agriculture* (Zkme-1B, 2014) was accepted. The amendment to the Law on agriculture set a 45-day maximum payment period for perishable foodstuffs and 90 days for other foods. It also identified illicit conduct and provided a legal basis for the appointment and functioning of a food supply chain relationships ombudsman. The first ombudsman was appointed on 3 January, 2015, for a period of five years. The ombudsman's task is to monitor the behaviour of stakeholders in the food supply chain, to publish examples of good business practices and to notify the Public Agency for the Competition Protection of any prohibited practices, whereby the Public Agency has to protect personal information and business secrets of the parties.

On the basis of the analysis of the results obtained by the method of participatory active research, the author of the article divided the allegations of unfair practices and illicit conduct, made by individual participants, into five groups, without making any direct references to the participants in order to prevent the revelation of the trade secrets of individual stakeholders.

biomass of wheat. Results revealed that germination percentage was neither affected by different plant parts nor by extract concentrations. Interaction between plant parts and concentrations were also non-significant. Germination (%) was maximum (91.8) in control conditions. Slighter decrease in germination was observed in petri-dishes treated with different extract concentrations of different plant parts; however, the differences among means of concentrations and plant parts for germination percentage were insignificant and they ranged between 89.7 - 90.9 % which did not differ significantly from 91.8 % in control (Table 1).

3.1 Retail chain 1:

In this chain, the following allegations of unfair practices have been found:

- the imposition of additional discounts and rebates amounting to over 20 % according to the value of delivered food products.

3.2 Retail chain 2:

In this chain, the following allegations of unfair practices have been found:

- the imposition of additional discounts in the form of charging superrabates (different rates depending on the supplier), additional payments for marketing up to 2 % of current output,
- the requirement for agreed action prices of items for products in the weekly specials for the period from 16 days before the special (also for food products with a shorter shelf life) and

up to 7 days after the completion of the special, which means that they require the action price for the article in a weekly special for the period of 1 month,
- the return of already delivered and absorbed goods - the transfer of risk to suppliers,
- the signing of a contract with the company for transferring money, recommended by the retail chain (the company for transferring money is through the founders associated with the retail chain), and billing the service of transfer up to 1 % in relation to payments made,
- the dictation of action prices that are lower than the prices in comparable neighboring countries, with the possibility of immediate loss of business in the case of the supplier's disagreement with the proposed price,
- the application of fines and penalties on the delayed or failed delivery of a certain item, in no proportion to the damage caused,
- the increase of various discounts in the case of the exclusion of the supplier of this trading system and his re-integration into the network of suppliers.

3.3 Retail chain 3:

In this chain, the following allegations of unfair practices have been found:
- the imposition of individual contracts on individual suppliers to pay up to 5 % of the total turnover of the previous year,
- contractual restriction of the right to charge default interest on the late payment of invoices and transfer of commercial risk to the supplier in the case of sales actions,
- bound trade at non-competitive prices in the case of franchises,
- opaque rejection of goods due to possible defects in food products or crops,
- exclusion of one's own-brand products which are in other retail chains marketed by the same suppliers under the commercial brand of a competitive retail chain.

3.4 Retail chain 4:

In this chain, the following allegations of unfair practices have been found:
- the transfer of the business risk to the supplier in the case of action sales,
- payment defaults and late payments for nutritional products, the lack of consent to the sale of receivables overdue to the purchasers of receivables,
- contractual restriction of the right to charge default interest on the untimely paid accounts,
- late payments (in some cases more than 90 days after currency),
- bound trade at non-competitive prices in the case of franchises.

3.5 Retail chain 5:

In this chain, the following allegations of unfair practices have been found:
- the coercion of small suppliers into the exclusive sale of their products only through a particular retail chain.

The list of suspected unfair practices and illicit conduct is unfortunately being constantly updated. During the action, taken by the food supply chain relationships ombudsman in one of the retail chains, compulsory payments for early payment of invoices (paid within 20 days instead of envisaged 45 days) were replaced with compulsory promotional rebate, which is probably due to the alertness to a usurious interest rate in the case of pre-payment of bills. It should be noted that suppliers continue to pay special promotions according to the price list of the retail chain.

4 CONCLUSIONS

Unfair trade practices are recognized throughout Europe and they are quite common. In the pan-European survey, conducted among suppliers in the food chain, 96 % of suppliers stated that they met with at least one form of unfair trade practices (Evropska komisija, 2014). However, there are considerable differences among individual EU countries. Thus, some national studies have shown different shares of the existence of unfair trade practices. In Spain, the national survey has found that 56 % of suppliers experienced retroactive changes in the contract terms. In Italy, a survey has shown that 57 % of producers often or always accept unilateral retroactive changes, from fear of commercial retaliation in the case of the rejection of changes. The overall impact of unfair trade practices is difficult to assess and quantify in quantitative terms, however, as a result of these practices, those parties are directly and negatively affected. Because of the unfair trade practices, the income of suppliers is undoubtedly reduced.

The unfair practices and illicit conduct in the food supply chains are dealt with by various European countries in different ways. The Czech Republic applies the *Act on abuse of a dominant market position in the market of agricultural and food products* (Official

Journal of the EU, 2013). The law in question among others prohibits the sale of a product at a price lower than its purchase price and only the net price is to be considered, without any additional discounts and rebates. In Italy, they use the *Regulation on economic relations in the sale of food and agricultural products* (Oggetto, 2016), which specifies mandatory and prohibited elements in contracts. The control over the implementation of the regulation is kept by the *Authority for the competition and the market*, which can also rely in special cases on the operational support of the Financial Guard (Guardia di Finanza). In neighboring Hungary, it is the *Trade act* (RS Government, 2013) which governs the relations in the food supply chain and which defines the abuses committed by traders with the dominant market power. The law should protect the suppliers and it is based on the protection of free trade and entrepreneurship. From 2012 onwards, the law no longer applies to food products. Therefore, the chain of food products and the prevention of unfair practices are today regulated by the *Trade act* and the *Law on unfair distribution practices*. The supervision of the implementation of the legislation is in the domain of the State Office for the safety of the food chain.

To sum up, the food supply chain in different EU countries is regulated in different ways. All the countries, however, share a common interest in regulating the relations existing in this chain and endeavour to prevent the exploitation of negotiating superiority achieved by individual stakeholders. Therefore, this problem, in addition to the food supply chain relationships ombudsman**,** is dealt with by the *Public Agency of the Republic of Slovenia for Protection of competition*, which was notified of the suspicions of unfair practices and illicit conduct by the ombudsman in January 2016. Nevertheless, it is likely that in Slovenia it will also be necessary to adopt a legislative framework through which the relations in the food supply chain will be regulated and unfair practices prevented, especially the pressure exerted on weaker negotiators by the parties with significant market power.

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Natural incidence of bean viruses in the northwest of Iran

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ABSTRACT

Bean is considered as one of the most important legumes around the world. Viral diseases are a major yield reducing factor in bean production. Bean samples with virus-like symptoms like severe or mild mosaic, vein banding, leaf curling, blistering and necrosis were collected from different bean fields in Urmia (Northwest of Iran) during the growing seasons of 2013 and 2014. *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Bean yellow mosaic virus* (BYMV), *Cucumber mosaic virus* (CMV), *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV) and *Tomato yellow leaf curl virus* (TYLCV) were detected by double antibody sandwich enzyme-linked-immunosorbent assay. Mixed infection of BCMV and BCMNV were found. BCMNV was the most frequent virus in this region whereas BYMV and TYLCV were each detected just in one sample. This is the first report of BCMNV, BCMV, BYMV, TSWV, TMV and TYLCV incidence on bean in Urmia, Iran.

Key words: BCMV; BYMV; BCMNV; TYCLV; TMV; TSWV; Iran; ELISA

IZVLEČEK

RAZŠIRJENOST VIRUSOV FIŽOLA V SEVEROZAHODNEM IRANU

Fižol je ena izmed najpomembnejših stročnic širom po svetu. Virusne bolezni so najpomembnejši dejavnik zmanjševanja njegovega pridelka. Vzorci fižola z znamenji virusnih ukužb kot so blagi ali izraziti mozaik, obžilna razbarvanja, zvijanje, mehurjavost in nekroza listov so bili nabrani z različnih fižolovih polj v okolici Urmie (severozahodni Iran) v rastnih sezonah 2013 in 2014. Virus navadnega mozaika fižola (BCMV), virus navadnega mozaika in nekroze fižola (BCMNV), virus rumenega mozaika fižola (BYMV), virus mozaika kumar (CMV), virus pegavosti in uvelosti paradižnika (TSWV), virus mozaika paradižnika (ToMV) in virus rumenenja in kodravosti paradižnika (TYLCV) so bili ugotovljeni z ELISA testom. Najdena je bila mešana okužba z BCMV in BCMNV. BCMNV je bil najpogostejši virus na tem območju, medtem, ko sta bila BYMV in TYLCV ugotovljena samo v po enem vzorcu. To je prvo poročanje o pojavljanju BCMNV, BCMV, BYMV, TSWV, TMV in TYLCV na fižolu v Urmiji, Iran.

Ključne besede: BCMV; BYMV; BCMNV; TYCLV; TMV; TSWV; Iran; ELISA

1 INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the major food legumes produced on over 26 million hectares worldwide (Mavrič and Šustar-Vozlič, 2004; Loebenstein et al., 2009). Virus diseases are a major yield reducing factor in bean production. Thirty four different virus species have been reported to infect common bean naturally (Arli-Sokman et al., 2016). Economically the most important viruses are potyviruses including *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV) and *Bean yellow mosaic virus* (BYMV). Other important viruses on bean are *Cucumber mosaic virus* (CMV), *Southern bean mosaic virus* (SBMV), *Tobacco streak virus* (TSV) and *Tomato aspermy virus* (TAV) (Brunt et al., 1996; Kumar et al., 1994). These viruses are transmitted by insect vectors, mainly aphids with the exception of TSV that is transmitted by thripses and SBMV that is transmitted by been leaf beetle. Some of them can be transmitted by seed (Loebenstein et al., 2009). Insect transmission is very important for virus spread on short distances while seed transmission is the most important factor in the spread of viruses around the world. Because of the high seed transmission rate (up to 83 %), BCMV and BCMNV are economically the

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most important viruses of common bean (Mavrič and Šustar-Vozlič, 2004). For example, BCMV can reduce yield up to 24 % (Kumar et al., 1994). BCMV, BYMV and BCMNV have been reported from bean-growing regions of Iran (Kaiser and Mossahebi, 1974; Shahraeen et al., 2002; Shahraeen et al., 2005; Peyambari et al., 2011; Ghasemzadeh et al., 2012; Ghobakhloo et al., 2012; Salari et al., 2013). Other viruses reported on bean from Iran include *Alfalfa mosaic virus* (AMV), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), *Bean leaf roll virus* (BLRV), *Soybean mosaic virus* (SMV), *Bean pod mottle virus* (BPMV), *Bean curly top virus* (BCTV), *Broad bean wilt virus* (BBWV), *Broad bean stain virus* (BBSV) and *Faba bean necrotic yellows virus* (FBNYV) (Kaiser and Mossahebi, 1974; Shahraeen et al., 2005; Alavi and Massumi, 2014). BCMV has been recognized as a major constraint on bean production in Iran. Bean is one of the most important cultivated crop in West Azarbaijan province covering around 1346 ha with the mean yield of 2 t ha^{-1}, but until now there was no data on viral diseases of this crop in this region. The occurrence, frequency and distribution of seven viruses including BCMV, BCMNV, BYMV, CMV, *Tomato spotted wilt virus* (TSWV), *Tomato mosaic virus* (ToMV) and *Tomato yellow leaf curl virus* (TYLCV) in bean growing areas of Urmia, the largest bean growing region in West Azarbaijan province were studied in 2013 and 2014 and are presented in the article.

2 MATERIALS AND METHODS

2.1 Plant material

A set of 195 symptomatic samples of bean showing severe or mild mosaic, vein banding, leaf curling, blistering and necrosis on leaves was collected from bean growing areas of Emamzadeh-Gharachelar, Ghafar Behi, Jabal, Balo, Nazloo (Urmia University campus) and Aliabad regions around the city of Urmia during the 2013 and 2014 growing seasons. Each sample was put in a plastic bag, labelled and stored at 4 °C.

2.2 Virus detection

Double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) was used for detection of BYMV, BCMV, BCMNV, TSWV, CMV, ToMV, TYLCV according to Clark and Adams (1977) method with minor changes using commercial polyclonal antisera obtained from DSMZ (Braunschweig, Germany). ELISA plates were coated with coating buffer containing anti-BCMV, BYMV, BCMNV, TSWV, CMV, ToMV and TYLCV polyclonal antibodies (1:1000) and the plates were incubated at 37 °C for 3 h. Each sample was diluted 1:5 with extraction buffer (8 g NaCl, 0.2 g KH_2PO_4, 0.2 g NaN_3, 0.5 ml Tween 20 and 2 % PVP in 1 l of distilled H_2O, pH 7.4). The diluted plant sap extracts were added to the wells (100 µl). Two replicates were used for each sample. Plates were kept at 4 °C overnight, then rinsed three times with washing buffer. Conjugated polyclonal antibody was diluted (1:1000) in conjugate buffer and was loaded into each well. The plate was incubated at 37 °C for 3 h. Finally, 10 mg of p-nitrophenyl phosphate (Sigma) in 10 ml of substrate buffer was added to the wells and incubated at room temperature for 30–60 min. Absorbance values were read at 405 nm (A405) using a microplate reader (BioTek ELX-808, USA). Healthy plants were used as negative controls and samples were considered to be positive when the absorbance values at 405 nm values exceeded at least tree time the mean of the negative controls.

2.3 Maintenance of the viruses

Bean plants were planted in pots in a greenhouse and inoculated with virus isolate prepared from systemically infected leaves macerated in a chilled sterilized 0.01 M cold phosphate buffer (K_2HPO_4 + KH_2PO_4), pH 7.0 (1 : 6 [w/v] tissue : buffer). Inoculation on plants at the three-leaf stage was done using the rub method on carborundum-dusted leaves. The plants were observed weekly for symptom development for 5 weeks and samples for DAS-ELISA were taken at each observation time.

3 RESULTS AND DISCUSSION

During summer of 2013 and 2014, 195 bean samples with virus-like symptoms such as mosaic on leaves, leaf distortion, downward curling, mottling, vein necrosis and local lesions (Figure 1) were collected from bean farms in different areas and villages around Urmia and subjected to DAS-ELISA that revealed the presence of all tested viruses as shown in Table 1. 96 samples were collected in 2013 and 99 samples in 2014. 12 samples collected in 2013 and 17 collected in 2014 were infected with BCMNV, 8 and 2 with BCMV, 1 and 0 with

BYMV and TYLCV, 2 and 1 with ToMV and CMV and 2 and 0 with TSWV (Table 1). DAS-ELISA results showed the presence of BCMNV and BCMV in all bean-producing parts around Urmia where samples were taken. BYMV, TYLCV and TSWV were only detected in Aliabad, the largest bean-growing area in Urmia. ToMV was detected in Emamzadeh-Gharachelar and Nazloo and CMV was detected in Emamzadeh-Gharachelar and Ghafar Behi regions. Mixed infection of BCMNV and BCMV were also detected in two samples from Aliabad region. According to DAS-ELISA results BCMNV was the dominant virus in Urmia, so one of the isolates was inoculated and monitored on *Phaseolus vulgaris* L. in the greenhouse. The necrotic symptoms were seen 5 to 6 days after inoculation (Figure 2) and the presence of the virus was confirmed by DAS-ELISA.

Table 1: Incidence of seven viruses in common bean plants collected in Urmia

Place of sampling	Year of sampling	No. of samples	BCMNV	BCMV	BYMV	TYLCV	ToMV	CMV	TSWV
Emamzadeh-Gharachelar	2013	15	4	2	-	-	1	1	-
	2014	16	5	1	-	-	-	-	
Ghafar-Behi	2013	12	2	1	-	-	-	1	-
	2014	15	3	1	-	-	-	-	-
Jabal	2013	14	2	1	-	-	-	-	-
	2014	16	3	-	-	-	-	-	-
Balo	2013	12	1	1	-	-	1	-	-
	2014	13	-	-	-	-	-	-	-
Nazloo	2013	12	1	1	-	-	-	-	-
	2014	10	1	-	-	-	1	-	-
Aliabad	2013	31	2	2	1	1	-	-	2
	2014	29	5	-	-	-	-	1	-
total	2013	96	12	8	1	1	2	2	2
	2014	99	17	2	-	-	1	1	-
Rate of infection (%)	2013	-	12.5	8.3	1.04	1.04	2.1	2.1	2.1
	2014	-	17.2	2.1	-	-	1.04	1.04	-

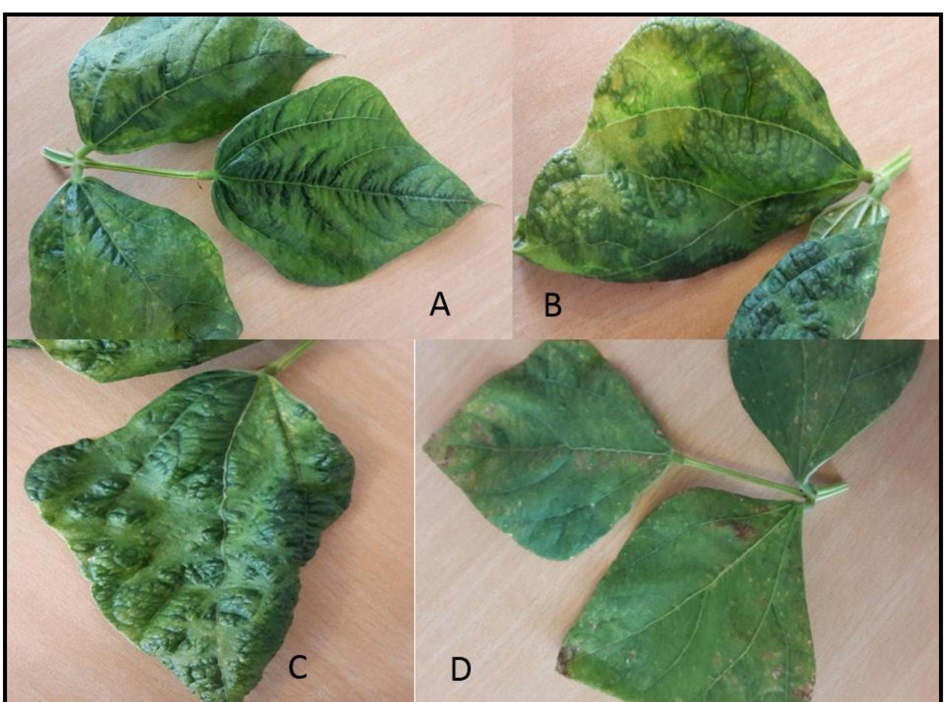

Figure 1: Symptoms of A, vein banding, B, blistering, C, mottling and D, necrosis on virus-infected bean plants

Figure 2: Necrosis induced by BCMNV after inoculation on bean

The incidence and distribution of BCMV, BCMNV, BYMV, TYLCV, TMV and TSWV in bean growing areas of Urmia were not similar to each other. BCMNV infection was the highest. As in Urmia, BCMV and BCMNV are the most prevalent viruses in common bean also in other areas of the world. Since both viruses were found in the same areas and even in the same plant, recombinations between them are possible and can lead to creation of new strains or even new pathotypes (Vallejoes et al., 2006) in Urmia.

Previous studies reported that the incidences of BCMNV strains is lower than those of BCMV strains in most bean production areas of the world (Berger et al., 1997; Kostova et al., 2003; Petrović et al., 2010), but our two year survey in Urmia bean-producing regions indicated that BCMNV is prevalent in most regions.

In most visited areas, especially in Aliabad region, high rate of viral infection was observed. DAS-ELISA test showed different infection rates in various places. The highest infection rate of BCMNV was found in Emamzadeh-Gharachelar and Aliabad probably due to virulence of this virus, high population of the vectors in these areas and suitable weather condition for infection.

TYLCV was found on common bean with a disease incidence of 50 to 70 % showing thickening and crumpling of leaves and stunting in northern Anhui Province, China (Ji et al., 2012). Hosseini and Eini Gandomani (2014) detected gemini viruses on bean in Zanjan province, Iran. We detected TYLCV in only one symptomatic sample in Aliabad region.

BCMNV was reported for the first time and in high abundance in Urmia indicating the spread of viral diseases with climate change and global warming. There has not been any data on occurrence and importance of viral diseases on bean and their spread in nature in the province since now. Investigation on occurrence, spread and determination of dominant viruses of bean are very important especially for implementation of proper diagnostic methods and management techniques, especially breeding for resistance. According to the local observations, symptoms of viral diseases on bean farms of this province are increasing considerably in recent years. Our results also showed high rate of virus infection in the area and confirmed these observations.

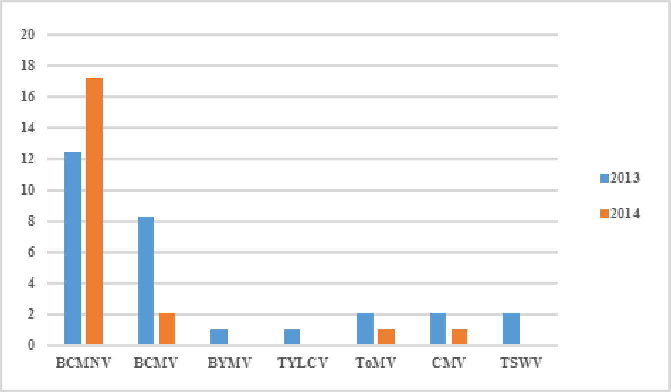

Figure 3: Percentage of incidence of BCMNV, BCMV, BYMV, TYLCV, ToMV, CMV and TSWV in bean fields of Urmia during 2013-2014 growing seasons

4 CONCLUSION

Viruses are known to greatly reduce bean yield. BCMV and BCMNV, two economically very important bean viruses transmitted by several aphid species and by seed, were found in high incidence in West Azarbaijan province, Urmia region using DAS-ELISA. Most of the mosaic and systemic necrosis symptoms observed in bean plants in the field could be attributed to these two viruses. Additionally, the presence of BYMV, TYLCV, ToMV, CMV and TSWV was confirmed. To our knowledge this is the first report of TSWV incidence on bean in Iran and the first report of the presence of BCMNV, BCMV, BYMV, TYLCV, ToMV, CMV and TSWV in West Azarbaijan province, Urmia (Iran). Results of this study are the base for further work on ecology, epidemiology, diversity and breeding for resistance to bean viruses in Urmia and also in Iran.

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The analysis of technical suitability of the equipment for application of plant protection products in Southeastern Slovenia

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ABSTRACT

Technical testing of the equipment for the application of plant protection products (PPP) was performed in Southeastern Slovenia in the period from 2004 to 2013. The technical conditions of boom or orchard sprayers was examined in details and the following parts were checked: drive, anti-drip valves, nozzles, filters, pipes and tubes, manometers, all valves, pressure regulators, agitator, pump, liquid discharge, spray solution reservoir and spray boom or fan system. The analysis revealed a poor condition of most devices in the initial years. However, technical suitability improved drastically until 2013. Technical condition was not directly related to the region of inspection although the lowest number of defective sprayers was recorded in the Posavje region. This can be linked to higher frequency of use and maintenance of the sprayers in this region as it stands out as the area with larger farms. Occasional technical disorders of the equipment can be recorded each year. It is therefore essential to continue with regular technical inspection to ensure optimal and accurate functioning of the sprayers.

Key words: boom sprayers; orchard sprayers; technical conditions; cross application

IZVLEČEK

ANALIZA TEHNIČNE USTREZNOSTI OPREME ZA UPORABO SREDSTEV ZA ZAŠČITO RASTLIN V JOGOVZHODNI SLOVENIJI

V letih od 2004 do 2013 smo opravljali tehnične preglede za nanos FFS na območju jugovzhodne Slovenije. Pregledovali smo dve vrsti naprav in sicer škropilnice in pršilnike. Ugotavljali smo njihovo tehnično brezhibnost. Na vsaki napravi so bili pregledani sklopi kot so pogon, protikapni ventili, šobe, filtri, cevi, manometri, pipe in zasuni, regulatorji tlaka, mešalo, črpalka, praznjenje, rezervoar ter škropilne letve oziroma puhala pri pršilnikih. Analizirali smo podatke o napakah in ugotovili, da je bilo tehnično stanje naprav v začetnem obdobju, zelo slabo. Tehnično stanje naprav se je do leta 2013 zelo izboljšalo. Ugotovili smo tudi, da tehnično stanje naprav ni odvisno od območja njenega nahajanja. Je pa območje Posavja izstopalo po najmanjšem deležu okvarjenih naprav. Razlog za to vidimo predvsem v tem, da se naprave nahajajo na velikih kmetijah, kjer jih redno uporabljajo in vzdržujejo. Ker pa se okvare in napake, sicer v manjši meri, pojavljajo vsakoletno sklepamo, da je s pregledi potrebno nadaljevati, če hočemo obdržati doseženo stanje tehnične brezhibnosti, kajti naprave se ob uporabi prej ali slej obrabijo.

Ključne besede: škropilnice; pršilniki; fitofarmacevtska sredstva; tehnično stanje; prečni nanos

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1 INTRODUCTION

Devices used for the application of plant protection products (PPP) are machines, which enable uniform application of PPP to the plant surface. Devices usually utilize water to dissolve and apply PPP and can be categorized as sprayers and air assisted sprayers (Mrhar, 1997). Phytopharmaceutical products, which are applied via sprayers, destroy, suppress, control or deter harmful organisms and prevent their negative effect on plant growth and development or storage of plant products (Blažič, 2009).

Technical flawless of the spraying equipment is crucial for optimal distribution of PPP. The quality of the sprayer is important but it does not guarantee good results if the device is not properly managed and maintained. Up-to-date development of spraying equipment and its technical assistance and inspection are generally focused to ensure accurate PPP application (exact dosage, uniform distribution) and ameliorate other factors (such as working speed) (Roettele et al., 2011). Sprayers must provide exact application and uniform dosage of PPP during the entire lifespan of the device. To ensure their proper functioning, sprayers must be regularly tested and potential technical faults eliminated (Ganzelmeier, 2004a).

SIST EN 13790-1 (2004) and SIST EN 13790-2 (2004) standards were implemented in EU member states in order to unify the technical demands for testing of the devices for PPP application. The purpose of the standards is to ensure comparable testing conditions throughout the EU. Their contents can be summarized into the following significant points (Ganzelmeier, 2004b):
(1) unification of different procedures, findings and technical demands, previously implemented in specific EU member states;
(2) methodology and technical requirements are based on successful practices, previously implemented in specific EU member states;
(3) high technical level is ensured with minimum time and funding;
(4) EU member states are obliged to accept and implement new standards and withdraw old standards;
(5) standards represent the basis for the unification of technical testing in the EU and serve as potential interactive tool in the future;
(6) standards set technical requirements but do not regulate the decisions of specific EU member states.

In case of Poland, technical testing of the devices for the application of PPP started in 1995 and became mandatory in 1999. Holownicki et al. (2004) reported similar national standards to that of SIST EN 13790-1 (2004) and SIST EN 13790-2 (2004) prior to their implementation. However, several parts of the spraying equipment were tested according to a less strict methodology, which frequently only included visual assessment.

Norway began technical testing of spraying equipment in 1990 (boom sprayers) and in 1995 (air assisted sprayers) on a voluntary level. The testing became mandatory for all devices in 2000. Although national technical tests were very sophisticated, slight changes were made after the implementation of SIST EN 17390-1 (2004) and SIST EN 13790-2 (2004) (Bjugstad et al., 2004) standards.

Italy started voluntary testing of air assisted sprayers in the Bolzano region as far back as in 1980. Oddly, technical testing of spraying devices has not been performed in several other regions to this day. In 2004, only 9 of 20 Italian regions had developed a system for technical testing. Some technical testing stations were established in 1996 – 1999 but most were founded in 2002 – 2004 (Balsari et al., 2004). Up to now, 120 testing stations are active in Italy but the testing is only mandatory in Tuscany region. Most technical tests are performed by private institutions. Public testing stations are mostly focused on voluntary testing and experimental testing of the spraying equipment (Balsari et al., 2004).

Germany implemented technical testing practices of spraying devices in late 1960 (boom sprayers) and in the middle of 1980 (air assisted sprayers) on a voluntary level. About 1000 testing stations are located in Germany today, which cover 2000 different locations. Testing became mandatory in 1993 and until then approximately 30.000 devices were tested each year. Today, as many as 63.000 sprayers are tested yearly (Osteroth, 2004).

In Slovenia, technical testing of spraying equipment was already defined in 1994 Plant Health Act (Zakon o zdravstvenem …, 1994). Tests have been performed for the last two decades and the aim of the present paper is to present the testing results to a broader scientific community. The effects and results of the testing are discussed and strategies for their improvement suggested.

2 MATERIALS AND METHODS

Experimental data include the records of technical testing from 2004 until 2013. Inspections were performed at 86 different locations in Southeastern Slovenia, encompassing Agricultural advisory services Trebnje, Novo Mesto, Metlika, Črnomelj, Krško, Sevnica and Brežice. The research covers the area from Trebnje, Bela krajina, Novo Mesto, Škocjan, Šentjernej, Kostanjevica na Krki, Krško, Brežice to Bizeljsko. Micro locations (testing stations) varied each year (according to the suggestions of local communities) but this did not affect the groups of spraying devices subjected to inspection.

Technical testing was performed according to Rules on terms and procedures, which must be met by all authorized supervisory companies for regular inspection of PPP application devices (Pravilnik o pogojih …, 2000, Pravilnik o spremembi pravilnika …, 2002, Pravilnik o spremembah in dopolnitvi …, 2005). The details on technical inspection are specified in aforementioned Rules on terms and procedures.

2.1 Measuring devices and other equipment

- Measuring set Herbst ROT-650/60/40/10
- Measuring of pump flow
- Measuring of the working pressure and the pressure gauge
- Measuring burettes Herbst ED 16 ECO
- Mobile Electronic Sprayer Test Equipment SprayerTest 1000
- Wireless rectifier Linksys
- Computer IBM Lenovo R60
- Drip tray
- Aluminum tracks for the trolley
- Inspection protocol (printed)

Boom sprayers and air assisted sprayers were inspected separately.
The following sections of the boom sprayer were inspected: spray solution reservoir, liquid discharge, agitator, pressure gauge, gate valves and other valves, manometer, pipes and tubes, filters, nozzles, spray boom, anti-drip valves and drive.

Several parts of the air assisted sprayer are identical to the parts of a boom sprayer but some segments are different. The following segments of air assisted sprayers were inspected: spray solution reservoir, liquid discharge, agitator, pressure gauge, gate valves and other valves, manometer, pipes and tubes, filters, nozzles, anti-drip valves, drive and blower.

2.2 Data analysis

Data were statistically analyzed in program R, version 3.0.2. Regression models- linear mixed models were used to interpret the correlation between the year and rate of faults. The level of risk was 5 %.

3 RESULTS AND DISCUSSION

3.1 Technical condition of all spraying devices

Technical condition of spraying devices varied according to the year of inspection. Data is presented in Table 1.

Table 1: Data on inspected devices (data on boom sprayers and air assisted sprayers merged) in a particular year

Preglednica 1: Podatki o pregledanih napravah (škropilnice in pršilniki skupaj) v posameznem letu

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Inspected devices	1440	2037	799	1419	1012	2006	1013	1939	1056	1823
Defected devices	708	851	374	517	188	150	56	63	39	29
Share of defected devices	49.2%	41.8%	46.8%	36.4%	18.6%	7.5%	5.5%	3.2%	3.7%	1.6%

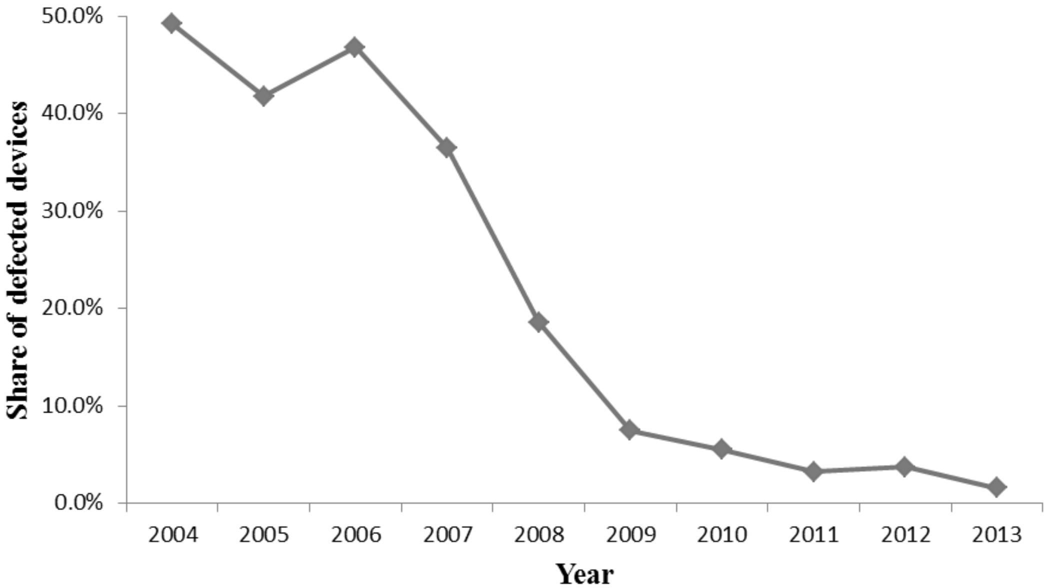

Figure 1: Share of defected devices in a particular year (data on boom sprayers and air assisted sprayers merged)

Slika 1: Delež okvarjenih naprav za obe vrsti naprav v posameznem letu

The share of defected spraying devices declined consistently from 2004 until 2013, with a slight increase detected in 2006. The greatest decline in the share of defected sprayers was observed between the years 2004 and 2009, when the share dropped to less than 10 %. The share of defected devices gradually declined until 2013 but in a smaller proportion compared to the initial experimental period.

3.2 Technical condition of boom sprayers

Data on boom sprayer technical testing is presented in Table 2.

Table 2: Data on inspected boom sprayers in a particular year

Preglednica 2: Podatki o pregledanih škropilnicah v posameznem letu

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Inspected devices	1080	1504	594	1085	713	1461	719	1381	722	1287
Defected devices	594	731	316	472	170	132	48	56	28	23
Share of defected devices	55.0%	48.6%	53.2%	43.5%	23.8%	9.0%	6.7%	4.1%	3.9%	1.8%

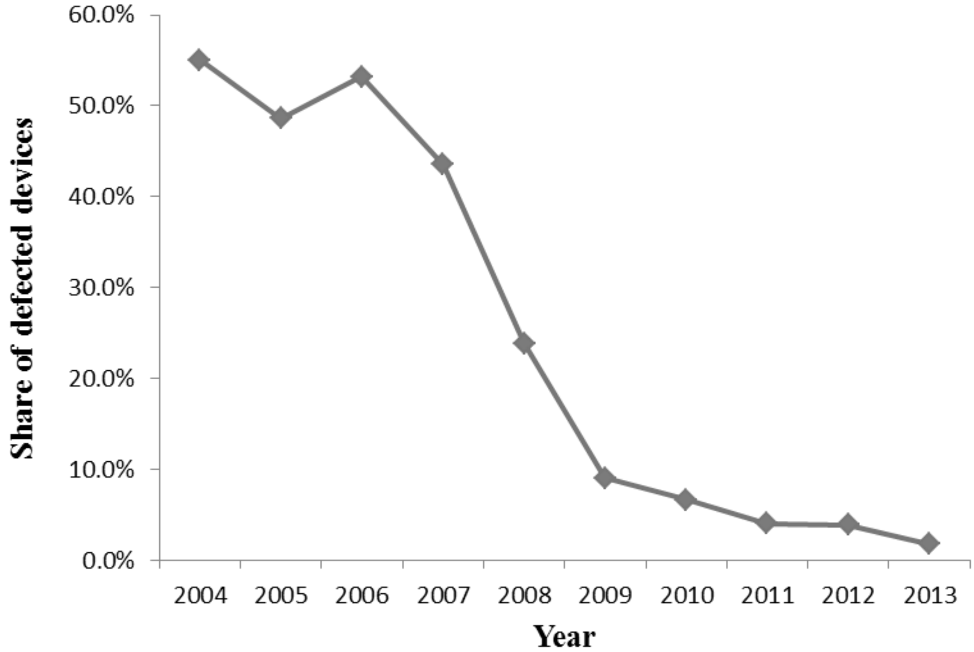

Figure 2: Share of defected boom sprayers in a particular year

Slika 2: Delež okvarjenih škropilnic v posameznem letu

We assumed that the share of defected boom sprayers decreased during the 10-year period. Data analysis was performed on several segments of the boom sprayer.

Table 3: Results of data analysis using the linear mixed model for several boom sprayer segments

Preglednica 3: Rezultati linearnega mešanega modela na podatkih za posamezne sklope - škropilnice

	Coefficient	Standard error	df	t	p-value
Intersect	0.079	0.016	116	4.863	0.0000
Year	-0.010	0.002	116	-4.895	0.0000

The negative value of the coefficient suggests that the defects on boom sprayers decreased in the examined period.

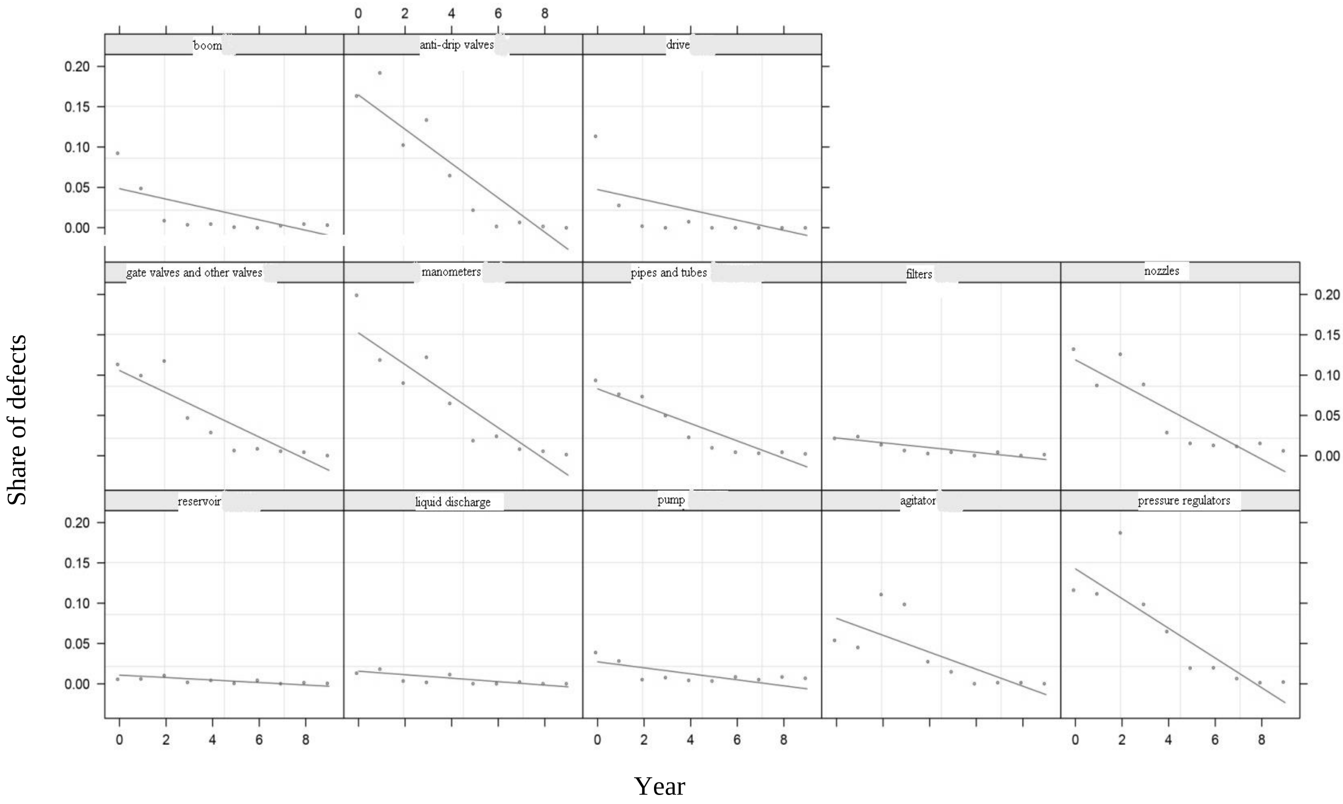

Figure 3: Share of defects on individual segments of a boom sprayer depending on the year – 0 marks year 2004

Slika 3: Delež napak za posamezen sklop v odvisnosti od leta - število 0 na abscisni osi ponazarja začetno leto 2004

Figure 3 depicts the decrease of share of devices with defects in all segments of boom sprayers in the period from 2004 to 2013. Some differences were minor but the condition of several segments was significantly improved.

3.3 Technical condition of air assisted sprayers

Differences in technical conditions of air assisted sprayers are reported in Table 4.

Table 4: Data on inspected air assisted sprayers in a particular year

Preglednica 4: Podatki o pregledanih napravah (pršilniki) v posameznem letu

Year	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Inspected devices	356	531	204	332	299	541	294	555	334	534
Defected devices	114	119	58	45	18	18	8	7	11	6
Share of defected devices	32.0%	22.4%	28.4%	13.6%	6.0%	3.3%	2.7%	1.3%	3.3%	1.1%

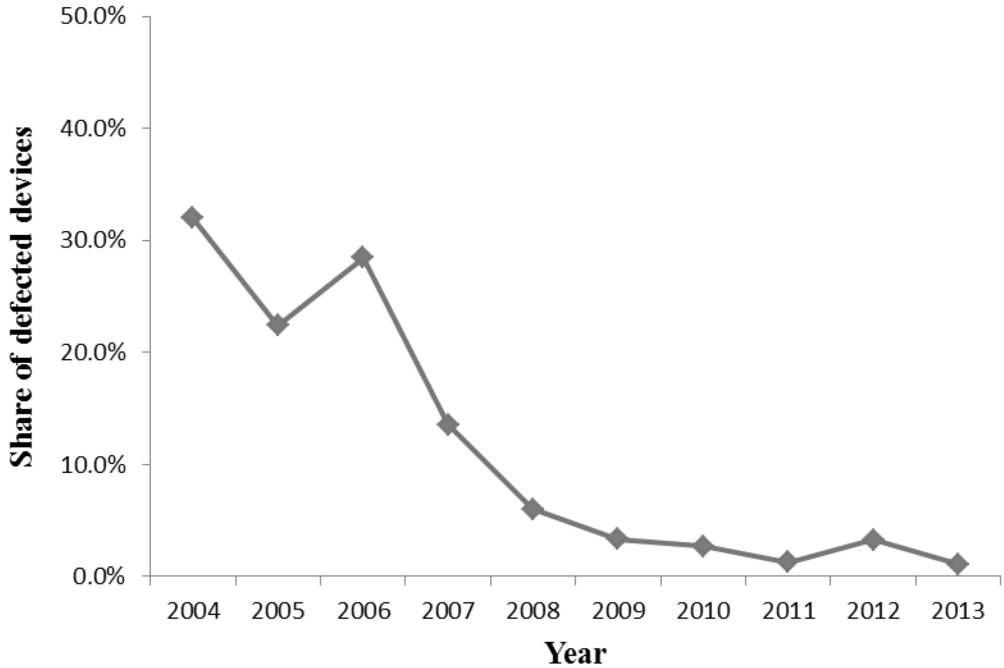

Figure 4: Share of defected air assisted sprayers in a particular year

Slika 4: Delež okvarjenih pršilnikov v posameznem letu

The share of defected air assisted sprayers ranged between 20 and 30 % in the period of 2004- 2006 and dropped to 13.6 % in 2007. The share was less than 10 % after 2008.

Data were statistically analyzed and a decrease of defected air assisted sprayers was expected in the 10-year period.

Analysis was performed on several segments of air assisted sprayers.

Table 5: Results of the linear mixed model analysing data of several air assisted sprayer segments

Preglednica 5: Rezultati linearnega mešanega modela na podatkih za posamezne sklope - pršilniki

	Coefficient	Standard error	df	t	p-value
Intersect	0.023	0.008	116	3.313	0.0012
Year	-0.004	0.001	116	-3.239	0.0016

The negative value of the coefficient denotes a decrease of defects in air assisted sprayers during the examined period.

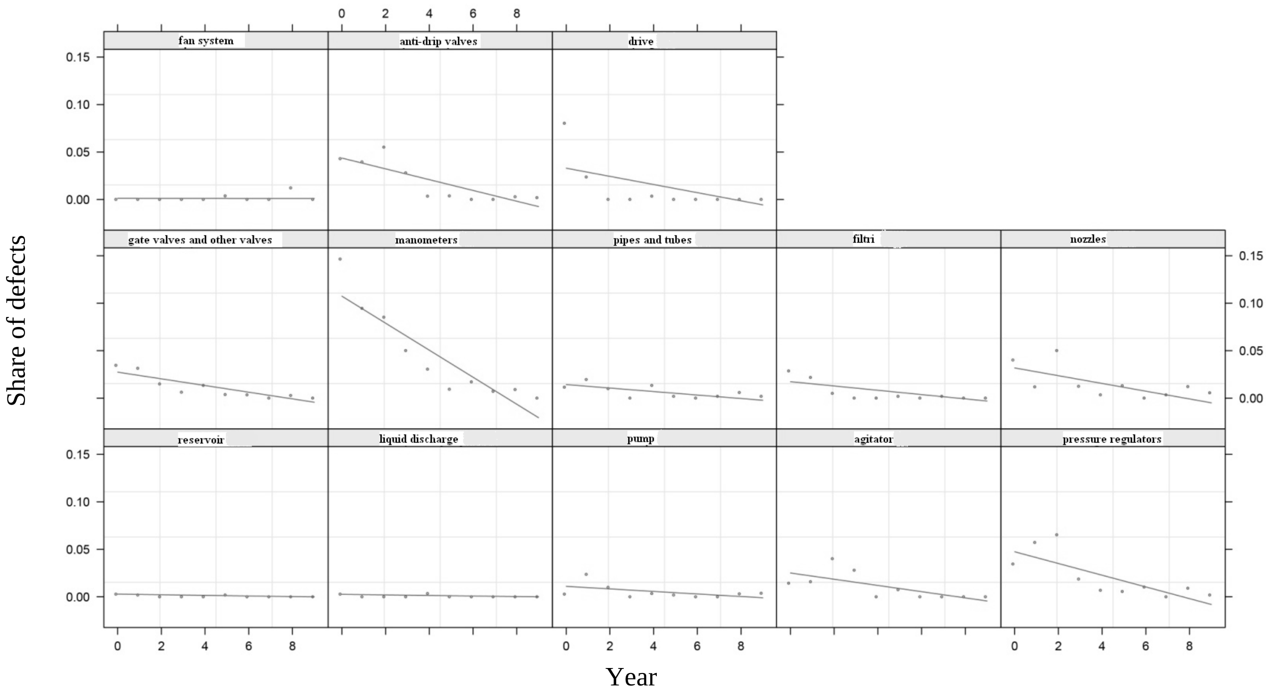

Figure 5: Share of defects on individual segments of an air assisted sprayer depending on the year – 0 marks year 2004

Slika 5: Delež napak za posamezen sklop v odvisnosti od leta - številka 0 na abscisni osi ponazarja začetno leto 2004

The share of defects on all segments of air assisted sprayers decreased in the period from 2004 to 2013. The only exceptions were defects on the air blower, which increased slightly from 2009 to 2012.

3.4 Technical condition linked to the region of the technical testing

The locations for technical testing were not the same each year and therefore, the area was divided into seven units, each corresponding to the jurisdiction of a single Agricultural advisory service (Trebnje, Novo Mesto, Metlika, Črnomelj, Krško, Sevnica and Brežice). Three main regions were formed based on geographical similarities for easier data analysis: (1) Osrednja Dolenjska (combining KSS Novo Mesto and Trebnje; NMTr), (2) Bela Krajina (combining KSS Črnomelj and Metlika; ČrMe) and (3) Posavje (combining KSS Brežice, Krško and Sevnica; BKKSe). Results report combined data on defects of both types of sprayers for each region.

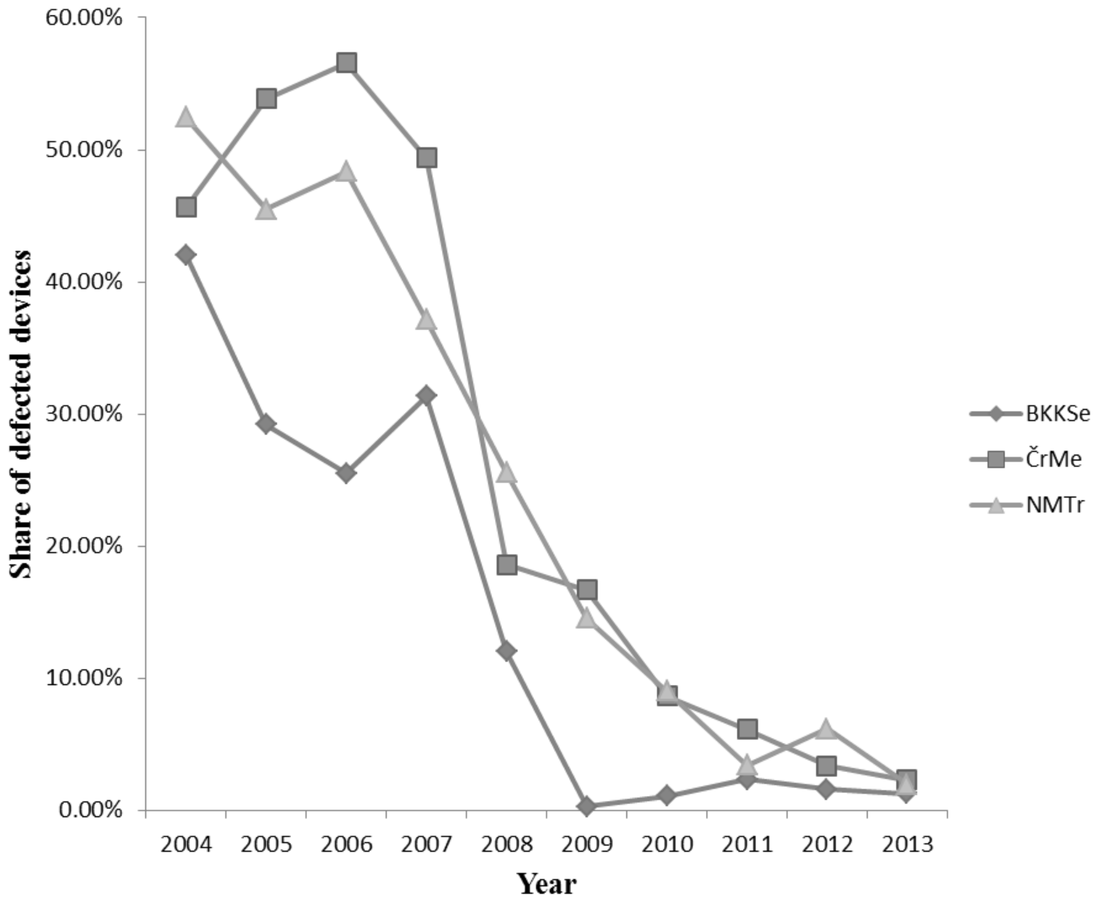

Figure 6: Share of defected sprayers in a specific region during the period of 2004-2013

Slika 6: Delež okvarjenih naprav v posameznem območju v letih 2004-2013

The share of defect sprayers was somewhat higher in Bela Krajina region in 2006 compared to 2004 and decreased significantly after 2006. A similar pattern was detected in Osrednja Dolenjska region, where the share of defected sprayers increased slightly in 2006 and decreased dramatically after that period. In Posavje region the pattern shows a significant decrease of defected sprayers from 2004 until 2007. In that year the share increased prior to a significant decrease of defected sprayers after 2008.

4 CONCLUSIONS

4.1 Technical condition of all spraying devices

As anticipated, technical condition of all spraying devices (boom sprayers and air assisted sprayers) increased during the examined time period.

Although technical inspection of PPP spraying devices in Slovenia began two decades ago, it only became mandatory in 2002. The initial technical condition of spraying devices was therefore poor, which can be ascribed to the general non-attendance at testing prior to 2002. Only environmentally conscious individuals and larger farmers tested their sprayers on a regular basis as they were aware of the importance of proper functioning of the devices. The latter are only effective in distribution of PPP if their technical condition is optimal. Frequently, leakage, poor distribution of the liquid solution and inadequate dosing (output) were recorded at testing, which resulted in reduced quality of application and economic loss. Every deviation from standard functioning of the sprayer inevitably leads to inefficient use of PPP.

Sadly, it seems that many farmers were not concerned with inferior performance of their sprayers as they only cultivate small areas of land and are thus satisfied with reduced performance - functioning of the devices. Small-scale farmers only require minor quantities of PPP and do not regard plant protection as a major economic cost of farming. This can be linked to a great number of defects on sprayers in the initial years of technical testing, i.e. in 2004 and 2005. Later, farmers were obliged to test their spraying equipment by law and a qualified mechanic serviced their devices on site. Many minor defects were corrected and users were simultaneously educated on a proper use and maintenance of the spraying equipment.

The results of this practice came to sight in the following years as the number of defects on sprayers greatly reduced. This proves the importance of regular technical testing for improved condition of spraying equipment in Slovenia.

However, defects can be recorded each year and it is therefore essential to proceed with technical testing of PPP sprayers in the future. Spraying devices have a limited lifespan and technical performance of every sprayer is reduced in time. For example, defected sprayers from the period of 2004-2008 were once in immaculate condition but because of their use and/or poor maintenance flaws occurred. That is why sprayers must be tested on a yearly basis to limit the use of faulty equipment on Slovenian farms and uncover hidden defects on the equipment such as inferior transversal distribution of spraying solution.

Technical condition of boom sprayers

As expected, technical condition of boom sprayers greatly improved in the experimental period. Many defects were recorded on different segments of boom sprayers in 2004 and 2005 and their number was reduced in later years.

Technical condition of air assisted sprayers

As in boom sprayers, technical condition of air assisted sprayers improved dramatically from 2004 to 2013. Most defects were similarly recorded in 2004 and 2005 and a superior condition of these devices was recorded in later years.

4.2 Technical condition linked to the region of the technical testing

Locations were grouped according to the jurisdiction of Agricultural advisory services and three main regions were formed based on geographical similarities of the area. We assumed that the technical condition of spraying devices is not defined by region and the hypothesis was confirmed. No significant differences were detected among the three regions. Nevertheless, smaller share of defected sprayers was recorded in the Posavje region which can be ascribed to several factors. Many large farms are active in this region and consequently, the farmers possess newer and better equipment for PPP application. The spraying devices are less prone to develop any defects and are also regularly serviced. Experiences show, that small-scale farmers frequently use defected sprayers on their land. The devices are old and poorly maintained but the farmers cannot afford new mechanization due to non-favorable economic calculation based on limited land use. The other reason for superior results of the Posavje region may be linked to better technical support of Agricultural advisory services and education on the importance of proper PPP use in this area. These practices should be inspected in detail and implemented in other areas.

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Pravilnik o pogojih in postopkih, ki jih morajo izpolnjevati in izvajati pooblaščeni nadzorni organi za redno pregledovanje naprav za nanašanje fitofarmacevtskih sredstev. (2000). Uradni list RS št. *12/*00

Pravilnik o spremembah in dopolnitvi Pravilnika o pogojih in postopkih, ki jih morajo izpolnjevati in izvajati pooblaščeni nadzorni organi za redno pregledovanje naprav za nanašanje fitofarmacevtskih sredstev. (2005). Ur. l. RS št. *97*/05

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In vitro allelopathic effect of aqueous extracts of sugarcane on germination parameters of wheat

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ABSTRACT

Allelopathy – interactions among plants for resources along with competition – is a composite phenomenon which has spacious potentials of application in agriculture. Understanding of interactions among plants, particularly cultivated crops, may be helpful in modifying crop cultivation pattern with consequent yields increments. In this study, we investigated the allelopathic effects of aqueous extracts of root, stem peels and leaves of sugarcane (*Saccharum officinale* L.) cultivar *51* at concentrations 0, 2.5, 5.0, 7.5 and 10.0 g/l on germination indices and seedling biomass of wheat (*Triticum aestivum* L.) cultivar *Pirsabak-2005*. Results demonstrated that higher concentration (10.0 g/l) of extracts of root, stem peels and leaves significantly decreased mean germination time (MGT) but increased shoot and seminal root growth and seedling dry biomass; however, germination percentage was affected neither by extract concentration nor by plant parts used in the study. Extract concentrations up to 7.5 g/l had no effect on the studied parameters of wheat. Our result suggests that sugarcane's allelopathy demonstrates healthy effects on wheat growth and that wheat could be cultivated in sequential rotation in field conditions.

Key words: allelopathy; sugar cane; common wheat; germination parameters; biomass; crop rotation

IZVLEČEK

***In vitro* ALELOPATSKI UČINKI VODNIH IZVLEČKOV SLADKORNEGA TRSA NA PARAMETRE KALITVE NAVADNE PŠENICE**

Alelopatija – interakcije med rastlinami za vire preko tekmovanja – je kompleksen fenomen, ki ima za uporabo v kmetijstvu velik pomen. Razumevanje teh interakcij med rastlinami, še posebej med gojenimi, lahko pomaga pri spreminjanju načinov pridelave z znatnim povečanjem pridelka. V raziskavi so bili preučevani alelopatski učinki vodnih izvlečkov korenin, stebel in listov sladkornega trsa (*Saccharum officinale* L.), kultivarja *51,* v koncentracijah 0, 2.5, 5.0, 7.5 in 10.0 g/l na kalitvene parametere in biomaso kalic krušne pšenice (*Triticum aestivum* L.), sorte *Pirsabak-2005*. Rezultati so pokazali, da so večje koncentracije (10.0 g/l) izvlečkov korenin, stebla in listov značilno zmanjšale povprečni čas kalitve (MGT), a povečale rast semenskih korenin in poganjkov ter biomaso kalic, na odstotek kalitve pa niti koncentracija izvlečkov niti rastlinski organ nista vplivala. Izvlečki v koncentracijah do 7.5 g/l niso imeli učinka na preučevane parametre pšenice. Rezultati nakazujejo, da ima sladkorni trs pozitivne alelopatske učinke na rast pšenice in da lahko poljščino gojimo v kolobarju neposredno za sladkornim trsom.

Ključne besede: aleopatija; sladkorni trs; krušna pšenica; biomasa; parametri kalitve; kolobar

1 INTRODUCTION

Allelopathy is a composite process occurring in natural habitats as well as in cultivated fields and is generally perceived as a mechanism of plants and microbes' capacity to maintain their dominance over others or at least to coexist in a given environment. Through allelopathy alone or in combination with competition, plants can influence survival capability of in-range plants and other microorganisms in a manner that they are constrained to either migrate to somewhere else or to remain in the habitat in a defensive mode. The

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influential capacity of one plant over the other is because of the metabolic compounds (known as allelochemicals) they release into the surrounding environments either as volatile substances, rain leachates, decomposed products or direct secretion to rhizosphere with potential negative or positive interactions with other plants and microbes (Barkosky et al., 2000; Barto et al., 2010; Rice, 2012). Allelochemicals are generally secondary metabolites present in different concentrations in different plant parts (leaves, stem, barks, flowers, seeds etc.) which upon release into the rhizosphere tend to modify the resource consumption capacity by several mechanisms i.e., alteration of cell membrane permeability, changing enzymatic activity, triggering genetic defects and disturbing photosynthesis of the competitor plants (Gonzalez and Estevez-Braun, 1997; Wu et al., 2000; Barto et al., 2010; Majeed et al., 2012). Interactions among plants for resources through allelopathy lead to physiological and biochemical modifications which may result in the establishment of a successful plant community by eliminating or restricting the susceptible species, although many plants exhibit positive allelopathic effects on the surrounding plants (Elijarrat and Barcelo, 2001; Maharjan et al., 2007; Hussain et al., 2010). Thus allelopathy may be successfully employed in agriculture for enhancing crop productivity and weed management (Fang et al., 2013).

Common wheat (*Triticum aestivum* L.) in the family Poaceae is an important agronomic crop widely cultivated for grains which are used in food and several other processed products. After maize and rice, wheat is ranked the third most widely produced grain crops in the world which has significant impact on meeting global food demands and dietary needs (Hou et al., 2014). The crop in Pakistan is generally cultivated in rotation with several other crops such as maize, rice, tobacco and sugarcane. Knowledge about allelopathic nature of preceding crops may be helpful in modifying cultivation pattern of wheat with other crops. During the last few years, allelopathic activities of several plants and agricultural crops including wheat have been rigorously worked out (Khaliq et al., 2013; Muhammad and Majeed, 2014). Sampietro and Vattuone (2006) observed root elongation of some selected weeds and crops at lower concentrations while declined root growth at higher concentration of aqueous extracts of sugarcane straw. Nikneshan et al. (2011) investigated aqueous extracts of air-dried leaf powder of eight cultivars of sunflower for allelopathic activities on wheat with decrease in germination indices at higher extract concentrations. Majeed et al. (2012) observed drastic effects of higher but stimulatory effects of lower concentrations of aqueous extracts of *Chenopodium album* L. on plant height, tillers, spike length and grain yield of wheat. Variable results were obtained for germination, plant height; fresh and dry mass of shoot and root of wheat under the allelopathic influence of three weeds species viz: *Asphodelus tenuifolius* Cav., *Euphorbia hirta* L., and *Fumaria indica* (Hausskn.) Pugsley (Jabeen et al., 2013). Ullah et al. (2013) recorded suppressive effects on germination of 20 varieties of wheat treated with aqueous leaf extracts of *Eucalyptus camaldulensis* Dehnh., *Acacia nilotica* (L.) Willd. ex Delile, *Helianthus annuus* L. and *Parthenium hysterophorus* L.

Although sugarcane's allelopathy has been worked out against weeds and some crops; however, reports on its allelopathic investigation against wheat are scarce in literature. The aim of this work was to study the allelopathic activity of root, stem peels and leaves of sugarcane on germination and seedling growth of wheat in laboratory conditions.

2 MATERIALS AND METHODS

2.1 Plant materials

Mature plants of Sugarcane (*Saccharum officinale* L.) cultivar *51* were collected at harvesting stage from cultivated fields in Naguman, Peshawar during 2013. Different plants parts i.e., leaves, stem peels and root were separated and dried under shade conditions. Dried parts were ground to powder with an electric grinder for further use. In order to get aqueous extracts of different concentration, 2.5, 5.0, 7.5 and 10.0 g of dried powder of each part i.e., leaves, stem peels and roots were soaked for 24 h in 1litre distilled water each at room temperature. The soaked materials were filtered through muslin cloth after 24 hours. Filtrate was again filtered through filter paper (Whatman No. 1) in sterilized flasks to get extracts of different concentrations. Aqueous extracts were stored at 4 °C in a refrigerator.

2.2 Bioassay

Seeds of wheat (*Triticum aestivum* L.) cultivar Pirsabak-2005 were obtained from Agricultural Research Institute, Tarnab, Peshawar. Seeds were placed on twice folded filter paper as seed beds in petri-dishes. Each petri-dish was provided with 10 ml of respective concentrated aqueous extracts. Control seeds were provided with the same volume of distilled water. Each Petri dish had 10 seeds; each treatment was further replicated 4 times. The experiment was arranged in a

Completely Randomized Manner at room temperature (20-25 °C) with 12 h photo period. The experiment was performed at Botany Department, Government Degree College Naguman Peshawar during 2013. After 72 hours, data on germination, seminal root and shoot length of seedling was recorded. Germination (%) was calculated as number of germinated seeds in each replicate after 72 hours till final reading. Mean germination time was determined as: MGT = $\Sigma (di \times ni) / \Sigma ni$; where n represents number of germinated seeds at i^{th} day and d is the number of days counted from the beginning till the completion of germination (Basra et al., 2005). Dry biomass of seedling was determined as previously described by Muhammad and Hussain (2012).

2.3 Statistical analysis

Results were statistically analyzed by the analysis of variance (ANOVA). Least significant difference (LSD) was used to determine significant mean values of the studied parameters at $p \leq 0.05$.

3 RESULTS

3.1 Germination (%)

In this experiment, the allelopathic activity of root, stem peels and leaves of sugarcane which were prepared as aqueous extracts in different concentrations were studied on germination parameters and dry biomass of wheat. Results revealed that germination percentage was neither affected by different plant parts nor by extract concentrations. Interaction between plant parts and concentrations were also non-significant. Germination (%) was maximum (91.8) in control conditions. Slighter decrease in germination was observed in petri-dishes treated with different extract concentrations of different plant parts; however, the differences among means of concentrations and plant parts for germination percentage were insignificant and they ranged between 89.7 - 90.9 % which did not differ significantly from 91.8 % in control (Table 1).

Table 1: Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on germination (%) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	Control 0	*2.5*	*5.0*	*7.5*	*10.0*	
Roots	91.8a	90.9a	90.7a	90.9a	91.4a	90.98a
Stem peel	91.8a	90.7a	91.1a	90.9a	91.0a	91.10a
Leaves	91.8a	90.8a	90.9a	90.7a	89.7a	90.64a
Concentration means	91.8a	90.8a	90.9a	90.83a	90.7a	

3.2 Mean Germination Time

MGT was calculated to assess the average number of days taken from the beginning of germination of seeds till completion of germination. MGT was significantly affected by plant parts as well as concentration of extracts. Interaction between plant parts and concentration was also recorded as significant. It was observed that lower extract concentrations up to 7.5 g/l of each plant part had no effect on this parameter; however, 10.0 g/l extract significantly minimized germination time particularly when roots and peel extracts were applied (Table 2). Leaf extract at the highest dose (10 g/l) significantly prolonged meant time of germination which was recorded as 5.8 days. In each plant part at highest extract concentration, MGT was lowest than control where it was 4.7 days except in leaf extract which revealed maximum value for this parameter.

3.3 Shoot growth

Analysis of variance (ANOVA) determined significant differences for values of shoot length of wheat seedlings at 10g/l concentrated extracts but insignificant effect at lower concentrations. Interactions were also significant. Shoot length was 15.9 mm in control plates which were treated with distilled water. There were no significant differences between values in extract concentrations 2.5, 5 and 7.5 g/l of respective plant parts which revealed results almost consistent with those of control; however, 10 g/l extract of root and stem peels significantly increased shoot length (16.8 and 16.7 mm respectively). Unexpectedly, the same concentrated extract of leaves had adverse effect on shoot growth which was significantly reduced (14.2 mm) as compared to control and other plant part extracts (Table 3).

Table 2: Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on mean germination time (MGT) (days) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	Control 0	*2.5*	*5.0*	*7.5*	*10.0*	
Roots	4.7a	4.3a	4.6a	4.8a	3.4c	4.36a
Stem peel	4.7a	4.4a	4.7a	4.9a	3.7b	4.48a
Leaves	4.7a	4.8a	4.8a	4.9a	5.8bc	4.4a
Concentration means	4.7a	4.5a	4.7a	4.86a	3.3c	

LSD (p≤0.05) for plant parts = 3.245; concentration = 2.769; interaction 3.120

Values in columns and rows with different alphabets are significantly different

Table 3: Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on shoot length (mm) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	Control 0	*2.5*	*5.0*	*7.5*	*10.0*	
Roots	15.9a	16.0a	15.9a	15.3a	16.8b	90.98a
Stem peel	15.9a	15.8a	15.7a	15.5a	16.7b	91.1a
Leaves	15.9a	15.4	15.6a	15.7a	14.2bc	90.64a
Concentration means	15.9a	15.8a	15.7a	15.5a	15.9a	

LSD (p≤0.05) for plant parts = 1.971; concentration = 3.963; interaction 4.7612

Values in columns and rows with different alphabets are significantly different

3.4 Seminal root length

Like other indices, seminal root length was significantly influenced by concentrations of the extracts as well as different plant parts. Lower concentration had no effect on RL whose values were similar to control with slight variations. Interaction between plant parts and concentrations at highest dose were significant. Control condition and extract concentrations up to 7.5 g/l recorded almost similar values of seminal root length which ranged between 27.3-27.9 mm. Maximum length (30.1 mm) of seminal root was observed in root extract at 10g/l concentration followed by the same extract concentration of stem peels and leaves which yielded consistent values 29.4 and 29.3 mm respectively (Table 4).

3.5 Seedling dry biomass

Dry biomass of seedling was calculated in each replicate of each treatment and then averaged to determine individual seedling's biomass. Results demonstrated significantly different values for different plant parts, 10g/l extract concentration and interactions but insignificant differences were observed for lower concentrations (2.5 - 7.5 g/l). Values of dry biomass slightly varied under lower extract concentrations of each plant part; however, they were statistically similar to control values. Root and stem peels extract at 10 g/l resulted in highest dry biomass of seedlings which were recorded as 40.1 and 39.2 mg respectively when compared to control (36.1 mg). The lowest value for dry biomass was observed in 10 g/l leaves extract which was 34.2 mg, significantly different from control as well as root and peel extracts at the same concentration (Table 5).

Table 4: Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on seminal root length (mm) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	Control 0	*2.5*	*5.0*	*7.5*	*10.0*	
Roots	27.8a	27.7a	27.6a	28.0a	30.1b	28.4a
Stem peel	27.8a	27.5a	27.7a	27.9a	29.4b	28.06a
Leaves	27.8a	27.3a	27.4a	27.8a	29.3b	27.9a
Concentration means	27.8a	27.5a	27.5a	27.9a	29.6b	

LSD ($p \leq 0.05$) for plant parts = 11.09; concentration = 7.939; interaction 1.890

Values in columns and rows with different alphabets are significantly different

Table 5: Effect of different concentrations of aqueous extracts of plant parts (root, stem peel and leaves) of sugarcane on dry biomass seedling^{-1} (g) of wheat

Plant parts	Concentration (g/l)					Plant parts means
	Control 0	*2.5*	*5.0*	*7.5*	*10.0*	
Roots	36.1a	35.8a	36.9a	36.5a	40.1b	37.08c
Stem peel	36.1a	36.4a	36.3a	36.9a	39.2b	36.98a
Leaves	36.1a	36.7a	36.4a	36.7a	34.2c	36.02a
Concentration means	36.1a	36.3a	36.5a	36.7a	37.8ab	

LSD ($p \leq 0.05$) for plant parts = 5.213; concentration = 1.7821; interaction 6.379

Values in columns and rows with different alphabets are significantly different

4 DISCUSSION

Germination is an important indicator which depicts the plants' response to changes in the environment, resources or any allelopathic stress induced as a result of allelochemicals released from donor plants (Hussain et al., 2010). Germination indices are generally used to detect potential stimulatory or inhibitory allelopathic activity of the test plant (Hussain and Reigosa, 2014). In our study, germination percentage was not affected by aqueous extract of different plant parts of *S. officinale*. Moreover, concentrations of the extracts were also unable to stimulate or inhibit the germination percentage of wheat. This is in contradiction with previous studies on the allelopathy of *Hypericum myrianthum* Cham. & Schltdl. (Fritz et al., 2007), *Eucalyptus camaldulensis* (Ahmed et al., 2008), *Prosopis juliflora* (Sw.) DC. (Siddiqui et al., 2009), *Dodonaea viscosa* Jacq. (Barkatullah et al., 2010) and *Halianthus annus* L. (Muhammad and Majeed, 2014) on germination of wheat and other target crops which revealed significant retardation of germination under the allelopathy of the respective plants at different extract concentrations. We used the highest concentration as 10 g l^{-1} which is relatively low as compared to extract concentrations used in other studies. Thus, non-responsiveness of seed germination to alleopathic stress in this study might be due to relatively low concentration or possible resistance exhibited by wheat seeds to potential allelopathic activity of sugarcane.

Mean germination time (MGT) is another important parameter which determines the energy of germination capacity of seeds in a stressed environment (Bonciarelli, 1995). MGT of target plants in a particular allelopathic assay may either be increased or decreased, depending on the concentration and type of allelopathic compounds. Phenolic compounds, in general, have been sought to prolong germination time as they may possibly interfere with seed dormancy and enzymatic activity necessary for rapid germinability. In this study, MGT was significantly reduced by root and peel extracts at 10 g/l concentration but prolonged under leaves extract. This suggest that root and peels extracts of sugarcane have some potent alleochemicals with stimulatory effect on germination time. On the other hand, leaves extract may possibly possess phenolics which had detrimental effect on this parameter.

Previously, Sampietro & Vattuone (2006) isolated phenolic compounds from straw of sugarcane which had deteriorating effects on germination and general growth of different weeds and crops.

Shoot and seminal root growth were significantly increased by root and stem peels extract at higher concentration which demonstrated stimulatory allelopathy of sugarcane. We assume that root and peel extracts of sugarcane may contain carbohydrates complexes and unknown diffusible allelochemicals which induced stimulatory response in wheat seedling with consequent increase in shoot and seminal root length. However, leaf extract showed detrimental effects on the lengths of shoot and seminal root. Previously stimulatory effect of rice hull extracts (Seyyednejad et al., 2010) but inhibitory effect of *Jatropha curcas* L. (Aburge and Sam, 2010), *Dodonaea viscosa* (Barkatullah et al., 2010) and oleander and walnut leaf extracts (Unal, 2013) on shoot length of different plants including wheat have been reported. Similarly, our results regarding reduced shoot and seminal root length under leaf extracts are in line with findings of Batlang and Shusho (2007), Singh et al. (2009), Sadehgi et al. (2010) and Unal (2013) who reported shoot and seminal root suppression of wheat in response to aqueous extracts of different allelopathic plants. Primary effect of allelochemicals may probably be alteration in cell membrane permeability of the target plant which can cause secondary effects such as changes in water and mineral absorptions potentials, changes in pH, enzymatic alterations etc. thus causing either stimulatory or inhibitory effects (Barkosky et al. 2000; Gatti et al., 2010; Majeed et al., 2012). Allelopathic stress may either elevate the level or induce the inhibition of carbohydrates and protein contents of target plant which build up more proline content as stress indicator; consequently plant growth is either increased or reduced (Batish et al., 2007; Al-Johani et al., 2012).

Increase in dry biomass was observed under allelopathic effects of 10 g/l root and peel extract which are supported by the findings of Mubeen et al. (2012) on barley and Han et al. (2013) on lettuce who documented increase in dry biomass in response to alleopathic aqueous extracts of diverse plants. Decrease in biomass of seedlings treated with leaf extracts in this study are in agreement with Singh et al. (2005), Jamil et al. (2009), Ullah et al. (2013) and Jabeen et al. (2013) who reported suppression in fresh and dry biomass of seedling in *Brassica* sp., wild oat and wheat under the influence of allelopathy of some weeds and crops. Differences in results of different studies may be due to different experimental conditions, extract concentrations and test plants they used. The increase in dry biomass of wheat seedlings treated with root and stem peel extract in the current study may probably be due to increase in photosynthetic rate and greater accumulation of photo-assimilate in the shoot, increase in thickness and length of seminal root, decrease in proline content and greater potassium ion uptake induced by allelopathic extracts of *S. officinale*; as in earlier studies some authors argued that botanical extracts could have positive influence on these phenomena thus stimulating biomass of the receptor plant (Rizvi and Rizvi, 1992; Gatti et al., 2010; Ibrahim et al., 2013). Similarly, reduced biomass, seminal root and shoot length under leaf extract suggests the presence of phenolic and growth inhibitory compounds in leaves of sugarcane and these reductions may be regarded as a result of induced oxidative damage and activation of cellular anti- oxidant systems which in turn changed permeability patterns of cell membrane, irregularities in mineral and ion uptake and the activation of stress enzymes (Oracz et al., 2007; Khaliq et al., 2012).

5 CONCLUSION

The present study suggests that different plant parts of sugarcane exhibit both stimulatory and inhibitory allelopathy against wheat in germination bioassay. Roots and stem peel extracts at higher concentration promoted germination time, shoot and seminal root growth and dry biomass of seedling while leaf extract had negative effects on these parameters. Pot culture study is underway to confirm our lab bioassay findings.

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Assessment of heritability and genetic advance for agronomic traits in durum wheat (*Triticum durum* Desf.)

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ABSTRACT

In order to evaluate the amount of heritability for desirable agronomic characteristics and the genetic progress associated with grain yield of durum wheat (*Triticum durum* Desf.), a split plot experiment was carried out with four replications during three cropping seasons (2009-2012). Three sowing dates (as environmental factor) and six durum wheat varieties (as genotypic factor) were considered as main and sub factors respectively. Analysis of variance showed interaction effects between genotypes and environments in days to ripening, plant height, spike length, number of grains per spike, number of spikes per unit area, grain mass and grain yield. The grain yield showed the highest positive correlation with number of grains per spike also grain mass (91 % and 85 %, respectively). A relatively high heritability of these traits (82.1 % and 82.2 %, respectively) suggests that their genetic improvement is possible. The maximum genetic gain (19.6 %) was observed for grain mass, indicating this trait should be a very important indicator for durum wheat breeders, although the climatic effects should not be ignored.

Key words: durum wheat; grain yield; plant genetics; yield components

IZVLEČEK

OVREDNOTENJE DEDNOSTI IN GENETSKE PREDNOSTI AGRONOMSKIH LASTNOSTI TRDE PŠENICE (*Triticum durum* Desf.)

Z namenom ovrednotenja dednosti željenih agrononmskih lastnosti in genetskih procesov povezanih s pridelkom zrnja trde pšenice (*Triticum durum* Desf.) je bil izveden poskus z deljenkami s štirimi ponovitvami v rastnih sezonah 2009-2012. Tri datumi setve kot okoljski dejavniki in šest sort trde pšenice kot genetski dejavnik so bili uporabljeni kot glavni in podrejeni dejavniki. Analiza variance je pokazala interakcijske učinke med genotipi in okoljem v dnevih do zrelosti, višini rastlin, dolžini klasov, številu zrna na klas, številu klasov na enoto površine, masi zrn in pridelku zrnja. Pridelek zrnja je pokazal največjo pozitivno korelacijo s številom zrn na klas in maso zrnja, 91 % in 85 %. Relativno velika dednost teh lastnosti, 82.1 % in 82.2 % nakazuje, da je možno njuno genetsko izboljšanje. Največja genetska pridobitev (19.6 %) je bila opažena pri masi zrnja, kar nakazuje, da bi morala biti ta lastnost zelo pomemben kazalnik za žlahtnitelje trde pšenice, čeprav tudi podnebni dejavniki ne bi smeli biti zanemarjeni.

Ključne besede: trda pšenica; pridelek zrnja; rastlinska genetika; komponente pridelka

1 INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is cultivated on 21 million hectares, about 10 % of all cultivated areas in the world. Durum wheat is an important and popular crop in the Mediterranean region and is used for food products as couscous, bulgur, and pasta (Gisslen, 2001). Durum wheat genotypes had shown better adaptation to varying environments than common wheat (*T. aestivum* L.) (Khazaei et al., 2013). So, selection of the stable durum wheat genotypes for achieving both high grain yield and good quality is very important. It is important to use appropriate selection method and selection intensity for traits of interest, proper statistical assessment of genetic variation, the magnitude of heritability (usually represented by h^2), genetic coefficient of variation, and response to selection.

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Genetic variability is an important factor in hybridization program for producing high yielding progenies. The effective selection depends on the amount of genetic variability and amount of heritability indices (Heidari, 2010).

For having a good response to selection, high genetic variation and high heritability are needed (Shukla et al., 2006). There is a direct relationship between heritability and response to selection which is referred to as genetic progress. The expectation of a response to selection is called genetic advance (G.A.). High genetic advance coupled with high heritability estimate offers the best effective condition for selection (Larik et al., 2000). Therefore, genetic advance is an important indicator associated with selection that aids plant breeder in his work (Shukla et al., 2006; Memon et al., 2005). High genetic variation for traits under selection as well as high heritability, are crucial for having good response to selection (Shukla et al., 2006). Manal (2009) reported high heritability accompanied by high genetic advance for spike length and 1000 grain-mass in his study of heritability and genetic advance of yield traits in common wheat (*T. aestivum*) under drought condition. This fact suggests that selection should lead to a fast genetic improvement of trait. The purpose of this study was to identify the traits which can be used as selection markers and can also help to predict the grain yield of durum wheat.

2 MATERIALS AND METHODS

The experiments were conducted during three crop seasons (2009-2012), at the experimental field of the Islamic Azad University, Qaemshahr Branch, Mazandaran Province of Iran (36 ° 30 ′ N, 52 ° 48 ′ E, 28 m above sea level). The experiments were designed as split-plots based on randomized complete block design with four replicates. Three sowing dates (as environmental conditions) were 25 October, 25 November and 25 December and were randomized as main plots. Six durum wheat genotypes ('Yavaros', 'Tarro-3', 'Shwa/Mald', 'Stork', 'Behrang' and 'Syrian-4') from different origin (CYMMIT and ICARDA) were used as subplots.

Each plot included fifteen rows 5 m long and 0.18 m apart. The seed rate was 500 viable seeds per one square meter. Based on soil test, urea fertilizer (46, 0, 0; N. P. K.) as source of nitrogen, and triple superphosphate fertilizer (0, 46, 0; N. P. K.) as source of phosphorus were used. Herbicide, fungicide and insecticide were used as usual. The area of 3 square meters was harvested to estimate grain yield and related traits (including the number of days to ripening, plant height, spike length, number of grains per spike, number of spikes per square meter and grain mass) at when plants were mature.

Analysis of variance and combined analysis of variance (Steel and Torrie, 1980) were conducted on data by using the statistical SAS program (SAS Institute, 2008), in according to following statistical model. Years with random effect as well as treatments and sowing dates with fixed effects had considered in this model (Yazdi Samadi et al., 1997). Mean comparisons by using Duncan's Multiple Range Test.

$X_{ijkl} = \mu + G_i + B_{ijk} + D_k + Y_l + GD_{ik} + GY_{il} + DY_{kl} + GDY_{ikl} + E_{ijkl}$ (Steel and Torrie, 1980)

Where, X_{ijkl} is the amount of each trait in a plot. The mean of trait, the effect of genotype, the effect of repetition, the effect of sowing date, the effect of binary interaction (respectively, including the effect of genotype × sowing date, genotype × year, planting date × year), the effect of triplet interaction (genotype × sowing date × year) and residual effect or experimental error, have been shown from left to right, respectively.

Phenotypic coefficient of variation (δ^2_p) depends on genetic variation (δ^2_g), environmental variation (δ^2_e) and their interactions.

$\delta^2_p = \delta^2_g + \delta^2_{gy} + \delta^2_{gd} + \delta^2_{gyd} + \delta^2_e$

$\delta^2_{gd} = (M5 – M6 – M7 + M8) / rdy$

$\delta^2_{gy} = (M7 – M8 + M9) / rd$

$\delta^2_{gd} = (M7 - M8 + M9) / ry$

$\delta^2_{gyd} = (M9 - M8) / r$

$\delta^2_e = M9$

Also the following formulas are used for calculating the coefficients of variation (Burton, 1952).

$G.CV. = (\sqrt{\delta^2_g} / \bar{Y}) \times 100$

$P.CV. = (\sqrt{\delta^2_p} / \bar{Y}) \times 100$

$E.CV. = (\sqrt{\delta^2_e} / \bar{Y}) \times 100$

P.C.V.; G.C.V. and E.C.V., were coefficient of variation of phenotypes, genotypes and environments, respectively. Also, $\bar{Y}$ was considered as a mean of trait. The following formula was used to predict the amount of broad sense heritability (h^2_b) (Marwede et al., 2004).

$h^2_b = (\delta^2_g / [(\delta^2_g + (\delta^2_e / ry) + (\delta^2_{gy} / y)]$

where: g, r, y, d, δ^2_g, δ^2_e, $\delta^2_{g\times e}$, δ^2_p and h^2_b refer to the number of genotypes, number of repetitions, number of years of experiment, genotypic variance, environmental variance, the variance of genotype-environment interaction, phenotypic variance and broad sense heritability, respectively. Amount of g, r, y and d was 6, 4, 3 and 3, respectively. The amount of variance (δ^2) was obtained base on mathematical expectancy factors (Table 1).

The following formula was used to estimate the average genetic improvement (Johnson et al., 1955).

Genetic gain $(G.G.) = K \times \delta_p \times h^2_b$

In this study, selection intensity was calculated at 5 % level.

K = 2.06 (Allard, 1960)

Also following formula was used to estimating the genetic advance of traits

$G.A.\ \% = (G.A. / \bar{Y}) \times 100$

3 RESULTS AND DISCUSSION

The analysis of variance (Table 1) showed a substantial variation in grain yield for years, sowing dates, and genotypes. A significant interaction effect was found between sowing date and amount of traits (P ≤ 1 %). This result confirms the reports of Rahman et al. (2009). Also a significant interaction was established between genotypes and environment effects. It shows that, in addition to genotypic factor (genetic structure), environmental factors influenced the studied traits too. So, farm management has to consider the date set of sowing for each durum wheat genotype (Table 1).

Table 1: Combined analysis of variance for some durum wheat traits

Source of variation	D.F.		Mean Square						
			Days to ripening	Plant height (cm)	Length of spike (cm)	Grains per Spike	Spikes per m^2	Mass of 1000 grains (g)	Grain yield (1000 kg ha^{-1})
Sowing date(D)	2	M1	41586.77**	1714.66**	14.78**	191.77**	10924.3**	350.93**	19.21**
Year (Y)	2	M2	0.02^{ns}	0.16^{ns}	99.11**	5.87**	1804.8**	0.23*	0.03^{ns}
Y×D	4	M3	0.06^{ns}	0.57^{ns}	0.41^{ns}	1.90^{ns}	115.1**	0.05^{ns}	0.01^{ns}
R(Y×D)	27	M4	0.03^{ns}	0.56^{ns}	0.04^{ns}	1.16^{ns}	10.1^{ns}	0.08^{ns}	0.02^{ns}
Cultivars (G)	5	M5	29.97**	1818.82**	8.22**	441.05**	6938.0*	474.99**	26.66**
G×D	10	M6	18.11**	114.43**	0.28**	16.95**	2059.8**	20.19**	0.65**
G×Y	10	M7	0.05*	1.14^{ns}	1.79**	1.52^{ns}	227.0**	0.18**	0.02^{ns}
G×Y×D	20	M8	0.05*	0.83^{ns}	0.25**	1.57*	136.7**	0.14**	0.02^{ns}
Error	90	M9	0.02	0.66	0.09	0.95	8.0	0.06	0.01

ns = non-significant, * significant in 5 %, ** significant in 1 %

The grain yield amount in early sowing (25 October) was 3.8 % ((4225 - 4062) / 4225) as well as in late sowing (25 December) was 21.8 % ((4225 - 3302) / 4225) lower than in optimum sowing date (25 November), according to data in table 2.

Table 2: Mean comparisons of effect of sowing dates on grain yield of wheat varieties

Sowing date	Grain yield (kg h^{-1})							
	Yavaros	Tarro-3	Shwa/Mald	Stork	Behrang	Syrian-4	Zardak	Average
25 October	3997b	4099a	3924a	5172a	5083b	3833b	2324b	4062^b
25 November	4353a	4269a	3772a	5163a	5315a	4149a	2553a	4225^a
25 December	3066c	3743b	3293b	3654b	4414c	3113c	1833c	3302^c

a, b, c Means within columns indicated by the same letter are not significantly different at P ≤ 0.01 according to Duncan's multiple range test.

Previous studies confirmed the importance of considering appropriate sowing date in each region (Rahman et al., 2009). The management should choose the optimal sowing date because it is crucial in order to optimize grain yield in such an environment (Radmehr et al., 2003 and Turner, 2004).

Bassu et al. (2009) and Bannayan et al. (2013) reported increasing of yield in early sowing and also its reduction in late sowing. One of the advantages of late sowing is the reduction of frost risk at anthesis. However the delay in sowing does not only affect the yield but also other aspects of growth and development of wheat, as well as yield components. It is generally related to reduced grain mass, number of grains per spike, number of spikes per plant, number of spikes per unit area, harvest index, and leaf area index (Radmehr et al., 2003). Accurate knowledge of the sowing time of any particular variety at a particular location is critical to achieve high grain yield (Andarzian, 2015). The highest and the lowest coefficients of phenotypic variation were determined for the number of spikes (84 %) and the number of days to grain maturity (0.4 %), respectively; while the highest heritabilities were calculated for grain mass, number of grains per spike and plant height (87.7 %, 82.2 % and 82.1 %, respectively) (Table 3).

Table 3: Components of genotypic variation, phenotypic variation, heritability and genetic advance for studied traits

Characters	Range	Mean±SD	GCV (%)	PCV (%)	δ^2_g	δ^2_p	h^2_b (%)	Genetic Advance (%)	Genetic Gain (%)
Days to ripening	192.0-242.0	218.5±18.2	0.2	0.4	0.3	0.8	38.3	0.7	0.3
Plant height (cm)	77.2-115.0	92.22±8.12	7.4	8.2	47.3	57.6	82.1	12.8	13.9
Length of spike (cm)	5.2-10.6	7.7±1.2	5.5	8.8	0.2	0.4	39.1	0.5	7.1
Grains per spike	17.1-35.61	26.8±3.8	12.8	14.1	11.7	14.3	82.2	1.5	5.6
Spikes in m^2	362.3-461.5	407.2±19.6	32.6	84.0	132.9	342.3	38.8	14.7	3.6
Mass of 1000 grains (g)	23.0-40.7	34.8±3.9	10.2	10.8	12.6	14.3	87.7	6.8	19.6

SD: standard deviation; GCV: genetic coefficient of variation; PCV: phenotypic coefficient of variation; δ^2_g = genotypic variance; δ^2_p = phenotypic variance; h^2_b = broad sense heritability

Coefficients of correlation were calculated using the method of Snedecor and Cochran (1989) with n-2 degrees of freedom at 5 and 1 percent levels. The highest positive correlation coefficient were determined between grain yield and number of grains per spike, grain mass, number of spikes per unit area and days to ripening (Table 4). Also, a significant negative correlation was found between plant height and grain yield and thus confirming the finding of Siahpoosh et al. (2003) and Rashidi et al. (2013) reports. They also reported a positive correlation between plant height and grain yield in some of their investigations. The plant height, therefore, need not to be considered as an indicator for selection of genotypes with high grain yield. Statistical analysis for agronomic traits in durum wheat showed that grain yield depended on the number of grains per spike and grain mass (r = 0.91** and r = 0.85**, respectively) (Table 4).

Table 4: Correlation coefficient of studied durum wheat traits

	Days to ripening	Plant height (cm)	Length of spike (cm)	Grains per spike	Spikes per m^2	Mass of 1000 grains (g)
Days to ripening	1					
Plant height	0.58**	1				
Length of spike	0.15**	0.55**	1			
Grains per spike	0.14**	-0.47**	-0.08^{ns}	1		
Spikes in m^2	0.30**	-0.17**	0.22**	0.36**	1	
Mass of 1000 grains	0.39**	-0.42**	-0.03^{ns}	0.63**	0.40**	1
Grain yield	0.34**	-0.52**	-0.01^{ns}	0.91**	0.57**	0.85**

ns = non-significant, ** significant in 1 %
+ , - : positive and negative correlation

The results of this study conforms Evans (1993), Satorre and Slafer (1999) reports. They reported that the increase of number of grains per spike is the most important indicator for high grain yield. In this way, this trait can be considered as a reliable indicator for selection of genotypes with higher grain yield. High grain mass is also an important indicator in selection of superior genotypes. Therefore, selection of genotypes based on a higher number of grains per spike and higher grain mass will improve the selection efficiency for more grain yield.

4 CONCLUSIONS

High and significant positive correlation between grain yield and number of grains per spike, and grain mass in durum wheat suggests that these traits can be considered as two important factors in selection for genotypes with higher grain yield. Because of high heritability of these traits, genetic improvement of grain yield in durum wheat is possible. The maximum genetic gain (19.6%) determined for grain mass suggests that this trait can be a very important indicator in durum wheat breeding, although the environment effects should not be ignored.

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Evaluation of an organic package of practice towards integrated management of *Solanum tuberosum* and its comparison with conventional farming in terms of yield, quality, energy efficiency and economics

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ABSTRACT

A study was taken up during 2014-16 for evaluating the potential of an organic package of practice towards integrated crop production (green farming) in comparison to conventional farmers' practice in West Bengal, India. Under green farming, compost was integrated with chemical fertilizer for soil management while organic plant/ pest management was undertaken utilizing Inhana Rational Farming (IRF) Technology. The study indicated higher yield (9.7 %), higher nutrient use efficiency and economic sustainability under green farming irrespective of study area or potato variety. Higher qualitative expression in terms of starch content, pulp pH, vitamin C etc. under green farming might be due to the organic plant management aimed at energization of plant biochemical functions. Soil quality development as noted under green farming might have been influenced by the on-farm produced compost containing rich self- generated micro flora (in order of 10^{16} per colony forming unit.). The study indicated that green farming may serve as an efficient substitute of conventional farming towards yield sustenance, abatement of food toxicity and quality end product; through higher use of renewable energy and activation of plant physiological functions.

Key words: green farming; organic plant management; energy use efficiency; soil quality; potato

IZVLEČEK

OVREDNOTENJE NABORA EKOLOŠKIH POSTOPKOV V INTEGRIRANI PRIDELAVI KROMPIRJA (*Solanum tuberosum* L.) V PRIMERJAVI S KONVENCIONALNO GLEDE NA PRIDELEK, KAKOVOST, ENERGETSKO UČINKOVITOST IN EKONOMIČNOST

Za ovrednotenje nabora praks ekološkega kmetovanja v integrirani pridelavi ("zeleno kmetovanje") v primerjavi s konvencionalno je bila v sezonah 2014-16 opravljena raziskava v Zahodni Bengaliji, Indija. Za pripravo tal je bil pri integrirani pridelavi uporabljen kompost skupaj z mineralnimi gnojili, pri ekološkem kmetovanju sta oskrba rastlin in nadzor škodljivcev sledila "Inhana" tehniki umnega kmetovanja (IRF). Raziskava je pokazala, da so bili večji pridelek (9.7 %), večja učinkovitost izrabe hranil in večja ekonomska vzdržnost doseženi pri integrirani pridelavi ne glede na mesto raziskave in sorto krompirja. Večje vrednosti kakovostnih kazalcev kot so vsebnost škroba, vitamina C, pH int. pri integrirani pridelavi bi lahko bile posledica ekološke obravnave rastlin, ki je ugodno vplivala na biokemične procese v rastlinah. Izboljšanje kakovosti tal, ki je bilo opaženo pri integrirani pridelavi, bi lahko bilo posledica uporabe na kmetijah pridelanega komposta, ki je vseboval bogato, samovzniklo mikrofloro (10^{16} na enoto kolonije). Raziskava je pokazala, da lahko integrirana pridelava služi kot učinkovit nadomestek konvencionalnemu kmetovanju glede stalnosti pridelka, preprečevanja zastrupitve hrane in kakovosti končnih proizvodov preko večje uporabe obnovljive energije in vzpodbujanja fizioloških procesov v rastlinah.

Ključne besede: integrirana pridelava; ekološka oskrba rastlin; učinkovitost izrabe energije; kakovost tal; krompir

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1 INTRODUCTION

Potato (*Solanum tuberosum* L.) has emerged as fourth most important food crop in India after rice, wheat and maize (Ojha & Saha, 2014); sharing 20.32 million ha area with an average production of 46.61 million metric tons. However, with the changing climatic patterns and declining soil productivity indications are ripe that use of only chemical fertilizers cannot ensure sustained production of the crop (Mollah et al., 2011). At the same time producing adequate quantity of good quality organic manure to support crop nutrition is a challenging task under large scale organic farming and practically unviable for resource poor farmers.

Integration of organic manure and inorganic sources of nutrient is necessary for sustainable agriculture as it not only provides greater stability in production, but also helps in restoration and maintenance of soil fertility (Nambiar, 1998). Considering that synthetic compounds especially pesticides have detrimental impact on soil and surrounding ecology; reducing dependency on fertilizers and adopting alternative means for control of pest/disease will help to control over the issues like residual toxicity, ecological imbalance, destruction of soil micro/macro fauna, etc..

Inhana Rational Farming (IRF) Technology, an organic package of practice developed by an Indian Scientist, Dr. P. Das Biswas, has been widely adopted in reputed organic tea estates in India and has shown its effectiveness towards both yield sustenance and soil quality development (Barik et al., 2014b; Chatterjee et al., 2014 and Bera et al., 2014a). Simultaneously conventional tea estates have adopted the technology towards reduction of chemical/ pesticide load and management of recurrent disease problems. In the agriculture sector, the technology has been successfully used for organic production of a variety of crops like paddy, baby corn, green-gram, cabbage, okra, tomato and black-gram, etc. In the present study, the potential of IRF Technology was evaluated towards integrated potato cultivation. Here integrated crop management has been termed as green farming as synthetic inputs for plant application that are the major cause of health hazards and disfunctional ecology; could be completely avoided through adoption of IRF plant management schedule. Performance in terms of crop productivity, quality, energy consumption and economics was adjudged in comparison to conventional crop management.

2 MATERIALS AND METHODS

2.1 Study area

The present study was undertaken during the crop season of 2014- 15 and 2015- 16 at two pilot sites. Pilot Area I- Mathurapur village of North 24 Paraganas district (Figure 1) and Pilot Area II- Bhabanipur village of Nadia district (state West Bengal, India). At initiation, farmers' meetings were conducted to select progressive volunteer farmers (Figure 2). Local governing bodies also extended their support in terms of infrastructure and other necessary components that were required for successful completion of the study.

Figure 1: Project farmer in green farming plot in Pilot area- II.

Figure 2: Prof. Kajal Sengupta (Head, Dept. of Agronomy, BCKV, State Agricultural University) and the Project farmers in the study area.

2.2 The hypothesis

Organic soil management is necessary for restoring soil health, which is pre-requisite for sustenance of yield. But it will take time until the desired developments are obtained, considering that resource scarcity forms the major bottle neck towards its adoption in wider scale. In this aspect adoption of organic plant managements towards re-activation of bio-chemical functions can directly compliment plant health while indirectly ensuring lesser pest interference; as pest starve on healthy plants (Paull, 2007).According to the trophobiosis theory of F. Chaboussou (Chaboussou, 2004), pest and disease set in with increase in free amino acids and reducing sugars pools in plant cell sap. The phenomenon usually occurring whenever plant metabolism becomes impaired due to biotic and abiotic factors, excess fertilization specially with nitrogenous fertilizers and application of systemic fungicides/pesticides etc. (Dias, 2012).Hence, better plant health *vis-a-vis* lesser pest incidence along with supportive soil functions can ensure the crop objective and economic balance in agriculture (Figure 3).

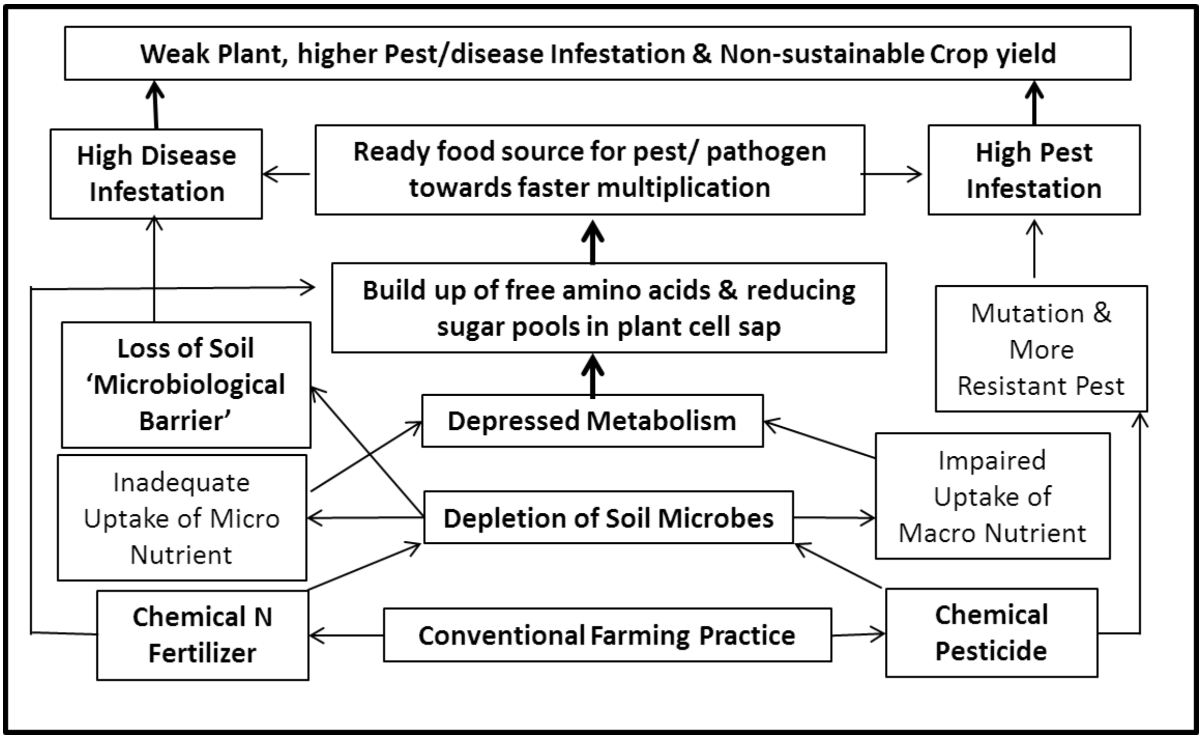

Figure 3: Flow diagram of plant - pest/disease relationship under conventional crop management as per Trophobiosis Theory of F. Chaboussou

2.3 Inhana Rational Farming Technology

Inhana Rational Farming (IRF) Technology developed by an Indian scientist (Dr. P. Das Biswas), associated with organic research for the last 15 years; has a holistic approach where interrelated and integrated relationships of all components of the ecosystem i.e. soil, plant and the surrounding environment are taken into consideration. It provides the right environment for all the components, be it a plant or soil, which leads to ecological improvement thereby ensuring economic sustainability (Barik et al., 2014a). The technology strives towards (i) energization of soil system i.e., to enable soil to function as an effective growth medium for plants and (ii) energization of the plant system i.e., to enable efficient extraction, utilization and assimilation of nutrients and enhancement of biochemical and structural defense leading to activation of the plants' host defense mechanism (Bera et al., 2014a).

2.4 Cultivation details

Three potato (*Solanum tuberosum* L.*)* varieties 'Kufri Chandramukhi', 'Super – 6' and 'Kufri Jyoti' were taken for the study. The experiment was laid out in randomized block design (RBD) and potato varieties Super – 6 and Kufri Jyoti were used in pilot Area-I (Mathurapur village) while 'Kufri Chandramukhi' and 'Kufri Jyoti' were used in Pilot Area-II (Bhabanipur village) (Figure 1). Tubers were planted during 10^{th} to 20^{th} November (2014- 15) and harvested during 20^{th} February to 2^{nd} March (2015– 16). Planting was done at the rate of 750 kg tuber ha^{-1}. A distance of 60 cm from row to row and 25 cm from plant to plant was maintained. One seedling was planted in each hill. In the conventional crop management (CCM) plots, 1500 kg complex fertilizer (10: 26: 26), 375 kg urea and 225 kg muriate of potash (MOP) were applied per hectare as per recommended practice. In case of green farming plots, 15 t of Novcom compost and 375 kg complex fertilizer (10: 26: 26) were applied per hectare.

In the green farming plots, full dose of compost and half dose of complex fertilizer were applied during final land preparation. Remaining complex fertilizer (10: 26: 26) was applied at the side row and covered with soil about 30-35 days post planting i.e., at the time of earthing up, followed by irrigation at 12-15, 30-35, and 45-50 DAP. In CCM plot, two third of total complex fertilizer (10: 26: 26), full dose of MOP and one- half of urea were applied at the time of final land preparation. Remaining urea, complex fertilizer and irrigation were applied as in green farming plots. Different intercultural operations were done in all the plots to ensure normal growth of the crops.

In case of green farming potato seed tubers were treated with Inhana seed solution. 1.5 l solution was used for 750 kg of tubers, for planting one hectare area. Mancozeb (75 % WP) was used for tuber treatment, 2.5g/l water/kg tuber under CCM.

Prophylactic spray for disease control i.e., late blight was carried out in case of both treatments. In CCM plots two rounds of copper oxychloride (50 WDP) was sprayed, 1 l/ha 60 and 75 days after transplanting. Under green farming ten rounds of spraying were given from 2 to 3 leaf stage using various Inhana Solutions (Table 1 and 2), at 7 to 10 days interval.

All the recommended cultural practices were adopted during the course of experimentation. The crop haulm was cut after 100 days of planting. The treatments were harvested after ten days of haulm-cutting to allow tuber curing in the field.

Table 1: Solutions used under IRF Plant Management Package for Green Farming

Sl. No	Solution Name	Dose & Dilution	Growth stage (time of application)
1.	Seed treatment solution	1.5 litre for tuber for planting one hectare, diluted in 1500 litre water.	Tubers were kept for 15-20 minutes in the diluted solution in shade for 5-10 minutes, followed by planting in the field.
Post Sprouting Plant Management			
1.	IB (Ag) - 2 + IB (Ag) - 7	(1.5 l + 1.5 l)/ hectare	To be sprayed on plants, at 7th day post sprouting.
2.	IB (Ag) - 12 + IB (Ag) - 7	(1.5 l + 1.5 l)/ hectare	14th day post sprouting.
3.	IB (Ag) – 4	1.5 l / hectare	21st day post sprouting.
4.	IB (Ag) – 11	1.5 l / hectare	28th day post sprouting.
5.	IB (Ag) – 1	1.5 l / hectare	35th day post sprouting
6.	IB (Ag) - 2 + IB (Ag) -7 + IB (Ag) - 11	(1.5 l + 1.5 l +1.5 l)/ hectare	42nd day post sprouting
7.	IB (Ag) - 3	1.5 l / hectare	49th day post sprouting
8.	IB (Ag) - 12 + IB(Ag) - 7	(1.5 l + 1.5 l)/ hectare	56th day post sprouting.
9.	IB (Ag)- 2 + IB (Ag)- 7	1.5 l + 1.5 l)/ hectare	63rd day post sprouting.
10.	IB (Ag) – 10	1.5 l / hectare	70th day post sprouting.

Note: Details regarding Solutions under IRF Package of Practice has been given by Chatterjee et al., (2014), Barik et al., (2014a; 2014b) and Bera et al., (2014a)

Table 2: Ingredients used in Inhana solutions and their role in crop production

1. **Seedtuber treatment solution:** The solution is biologically activated and potentized extract of *Calotropis procera* R. and *Tinospora crispa* Miers. It plays a major role in initiation of metabolic resources during germination, faster independence of plants from the tuber reserve, photosynthesis enhancement and increased uptake of organic and inorganic solutes through roots.
2. **IB (Ag) - 1:** The solution is biologically activated and potentized extract of *Hyoscyamus niger* L., *Ficus benghalensis* L. and *Dendrocalamus strictus* Nees. It acts as an organic growth promoter, activator and regulator. It energizes and stimulates the plant system for best use of soil nutrients (both applied and stored). It also regulates every stage of the grand growth period influencing growth correlation.
3. **IB (Ag) - 2:** The solution is biologically activated and potentiated extract of *Ocimum sanctum* L., *Calotropis procera* R. and *Cynodon dactylon* (L.) Pers. It activates plants' host defense mechanism through silica management providing structural defense against fungal pathogens. It also stimulates plants' immune system by activating the biosynthesis of different phenolic compounds having fungi-toxic property.
4. **IB (Ag) - 3:** The solution is biologically activated and potentiated extract of *Adhatoda vasica* Nees., *Zingiber officinale* Roscoe and *Embelia ribes* Burm. F. It acts as an organic solution for potassium absorption and utilization. It increases the efficiency of potassium uptake through energized root capacity so that gradual reduction in application is ensured. At the same time it activates suction pressure by influencing diffusion pressure deficit.
5. **IB (Ag) – 4:** The solution is biologically activated and potentiated extract of *Calotropis procera* R., *Dendrocalamus strictus* Nees. and *Bombax malabaricum* D.C. It ensures direct biological absorption and utilization of atmospheric-N by plant. It also balances the quantity of nitrogen within plant system at the right time, thereby preventing deleterious effect on quality of the produce.
6. **IB(Ag) - 7:** The solution is biologically activated and potentiated extract of *Ocimum sanctum* L. It stimulates root function, activates root growth/ penetration and energizes soil in the root zone; thus improving soil-plant relationship. It helps to develop soil CEC, energizes the production of micro-flora and bio-flora around the root zone, improves base saturation to the desired level and enhances root cation exchange capacity. It stimulates root growth and penetration by activating contact exchange capacity of root.
7. **IB (Ag) – 10:** The solution is biologically activated and potentiated extract of *Costus speciosus* Sm. and *Tylophora indica* (Burm. f.) Merrill. It helps to improve plant transport by delivering essential substances for various internal functions.
8. **IB(Ag) - 11:** The solution is biological activated and potentiated extract of *Solanum xanthocarpum* Schrad. & Wendl. and *Aristolochia indica* L.. It helps to improve the movement of solutions by providing systemic presence to give structural integrity.
9. **IB (Ag) - 12**: The solution is biological activated and potentiated extract of *Sida cordifolia* Linn. and *Berberis asiatica* Roxb. ex. DC . It helps to improve plant's capacity for starch synthesis.

2.5 Research methodology

Compost for green farming plots was produced on-farm within a period of 21 days; from poultry litter and cow dung; using Novcom composting method. Detailed procedure was documented by Seal et al. (2015) while studying quality of Novcom compost from poultry litter. Twelve compost quality parameters were studied as per the procedure described by Seal et al. (2012). Final compost samples appeared dark brown in colour with earthy smell, deemed necessary for mature compost (Epstein, 1997). Average moisture in compost samples varied from 58.76 to 65.15 percent (Table 3). pH values of the Novcom (poultry litter) compost samples varied from 6.89 – 7.23. Organic carbon content in the compost samples varied from 22.64 to 28.14 percent, qualifying the criteria for field application (16 to 38 percent) as per the standard range (Evanylo, 2006). Total nutrient content in terms of total NPK in the compost sample varied from 4.06 to 4.71 percent. C/N ratio varied from 12:1 to 15:1, which was within the reference range of ≤ 20 (FAI, 2007) as suggested for well-matured compost; indicating the compost was mature and suitable for soil application. Microbial population in terms of bacteria, fungi and actinomycetes (expressed in $\log_{10}$ value) in mature compost was 14.373 to 15.237. Stability of compost was examined through study of CO_2 evolution rate of compost samples, which was more or less within the stipulated range (2.0 - 5.0 mg CO_2 – C/g OM/day) for stable compost (Trautmann & Krasny, 1997). The phytotoxicity bioassay test value (0.89) indicated absence of phytotoxic effect in the final compost as suggested by Trautmann & Krasny, 1997.

Table 3: Quality parameters of Novcom compost

Sl. No.	Parameter	**Novcom** (*Poultry Litter*) **Compost**		
		Range Value	Mean	± Std. E.
1.	Moisture percent (%)	58.76 – 65.15	62.10	± 1.02
2.	pH_{water} (1: 5)	6.89 – 7.23	7.05	± 0.08
3.	Electrical conductivity (dSm^{-1})	2.13 – 2.39	2.21	± 0.09
4.	Organic carbon (%)	22.64 – 28.14	25.87	± 1.10
6.	Total NPK (%)	4.06 – 4.71	4.13	± 0.21
7.	C/N ratio	12:1 – 15:1	13:1	± 0.43
8.	Total bacterial count[2]	15.032 -15.237	15.201	± 1.01
9.	Total fungal count[2]	14.373 – 15.047	15.043	± 0.65
10.	Total actinomycetes count[2]	14.573– 14.843	14.763	± 0.53
11.	CO_2 evolution rate (*mgCO_2 – C/g organic carbon/day)*	2.13 - 3.58	2.71	± 0.08
12.	Germination index *(phytotoxicity bioassay)*	0.83 – 0.96	0.89	± 0.01

[2]*Microbial count :colony forming unit c.f.u. per g moist compost expressed as Log_{10} value*

For measurement of agronomic parameters, ten plants per plot were selected at random, and marked for height measurement. Plant emergence was recorded at 30 DAP. Observations on plant height, number of shoots and leaves per plant were recorded at 60 DAP. Height of the tallest shoot was measured from the soil surface to the base of last leaf unfolded at 60 DAP. Number and mass of total tubers were recorded at harvest. In order to find out if there were significant differences among treatments; Duncan's Multiple Range Test (DMRT) was used for analyzing data collected on apparent growth (plant height, number of leaves) and yield parameters (tuber count and tuber mass). Pre and post experiment surface soil samples (0 – 25 cm) were collected and analysed for soil physico-chemical properties, nutrient content and biological properties following standard methodologies (Black, 1965; Jackson, 1973).

Soil Development Index (SDI) was calculated taking eleven soil quality parameters *viz.* pH, organic carbon, available N, P, K and S, soil MBC, soil-FDA, qMBC, qCO_2 and qFDA as per following equation.

Soil Development Index (SDI) (Bera et al. 2013a)

$$= \frac{a}{n^2} \left\{ \sum_{n=1}^{n} \frac{100(X_1)}{C_1} + \frac{100(X_2 - C_2)}{C_2} + \ldots\ldots + \frac{100(X_n - C_n)}{C_n} \right\}$$

Where X = Value of Individual Soil Quality Parameter after Experimentation, C = Value of Individual Soil Quality Parameters before Experimentation; a = no. of Soil Quality Parameters showing increase over initial value. In case of qCO_2, a modification [-1 x $(X_n-C_n)/C_n$] in the formula is taken, as decrease of qCO_2 is considered to be good for soil health.

Different physicochemical analyses were done for determining potato quality. Specific gravity was determined by standard water displacement method (Raj et al., 2011). Starch and pH content was assessed as per method of Widmann et al., 2008 and Feltran et al., (2004) respectively. Titratable acidity and vitamin C were adjudged using the methods of Sadasivam & Manickam, 2011.

Energy equivalence of inputs and output was sourced from Khan & Hossain (2007); Zangeneh et al. (2010) and Zahedi et al. (2014). Energy use efficiency, energy productivity, specific energy, energy intensiveness and net energy were calculated as per the methodology of Banaeian et al. (2010) and Ghorbani et al. (2011).The economics of various treatments was also worked out following standard techniques (Gomez & Gomez, 1984).

3 RESULTS AND DISCUSSION

3.1 Growth and yield attributes

Emergence percent varied within 93.3 and 98.8 per cent in CCM plots and 96.3 to 99.8 in green farming plots (Table 4). Slightly higher (non significant difference) emergence in case of the latter may influence the sprouting potential of potato tubers. Planting of well sprouted healthy seed tubers, provided favourable condition for emergence (Bhatia et al., 1992).

Plant height and number of compound leaves per plant at 60 days of crop growth varied significantly under the studied farming systems. Maximum (average) plant height of 56.2 and 57.1 cm with a maximum (average) 56.6 and 59.3 leaves per plant under green farming was well above the values (49.6 and 50.7 cm plant height with 50.2 and 57.5 leaves per plant) as recorded for CCM plots. The results may indicate efficient plant metabolic functions and soil nutrient mineralization under organic plant management (using IRF Technology) and soil application of Novcom compost. Almost identical impact was documented by Barik et al. (2014a; 2014b) and Bera et al. (2014b), while working with other crops. Similar trend of results were also observed in case of tuber and number of tubers above processing grade (> 45 mm). As per Venkatasalam et al. (2012), addition of organic manure significantly influenced the beneficial microorganisms to colonize in rhizosphere and stimulate plant growth by providing necessary nutrients besides synthesizing some plant hormones; which may be the reason for increase in plant height, number of leaves and tuber mass, irrespective of potato variety.

Table 4: Growth attributes of potato under studied farming systems (as average of both seasons)

Treatment	Potato Variety	Emergence (%)	Plant height (cm)	No. of compound leaves /plant	No. of tuber (thousand/ha) Processing Grade (> 45 mm)	Total
Pilot Area I: Mathurapur village (district: North 24 Paraganas), West Bengal, India						
Conventional Crop Management (CCM)	KufriJyoti	93.3 – 96.8 (95.1^{c})[±4.91]	43.3 – 49.8 (45.5)[±2.53]	44.2 – 46.8 (44.6^{c})[±2.13]	294.69^{b}	539.7^{b}
	KufriChandramukhi	93.7 – 97.1 (95.6^{c})[±5.03]	46.7 – 54.1 (49.6)[±2.11]	48.3 – 54.4 (50.2^{b})[±2.37]	275.67^{c}	501.45^{c}
Green Farming utilizing IRF	KufriJyoti	97.5 – 99.8 (98.6^{a})[±4.13]	43.7 – 52.8 (48.1)[±2.05]	44.8 – 53.7 (50.3^{b})[±2.76]	325.53^{a}	586.74^{a}
	KufriChandramukhi	97.1 – 99.4 (97.7^{ab})[±4.11]	47.1 – 59.3 (56.2)[±2.09]	49.6 – 59.8 (56.6^{a})[±2.14]	287.25^{bc}	529.23^{b}
Pilot Area II: Bhabanipur village, (district: Nadia), West Bengal, India						
Conventional Crop Management (CCM)	KufriJyoti	94.3 – 98.8 (96.8^{ns})[±5.88]	44.3 – 51.8 (48.8^{d})[±2.09]	43.7 – 54.8 (47.5^{c})[±1.71]	263.27^{d}	494.44^{d}
	Super 6	95.2 – 98.4 (96.5^{ns})[±5.44]	49.3 – 53.8 (50.7^{c})[±2.01]	48.7 – 63.4 (57.5^{ab})[±2.23]	302.13^{b}	543.24^{b}
Green Farming utilizing IRF	KufriJyoti	96.5 – 99.2 (97.4^{ns})[±4.79]	46.2 – 59.5 (54.4^{b})[±2.12]	43.3 – 53.5 (46.5^{c})[±2.19]	285.46^{c}	530.13^{bc}
	Super 6	96.3 – 98.3 (96.3^{ns})[±4.75]	48.3 – 59.8 (57.1^{a})[±2.13]	50.4 – 65.3 (59.3^{a})[±2.12]	365.34^{a}	631.13^{a}

Note: Figure in parenthesis ( ) represents mean value & Standard Error [±]. The means marked with different letters in the same column were significantly different at P< 0.05 under Duncan's New Multiple Range Test.

Nutrient Use Efficiency (NUE) in terms of 'Partial Factor Productivity' of applied nutrients (PFP_{NPK}); were evaluated for both CCM and green farming plots. The advantage of PFP_{NPK} is that it quantifies total economic output from any particular factor/nutrient, relative to its utilization from all resources in the system, including indigenous soil nutrients and nutrients from applied inputs (Cassman et al., 1996). Decline in PFP_{NPK} may be attributed to nutrient imbalance, decline in indigenous soil-NPK supply, subsoil compaction, reduced root volume and increased incidence of pests and diseases (Karim & Ramasamy, 2000). Adoption of efficient NPK management practices is responsible for higher partial factor productivity (Yadav, 2003). The value was found to be significantly higher in green farming plots as compared to CCM (Table 5). 20.61 to 23.03 kg and 63.01 to 74.93 kg potato per kg NPK, were recorded under CCM and green farming plots respectively. Higher value in case of the later treatment may be attributed to adoption of organic plant management (using IRF).

Evaluation of grade wise tuber distribution revealed higher production of large (>75 g) and medium sized (50-75 g) tubers under green farming (13.6 and 25.5 % respectively). This may be due to cumulative effect of organic and inorganic fertilizers sources (Mandal et al., 2005) as well as organic plant management (Figure 4). Higher bulking of tubers under integrated soil management was corroborated with the finding of Sharma et al. (1996).

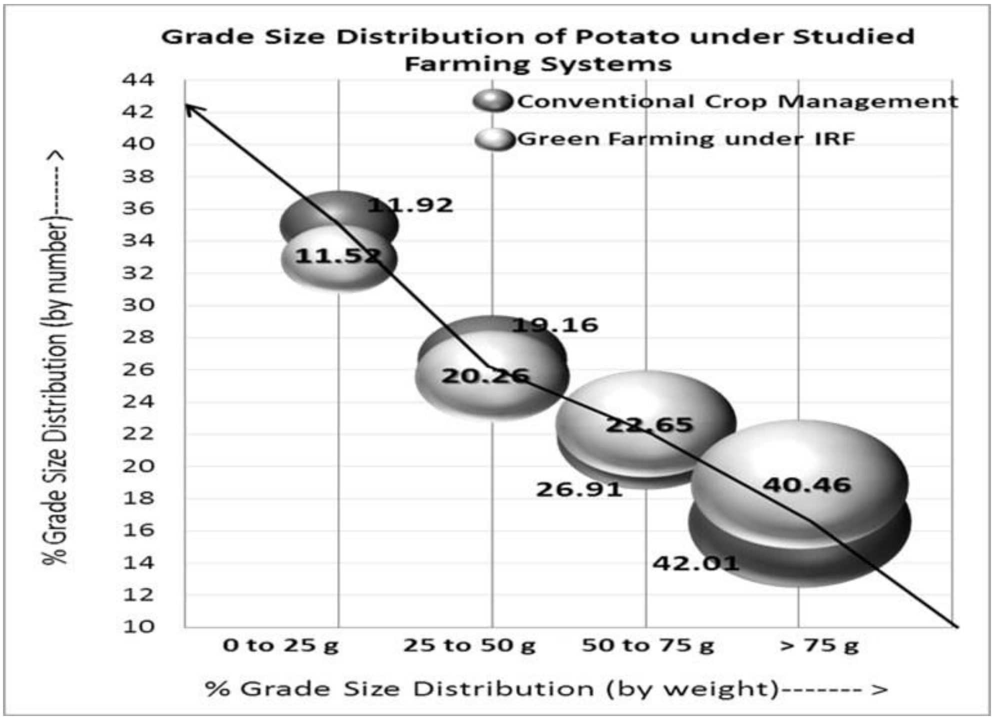

Figure 4: Grade wise distribution of potato tubers (number and mass) under CCM and Green Farming

Production of large sized tubers *vis-à-vis* higher yield would reflect efficient photosynthesis, phloem loading and translocation as well as synthesis of large molecular weight substances within storage organs (Singh, 1999). Hence, the observed phenomenon under green farming may indicate the positive influence of organic plant management (utilizing IRF) towards rapid bulking of tubers.

Maximum tuber yield was obtained from variety Super-6 (33.0 t/ha) followed by 'Kufri Jyoti' (30.00 t/ha) in green farming plots, at Pilot Area II and I respectively. In both the Pilot Areas minimum tuber yield was obtained in CCM plots irrespective of potato variety (Table 5). Mohapatra et al. (2008) and Kumar et al. (2011) also reported improvement in tuber yield of potato with application of organic manure. Hence better yield performance in the green farming plots may be attributed to the release of organic acids following compost application which led to proper nutrient mineralization (Kumar et al., 2011) *vis-à-vis* organic plant management, which enabled adequate nutrient uptake (Kumar et al., 2008).

Table 5: Nutrient Use Efficiency (NUE) and yield attributes under studied farming systems (as average of both seasons)

Treatment	Potato Variety	NUE* (kg/kg NPK)	Grade wise number [mass (kg) of the tubers/ha] 0 – 25 g	25 – 50 g	50 -75 g	> 75 g	Total tuber yield (t/ha)
Pilot Area I: Mathurapur village (district: North 24 Paraganas), West Bengal, India							
Conventional Crop Management (CCM)	KufriJyoti	22.42[c]	184577[bc] [3402]	141283[b] [5041]	113914[b] [7452]	99925[b] [11855]	27.75[b]
	KufriChandramukhi	21.21[c]	184478[bc] [3300]	135447[c] [5043]	105881[bc] [6668]	75644[d] [11239]	26.25[bc]
Green Farming utilizing IRF	KufriJyoti	68.12[a]	189001[a] [3457]	151320[a] [6549]	135021[a] [8543]	111398[a] [11451]	30.00[a]
	KufriChandramukhi	63.01[ab]	182950[d] [3396]	138214[bc] [5511]	118256[b] [7532]	89810[c] [11312]	27.75[b]
Pilot Area II: Bhabanipur village, (district: Nadia), West Bengal, India							
Conventional Crop Management (CCM)	Kufri Jyoti	20.61[d]	181900[bc] [3211]	134240[cd] [5221]	111017[d] [7219]	67283[d] [9849]	25.50[d]
	Super 6	23.03[c]	174445[d] [2927]	142003[b] [5352]	123804[b] [7718]	102987[b] [12503]	28.50[b]
Green Farming utilizing IRF	Kufri Jyoti	63.01[b]	183006[b] [3338]	138438[c] [5600]	117206[c] [7868]	91479[c] [10944]	27.75[bc]
	Super 6	74.93[a]	190115[a] [3401]	153617[a] [6331]	145917[a] [8918]	141480[a] [14350]	33.00[a]

**NUE was evaluated in terms of 'Partial Factor Productivity' of Applied Nutrient (PFP_{NPK})*
Note: The means marked with different letters in the same column were significantly different at P< 0.05 under Duncan's New Multiple Range Test.

Year wise comparison indicated an interesting pattern of crop yield under the studied farming systems (Figure 5). Under CCM, yield did not follow any consistent pattern during the two years study period. During 2nd year slightly lower yield was obtained under variety 'Jyoti', while just the opposite trend was recorded under varieties Chandramukhi and Super 6. In case of green farming however, yield increased in the 2nd year as compared to 1st year's performance; irrespective of experimental site and potato variety. During 2nd year while slightly lower (-1.79 percent) yield (on an average) was recorded under CCM, the same increased by about 7.16 percent, in case of green farming plots.

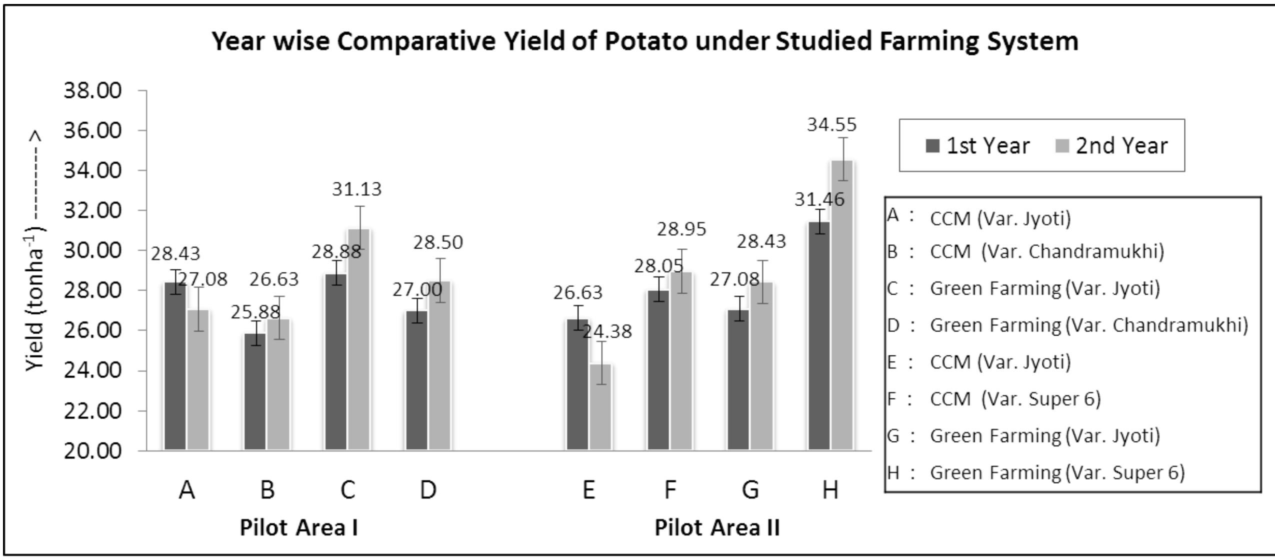

Figure 5: Comparative yield performance of potato under CCM and green farming

In case of green farming adoption of organic plant management (utilizing IRF) at different stages of plant growth; perhaps activated the plant metabolic functions leading to better nutrient uptake, assimilation, translocation and storage; finally culminating in higher tuber yield. Similar improvement in plant functioning under organic (IRF) plant management was documented in various other crops *viz.* tomato, black gram, green gram (Sengupta et al., 2011; Bera et al., 2014b; Barik et al., 2014b;) okra, cabbage (Barik et al., 2014a; 2014c) and rain-fed/ winter paddy (Mukhopadhyay et al., 2015).

3.2 Soil quality development

Variation in soil properties was studied before initiation of experiment and after harvest of potato tubers (Table 6). Analysis revealed that soil of all the experimental plots were slightly acidic in reaction, pH varying from 6.46 to 6.49. Electrical conductivity (EC), which reflects soil fertility (except in salt effected problematic soil) was found to increase in case of compost treated plots indicating steady nutrient mineralization. Increasing trend of organic carbon was noticed in green farming plots, where Novcom compost was applied during land preparation. Potato produces more dry matter than any other food crop and therefore, requires higher amount of nutrient inputs (Kushwah and Singh, 2011).The imbalanced and indiscriminate use of fertilizers in intensive cropping system without adequate restorative practices may pose threat towards sustainability of the system (Jatav et al., 2013). Soil fertility in terms of available - N, P_2O_5, K_2O and SO_4^- showed an increasing trend under both the studied farming systems.

Table 6: Change in soil quality under studied farming systems (as average of both seasons)

Treatments	Soil Physicochemical Properties						
	pH (1: 2.5)	EC (dSm^{-1})	Org.- C (%)	Av.- N	Av.- P_2O_5	Av.- K_2O	Av.- SO_4
				< ------------- $kgha^{-1}$ --------------- >			
T_1: Conventional Crop Management (CCM)	6.46^{*} (6.31)	0.034 (0.035)	0.75 (0.74)	354.21 (394.32^{**})	68.43 (73.45^{**})	241.06 (245.62^{*})	34.53 (35.67)
T_2: Green Farming utilizing IRF	6.49 (6.54)	0.031 (0.038^{**})	0.74 (0.79^{**})	357.69 (386.21^{**})	65.21 (68.79^{*})	237.51 (235.21)	36.32 (38.84^{*})
	Soil Biological Properties						
	MBC	SR	FDA	qMBC	qCO_2	qFDA	QR
T_1: Conventional Crop Management (CCM)	279.42 (271.31)	0.586 (0.623^{**})	26.31 (28.42)	3.73^{*} (3.67)	2.10 (2.30^{**})	0.351 (0.384^{**})	0.08 (0.09)
T_2: Green Farming utilizing IRF	278.16 (312.24^{**})	0.584 (0.591)	25.21 (39.23^{**})	3.76 (3.95^{**})	2.10^{**} (1.89)	0.341 (0.497^{**})	0.08 (0.08)

Note: MBC- Microbial Biomass Carbon (µg CO_2-C/g dry soil), SR- Soil Respiration, FDA- Fluorescein Di-acetate Hydrolysis (µg/g dry soil), qMBC- Microbial Quotient (%), qCO_2 – Microbial Metabolic Quotient, qFDA: Specific hydrolytic activity (%),QR- Soil Microbial Respiration quotient.
Figure in parenthesis indicate analytical values of post harvest soil samples taking average of the two seasons.
T – test for 2 dependent means (significant at $P<0.05$ and ** significant at $P<0.01$).*

Microbial activity is probably the most important factor that controls nutrient re-cycling in soil. Microorganisms participate in disintegration and decomposition processes leading to the release of nutrients trapped in plant, animal debris, rock and minerals; as well as synthesize and release hormones that are essential for plant growth (Gogoi et al., 2003). Anderson (2003) pointed out that the MBC/OC ratio and metabolic quotient (qCO_2) could be used as more sensitive indicators of soil microbial response to land use, soil management, and environmental variables. Microbial biomass carbon was found to increase under green farming, whereas slight reduction in value was noticed under CCM. Increase in soil respiration in green farming plots, might be due to increased microbial activity for utilization of added organic source. This was corroborated by enhanced FDA value, that represents overall enzymatic activity by soil microbes. Microbial quotient (qMBC) i.e., the ratio of C_{mic}/C_{org}, increased in green farming plots indicating increase in microbial activity. qCO_2 value was found to increase under CCM. In general, conventional agro-systems present higher values in comparison to organic or natural ecosystems; indicating comparatively more stressed conditions under the former (Dilly & Munch, 1998). Simultaneously qFDA (FDA per unit organic carbon) value of soil decreased in CCM plots. Decrease in qFDA under conventional practice, perhaps indicated stress on microbial community, thereby requiring higher energy for maintenance.

3.2.1 *Soil Development Index*

Soil Development Index (SDI) is a concept to quantify the extent of positive changes in different soil quality parameters for expression of overall soil development; to enable easy understanding of the end-users. (Bera et al., 2014a). Soil Development Index was noticeably higher under green farming plots (where Novcom compost was integrated with chemical fertilizer) as compared to CCM plots, receiving only chemical fertilizer (Fig. 6). The variation was especially significant in terms of soil biological properties, which was recordably higher in green farming plots as compared to plots receiving chemical fertilizers. Similar enhancement of soil quality under organic manure application was documented by Golabi et al. (2004), Gulshan et al. (2013), Das et al. (2013) and Bera et al. (2014).

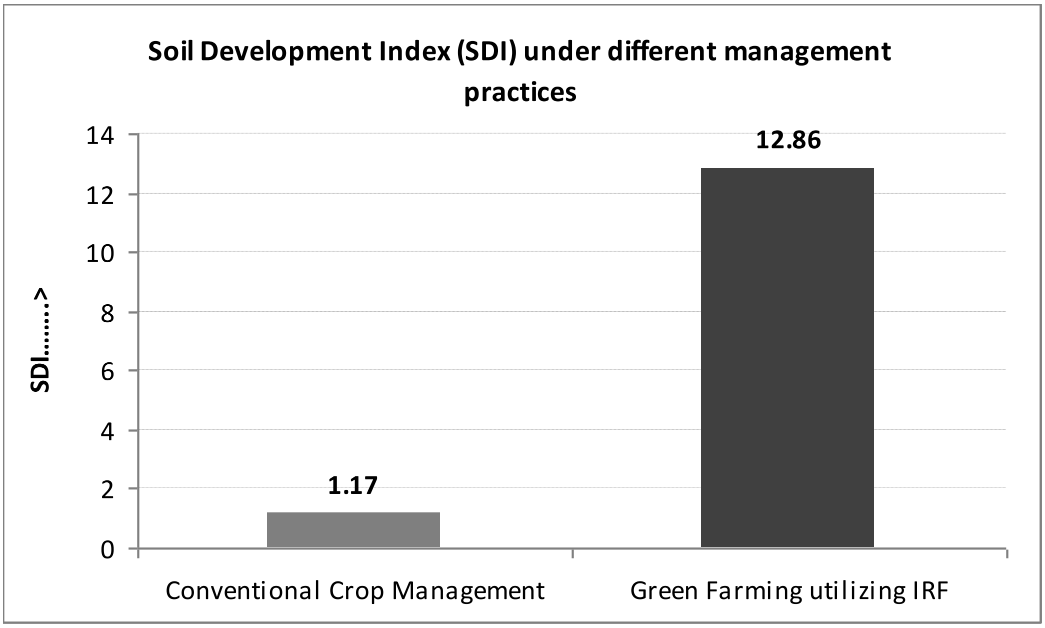

Figure 6: Soil quality development under CCM and green farming

3.3 Potato quality under studied farming systems

Analysis of different quality parameters of potato was done to assess qualitative differences in potato (if any) under varying management practice (Table 7). Mean specific gravity of potato was the highest (1.084) under green farming as compared to CCM (1.071). Specific gravity of potato positively correlated with dry matter and starch content, as was also noted by Marwaha (1997), Abbas et al. (2011) and Kaur & Agarwal (2014); but presented negative correlation with reducing sugar content (Iritani & Weller, 1974; Salamoni et al., 2000). Starch, comprising 65-80 % of dry matter content, is considered to be the main constituent of potato (Kadam et al., 1991a). Starch content (mean value 13.73 g / 100g tissue) was about 15.6 percent higher in case of potato grown under green farming as compared to those produced under CCM. Since starch comprises the largest part of dry matter, it has direct influence on technological quality, especially with regard to the texture of the processed products (Kadam et al., 1991b).

Table 7: Quality of potato grown under studied farming systems

Quality parameter	Conventional Crop Management (CCM)	Green Farming utilizing IRF
Specific gravity	1.045 – 1.112 (1.071 ± 0.012)	1.049 – 1.124 1.084** ± 0.011
Starch (g/ 100g tissue)	6.81 – 19.91 (11.87 ± 2.034)	7.66 – 22.56 (13.73** ± 2.061)
pH	5.99 - 6.10 (6.02 ± 0.016)	6.11 – 6.38 (6.17** ± 0.019)
Titratable acidity (%)	0.30 - 1.02 (0.51** ±0.115)	0.16 - 0.27 (0.23 ± 0.019)
Vitamin C (mg/ 100 mg tissue)	10.80 – 12.00 (11.40 ± 0.364)	12.00 - 14.40 (13.20** ± 0.318)

Figure in parenthesis indicate mean value and standard error, T – test for 2 independent means (* significant at P<0.05 and ** significant at P<0.01).

Factors that may interfere in a negative and/ or indirect way on the technological quality of tubers are pulp pH and total titratable acidity. The pH index determines deterioration potential by fermentation and the activity of enzymes (Cecchi, 1999).The phosphorylase enzyme acts predominantly on starch breakdown (Jadhav et al., 1991), with maximum activity at pH 5.5 (Iritani & Weller, 1973). In addition, pulp pH is variable and presents a negative correlation (-0.86) with reducing sugars accumulation (Iritani & Weller, 1973). Total acidity on the other hand quantifies organic acids present in foods and, in general, there is a tendency of reduction in their contents because of respiration and/or due to conversion into sugars (Chitarra & Chitarra, 1990), which could contribute toward browning of the fried product. Lower pH (6.02) value and higher titratable acidity (0.51 percent) in case of conventionally grown potato (as against those grown under green farming) indicated its inferior quality in terms of faster degradability and higher browning potential, when fried**.**

Potatoes are a steady reliable source of vitamin C (ascorbic acid) in human diet (FAO 2008).Although vitamin C is sensitive to heat, and breaks down to some degree on cooking, enough is still retained to make cooked potatoes a rich source of this nutrient. Vitamin C works as antioxidant which helps protect cells from free radical damage apart from its requirement for healthy skin, teeth, gums, muscles and bones. It also helps with the absorption of iron from plant foods, which is poorly available to the body otherwise. Vitamin C (13.20 mg/ 100 mg tissue) was about 15.7 percent higher in potatoes grown under green farming as compared to their conventional counterparts, which corroborated the findings of Hamouz et al. (1999), Hamouz et al. (2005) and Zarzyńska (2013). As per their observation ascorbic acid content in potato tubers was influenced by the method of cultivation; with higher levels in potatoes cultivated following ecological pathway i.e., without using any chemical protection as compared to those produced under conventional methods. According to Widmann et al. (2008) fertilizers used to increase potato growth represent an ecological risk because they penetrate potato's tissues, affect their metabolism and change the chemical composition of the tubers, reducing the content of their active principles. Therefore, potato grown under organic environment, tend to be qualitatively superior in comparison to its conventional counterpart.

3.4 Comparative energy use efficiency under studied farming systems

Conventional agriculture's energy inefficiency is directly tied to the high energy consumption of producing and transporting synthetic pesticides and fertilizers used to grow these crops. Whereas organic agriculture utilizes manure, legumes, and other natural sources of nitrogen, which replace the fossil fuels used for manufacturing synthetic nitrogen fertilizer; with natural biological process (Ziesemer, 2007).

Total energy input in the form of direct, indirect, renewable and non-renewable; are presented in Table 9. In case of green farming about 49 % of total energy input was in the form of direct energy, as compared to only 22 % in case of CCM plots.

Energy productivity or the potato output per unit energy application (Fig. 7) was more under green farming (1.26 kg MJ^{-1}) as compared to CCM (0.56 kg MJ^{-1}). This can be attributed to the differences in the level of technology and other characteristics of each treatment (Zangeneh et al., 2010). Specific energy was 55.6 percent less under green farming *vs*. CCM; indicating higher sustainability under the former. The result was well corroborated with 34410.09 MJ ha^{-1} excess gain in net energy under green farming as compared to CCM. Energy intensiveness was computed as energy consumed in crop production per unit market value of crop produced (Lockeretz et al., 1977). Lower energy intensiveness under green farming (0.09 $MJRs^{-1}$ *vs.* 0.21 $MJRs^{-1}$ under CCM) indicated towards the sustainable agriculture practice.

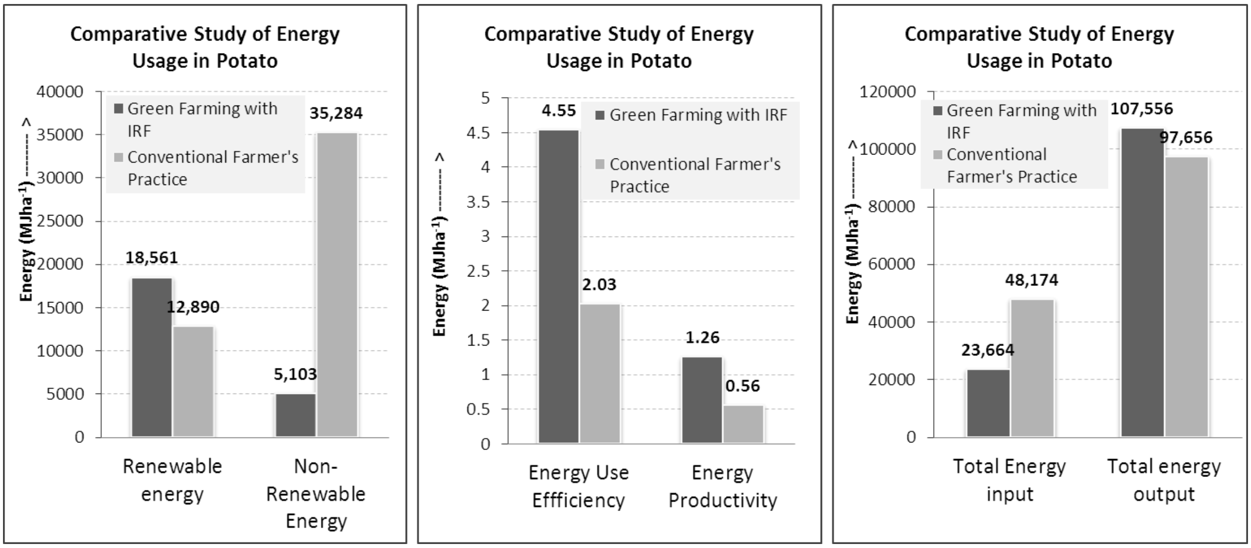

Figure 7: Comparative energy usage for potato cultivation under green farming and conventional crop management

3.5 Economics of potato cultivation under studied farming systems

Total cost of potato cultivation (Table 8) was found to be almost the same (Rs. 1,57,012 under green farming and Rs. 1,57,462 under CCM**)** under both the farming systems.

Net income under green farming was 30.98 percent higher as compared to CCM due to better tuber yields under the former. Green farming also led to higher economic gains as compared to CCM in terms of other financial components like net return, return per rupees invested and benefit cost ratio. The findings pointed towards the scope for financial security under this crop production system.

Table 8: Economics of potato cultivation under studied farming systems

Economics	**T_1: Conventional Crop Management (CCM)**	**T_2: Green Farming utilizing IRF**
Total cost (Rs./ha)	157462	157012
Gross income (Rs./ha)	229125	250875
Net income (Rs./ha)	71663	93863
Returns per rupees invested	0.49	0.60
Benefit cost ratio	1.49	1.60

Note: 100 Indian Rupee = 1.487US$ or 1.325€ or 1.050 British £ (as on 2016 June 14).

4 CONCLUSION

Green farming practice utilizing IRF Technology delivered positive results encompassing all the critical aspects of crop (in this case potato) cultivation i.e., yield, quality, energy investments and soil health; besides ensuring economic sustenance. Higher tuber yield and rapid bulking of potato tubers under green farming indicated the positive influence of organic plant management (utilizing IRF) towards efficient photosynthesis, translocation and synthesis of large molecular weight substances within storage organs; or in other words better plant metabolic functioning. The finding was corroborated by the better technological quality of potato tubers under green farming versus conventional crop management. The criteria of economic sustenance under changing climatic pattern was also better ensured under this farming, which showed higher withstanding capacity against production loss or fall in market price; as compared to conventional crop management. The study also revealed that complete cessation of chemical pesticides and other agrochemicals was possible even for an input intensive crop like potato, by undertaking initiatives towards activation of plant biochemical functions. Energy efficiency is an integral part of sustainable agriculture. As compared to conventional farming, green farming ensured higher use of renewable energy and significantly lower energy intensiveness for potato cultivation; thereby presenting the prototype for sustainable agriculture.

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Evaluation of different crop sequences for wheat and maize in sandy soil

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ABSTRACT

The objective of this paper was to assess four crop sequence system including wheat and maize grown in sandy soil of Upper Egypt with respect to the applied irrigation amount for each crop sequence, total production and water productivity. Two field experiments were conducted in Egypt during 2013/14 and 2014/15 growing seasons. Each experiment included four crop sequences: maize then wheat (CS1); maize, short season clover (SSC) then wheat (CS2); cowpea, SSC then wheat (CS3); cowpea intercropped with maize, SSC then wheat (CS4). The lowest amount of applied water was added to CS1 which resulted with low value of wheat and maize yield and the lowest water productivity. The highest amount of applied water was applied to CS2 and CS4 (similar values). The highest wheat yield and water productivity were obtained in CS3. The highest maize yield and water productivity was obtained from CS4. The highest total production (170.88 and 213.43 CU ha^{-1} in the 1st and 2nd season, respectively) and water productivity (0.093 and 0.114 CU m^{-3} in the 1st and 2nd season, respectively) for the studied crop sequences was obtained from CS3. In conclusion, higher water productivity for wheat in sandy soil can be attain by cultivating two legume crops before it (CS3); and for maize, it should be intercropped with a legume crop (CS4).

Key words: maize; wheat; short season clover; cowpea; cowpea intercropped with maize; cereal units; Assiut Governorate

IZVLEČEK

OVREDNOTENJE RAZLIČNIH KOLOBARJEV ZA KORUZO IN PŠENICO NA PEŠČENIH TLEH

Predmet te raziskave je bilo ovrednotenje kolobarja za pšenico in koruzo na peščenih tleh v Zgornejm Egiptu glede na obseg namakanja v posameznem kolobarju, celokupno produkcijo in učinkovitost izrabe vode. Izvedena sta bila dva poljska poskusa v rastnih sezonah 2013/14 in 2014/15. Vsak poskus je obsegal štiri sosledja poljščin v kolobarju in sicer : 1 - koruza nato pšenica (CS1); 2 - koruza, kratkosezonska detelja (SSC), potem pšenica (CS2); 3 - čičerka, SSC, potem pšenica (CS3); 4 - čičerka v medsetvi s koruzo, SSC, potem pšenica (CS4). Najmanjša količina dodane vode je bila v sistemu CS1, kar je rezultiralo v majhnem pridelku pšenice in koruze in najmanjši učinkovitosti izrabe vode. Največ vode je bilo dodano v kolobarjih CS2 in CS4 (enake količine). Največji pridelek in največja učinkovitost izrabe vode sta bila dosežena v kolobarju CS3. Največji pridelek koruze in največja učinkovitost izrabe vode sta bila dosežena v kolobarju CS4. Največja celokupna produktivnost (170.88 in 213.43 CU ha^{-1} v prvi in drugi rastni sezoni) in največja učinkovitost izrabe vode (0.093 in 0.114 CU m^{-3} v prvi in drugi rastni sezoni) sta bili za preučevane kolobarje deseženi v kolobarju CS3. Zaključimo lahko, da je večja učinkovitost izrabe vode za pšenico na peščenih tleh dosežena v kolobarju z dvema metuljnicama pred njo (CS3) in za koruzo z medsetvijo metuljnice (CS4).

Ključne besede: koruza; pšenica; kratkosezonska detelja; čičerka; medsetev čičerke v koruzo; žitne enote; Assiut upravna enota

1 INTRODUCTION

Maize and wheat are very important cereal crops all over the world (Valipour 2012a). The cultivated area of these important crops is under competition with other crops with higher economic values (Valipour 2016). In Egypt, there is a large gap between the production of these two crops and its consumption. Therefore, it is important to increase its cultivated area by cultivating low fertile soil on the edges of the Nile Delta and

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Valley. These soils are characterized by low fertility level and high water infiltration rate. Increasing productivity of unit land and water of these soils is a challenge under the prevailing situation of water deficiency and food insecurity in Egypt. For that reason, proper water management for the cultivated crops in such areas is essential. Accurate estimation of reference evapotranspiration is the main factor to attain that. Earlier studies compared different ETo equations for their accuracy revealed that Penman-Monteith equation is the most accurate because of its detailed theoretical base and its accommodation of small time periods (Valipour, 2014). It was found that air temperature and solar radiation contributed most to the temporal variation of ETo in the upper reaches, as well as solar radiation and wind speed were the determining factors for the temporal variation of ETo in the middle-lower reaches (Zhao et al., 2015). Furthermore, comparison between FAO Penman-Monteith with other methods that calculating reference evapotranspiration was done by several authors (Valipour, 2012b and 2014).

The prevailing crop sequence in these areas is two crops per year (a winter then a summer crop). One of the management that could be done to increase productivity of unit land and water in these soils is changing crop sequence from two crops per year to three crops per year, with inclusion of legume crop (early winter, winter then summer crops). The major benefit resulted from this practice is improvement in soil fertility and increased farmers' income (Sheha et al., 2014). In addition, implementing intercropping in one season can play a similar role in increasing productivity of unit land and water (Kamel et al., 2010). A very common crop sequence in Egypt, either on fertile or low fertile soils, is the cultivation of wheat then maize, where both are cereals and its cultivation in a year on the same piece of land leads to imbalance in soil nutrients and decline in the yield of both crops (Hamd-Alla et al., 2015). Previous research on the effect of crop sequence on wheat yield indicated that it was significantly improved when cowpea preceded it. The opposite occurred when maize preceded wheat, where wheat yield was reduced (Hamd-Alla et al., 2015). Under these circumstances, wheat benefited from the residual effect of legume, which positively affected wheat yield (Kumpawat and Rathore, 2003). Furthermore, cultivating short season clover in September before wheat cultivation in November proved to increase wheat yield (Sheha et al., 2014).

Another avenue to increase the productivity of unit land and water is intercropping, where one crop share its life cycle or part of it with another crop (Eskandari et al., 2010). This practice can be used as a way to improve soil fertility, increase land productivity and save on the applied irrigation water (Kamel et al., 2010). Furthermore, it increases water productivity as a result of using less water to irrigate two crops (Andersen, 2005). One example of intercropping systems is cowpea intercropped with maize, which has many advantages, such as increasing maize yield by 10% and reducing associated weeds (Hamd-Alla et al., 2014). Furthermore, no additional water will be applied to cowpea under this system (Kamel et al., 2016). In low fertile soil, such as in Egypt, legume/cereal intercropping system can increase soil fertility via raising its organic content and available nitrogen fixed by legume (Singh et al, 1986), which reduces fertilizer requirements for cereal crops, reduces costly inputs and ensure agricultural sustainability (Megawer et al., 2010). Furthermore, Banik et al. (2006) reported that intercropping can offer opportunity for stable agricultural production in low fertile soil, whereas mono cropping cannot ensure its stability.

Although many studies were done to determine the effect of crop sequence and intercropping systems on maize and wheat productivity in old fertile soil in Egypt (Sheha et al., 2014; Nofal 2012; Zohry 2005a; Zohry, 2005b), there were no previous studies on different crop sequences including wheat and maize in low fertile soil existed on the edges of the Nile value. Such a study can enhance our knowledge about the impact of different crop sequences on the applied irrigation water for these two crops in the whole crop sequence and its consequent total production. Application of such a study in Egypt is important for policy makers and can allow reduction of food gap of these two crops. Thus, the objective of this paper was to assess four crop sequences systems including wheat and maize grown in sandy soil of Upper Egypt with respect to the applied irrigation amount for each crop sequence, total production and water productivity. These crop sequences were: maize then spring wheat; maize, short season clover then spring wheat; cowpea, short season clover then spring wheat; and cowpea intercropped with maize, short season clover then spring wheat.

2 MATERIALS AND METHODS

Two field experiments were carried out at Arab El-Awammer Research Station; Agricultural Research Center; Assiut Governorate; Upper Egypt during two growing seasons of 2013/14 and 2014/15. Each experiment included four crop sequences as follows:

1. Maize then spring wheat (CS1);

2. Maize, short season clover then spring wheat (CS2);
3. Cowpea, short season clover then spring wheat (CS3);
4. Cowpea intercropped with maize, short season clover then spring wheat (CS4).

The soil of the experiment was sandy (sand, 89.9 %, silt, 7.1 % and clay, 3.0 %), with soil pH equal to 8.4, EC was 0.33 dS m^{-1} and $CaCO_3$ was 30.9 %. Furthermore, total N % was 0.008 and available P and K values were 8.31 and 64.00 mg kg^{-1}, respectively. The soil was cultivated for the first time with this experiment. Soil chemical analysis was determined according to Jackson (1958).

Irrigation water was applied each fourth day by using a solid-set sprinkler system. The rotary sprinkler (type Rc160) has 0.87 to 1.23 m^3h^{-1} discharge at 2.10 to 2.5 bars nozzle pressure, with spacing of 9 meters between laterals and 7 meters between sprinklers. A differential pressure tank was connected to the sprinkler irrigation system to inject fertilizer via irrigation water. The soil moisture constants (% per mass) in the depth of 0 - 60 cm were measured. Field capacity was 12.5 - 11.8 %, wilting point was 4.9 - 4.9 % and bulk density was 1.57 -1.55 g cm^{-3}. Reference evapotranspiration (ETo), crop evapotranspiration and irrigation schedule were determined using BISm model (Snyder et al., 2004) for weather data of 2013/14 and 2014/15 growing seasons. The model uses Penman-Monteith equation, as presented in Allen et al., (1989) to calculate ETo.

Table 1 presents weather data and ETo values in both growing seasons in the studied site. There is no rain occurrence in Assuit governorate because it is located in Upper Egypt region.

Table 1. Monthly weather data and ETo in 2013/14 and 2014/15 growing seasons in Assuit Agricultural Research Station

	2013/14 growing season						2014/15 growing season				
	SR	TX	TN	WS	ETo		SR	TX	TN	WS	ETo
Nov13	16.3	27.5	14.0	2.6	4.1	Nov14	16.5	25.6	12.2	2.9	4.1
Dec13	14.1	20.3	7.8	3.1	3.3	Dec14	14.3	22.5	9.1	2.5	3.3
Jan14	15.4	21.7	7.6	2.5	3.3	Jan15	15.2	19.0	5.7	2.7	2.9
Feb14	18.8	23.4	8.0	3.0	4.2	Feb15	17.5	21.8	7.8	2.9	3.8
Mar14	21.8	26.8	11.6	3.0	5.1	Mar15	18.1	26.9	11.9	3.2	5.0
Apr14	25.0	32.2	16.2	3.2	6.7	Apr15	25.9	29.2	12.8	3.6	6.6
May14	26.7	34.9	19.7	3.6	7.7	May15	27.8	34.7	18.9	3.5	7.8
Jun14	29.9	37.2	21.7	3.8	8.8	Jun15	26.8	35.7	21.0	4.0	8.7
Jul14	29.4	37.9	22.7	3.9	8.9	Jul15	29.4	37.7	22.6	3.5	8.6
Aug14	27.6	38.0	23.0	3.5	8.4	Aug15	25.5	40.2	25.4	3.9	9.3
Sep14	24.4	35.7	20.8	3.8	7.6	Sep15	24.2	35.5	20.6	3.6	7.8
Oct14	20.2	30.7	16.9	3.0	5.5	Oct15	20.0	30.5	16.7	2.8	5.8
Average	22.5	30.5	15.8	3.2	6.1	Average	20.9	29.9	15.4	3.3	6.0

SR = solar radiation ($MJ/m^2/day$), TX and TN = maximum and minimum temperature, respectively (°C), WS = wind speed (m s^{-1}), ETo = reference evapotranspiration (mm day^{-1}).

Land preparation was done by ploughing the land twice and then the land was leveled. The experimental design was spilt plot design, where the year was considered to be in the main plot and crop sequences were in the subplots. The size of single experimental plot was 21 m^2.

Regarding to maize, 'SC130' hybrid was sown on 12/5/2013 and 5/5/2014 in the first and second season, respectively using 27 kg of maize grains. Sole maize or intercropped with cowpea, *Vigna sinensis* 'Cream'), was planted with 100 % of its recommended planting density on one side of narrow furrows (70 cm width), 25 cm apart between plants. Nitrogen fertilizer was added at the rate of 360 kg N ha^{-1} of ammonium nitrate (33.5 % N). It was applied in five equal doses, after 15, 25, 35, 45 and 55 days from planting. Maize was also fertilized with 74.4 kg P_2O_5 ha^{-1} of calcium super phosphate, (15.5 % P_2O_5) and potassium sulphate (48.8 % K_2O) at the rate of 58.6 kg K_2O ha^{-1}, both were applied during land preparation, as recommended by Ministry of Agriculture and Land Reclamation in Egypt. Maize plants were harvested on 2/9/2013 and 25/8/2014 in the first and second season, respectively and maize grain yield was measured.

Cowpea seeds (‘Cream’) were planted in 12/5/2013 and 5/5/2014 in the first and second season, respectively using 15 kg of cowpea seeds. Sole cowpea was sown on one side of the narrow furrow (70 cm width), 36 cm apart between plants. Nitrogen fertilizer was added at the rate of 96 kg N/ha of ammonium nitrate (33.5 % N) with the second irrigation. The high applied rate of N fertilizer for cowpea is recommended by the Ministry of Agriculture and Land Reclamation in Egypt because it is known that, in these areas, the activity of soil bacteria could be limited. In addition, 74.4 kg P_2O_5 ha^{-1} of calcium super phosphate (15.5 % P_2O_5) was added during land preparation as recommended by Ministry of Agriculture and Land Reclamation in Egypt. First cut of cowpea was done on 12/7/2013 and 2/7/2014 in the first and second season, respectively. Second cut of cowpea was done on 22/8/2013 and 15/8/2014 in the first and second season, respectively.

Regarding to cowpea intercropped with maize, cowpea was sown on one side of the narrow furrow (70 cm width) and maize was planted on the other side of the narrow furrow with (50 % and 100 % of the recommended rate for cowpea and maize, respectively). No fertilizes was applied to cowpea under this intercropping system.

Short season clover seeds (*Trifolium alexandrinum*‘Fahl’) were planted with its recommended planting density in 15/9/2013 and 10/9/2014 in the first and second season, respectively using 60 kg of seeds. Nitrogen fertilizer was added at the rate of 72 kg N ha^{-1} of ammonium nitrate (33.5 % N), 20 days after planting as a result of low activity of the symbiosis bacteria in the soil. It was also fertilized with calcium super phosphate (15.5 % P_2O_5) as 37.2 kg P_2O_5 ha^{-1} during land preparation as recommended by Ministry of Agriculture and Land Reclamation in Egypt. Harvest was done in 20/11/2013 and 15/11/2014 in the first and second season, respectively.

With respect to wheat, *Triticum aestivum* ‘Sids1’, which is a common wheat cultivar was sown in 1/12/2013 and 25/11/2014 in the first and second season, respectively using 100 % of its recommended planting density (144 kg of grain yield). As recommended by Ministry of Agriculture and Land Reclamation in Egypt, nitrogen fertilizer was added as 288 kg N ha^{-1} in the form of ammonium nitrate (33.5 % N) in five equal doses, after 20, 40, 55, 70 and 85 days after planting. Phosphorus fertilizer was applied in the form of single super phosphate (15.5 % P_2O_5) as 74.4 kg P_2O_5 ha^{-1} and was incorporated into the soil during land preparation. Potassium in the form of potassium sulphate (48.8 % K_2O) as 58.6 kg K_2O ha^{-1} was applied during land preparation. Wheat was harvested on 20/4/2014 and 15/4/2015 in the first and second season, respectively, where wheat grain yields were measured.

For all the studied crops, seeds yield was recorded on the basis of experimental plot area by harvesting all plants, weighted, and then all the plots were combined together . The biomass of all studied crops was removed from the field after harvest. Dry mass of cowpea and short season clover were measured. In the second year experiment, the experiment was implemented on the same area used for the first year experiment.pART27 2mgfp5-ER 103 explants.

All the obtained data from the experiment of each season were subjected to the statistical analysis of complete randomized blocks design with four replications according to Gomez and Gomez (1984). Revised Least Significant Differences (LSD') at 5 % levels of probability was used for comparing means according to Waller and Duncan (1969).

Crop water productivity

Water productivity was calculated for each crop in the sequence, as well as for each crop sequence as a whole. Crop water productivity was calculated by dividing the obtained yield by applied water for each crop. To calculate water productivity for the whole crop sequence, calculation of Cereal Units (CU) (Brockhaus, 1962) for each crop in the sequence was done, then it was added together to obtain one value to represent the total yield from each crop sequence.

The CU has been used as a common denominator in German agricultural statistics for decades and is mainly based on the nutritional value for livestock. It is also an appropriate unit for the description of agricultural products (Brankatschk and Finkbeiner, 2014). Furthermore, Macak et al., (2015) used CU to evaluate productivity of different crop rotations. This methodology is widely used in Egypt to evaluate the production of different intercropping systems. Abou-Keriasha et al., (2013) reported that according to Brockhaus (1962) 100 kg of either wheat or maize is equal to 1.0 CU. Furthermore, 100 kg of short season clover or cowpea equal to 1.14 and 1.12 CU, respectively. Thus, water productivity (CU mm^{-1}) was calculated using the accumulated values of cereal units as numerator and the applied water in millimeters as dominator.

3 RESULTS

3.1 Applied water for crops and crop sequences

Table 2 indicates that water requirements for all the studied crops were higher in the second year compared to the first growing season, except for wheat. With respect to the four crop sequences, the lowest amount of applied water was added to CS1, where only two crops were cultivated. The value of the applied water to CS2 and CS4 were similar in each growing season and different in both growing season. Furthermore, this amount was the highest, compared to what was applied to the other crop sequences.

Table 2: Water requirements (WR, m^3 ha^{-1}) for the studied crops and crop sequences in both growing seasons

	WR 2013/14	WR 2014/15
Wheat	6267	6200
Maize	8440	8947
Short season clover	5400	5507
Cowpea	6853	6933
Cowpea intercropped with maize	8440	8947
Maize then wheat (CS1)	14707	15147
Maize, short season clover then wheat (CS2)	20107	20653
Cowpea, short season clover then wheat (CS3)	18520	18640
Cowpea intercropped with maize, short season clover then wheat (CS4)	20107	20653

3.2 Effect of crop sequence on wheat productivity

Table 3 shows that in all the studied crop sequences, there were significant differences between wheat yields ($P < 0.05$) in both growing seasons, where the lowest wheat yield was obtained when maize/wheat system was cultivated (CS1). In the second growing season, wheat yield was insignificantly higher. Furthermore, wheat cultivation after short season clover in CS2 increased wheat yield by 16 and 47 % in the first and second season, respectively, compared to maize cultivation before wheat system (CS1). The highest wheat yield was obtained when cowpea and short season clover were cultivated before it in both growing seasons (CS3), which increased its yield by 23 and 87 % in the first and second growing seasons, respectively. It can be also noticed, in all crop sequences, that wheat yield value was higher in the second growing season, compared to the first growing season.

Table 3 also reveales that the highest water productivity for wheat was obtained when cowpea and short season clover were cultivated before it in CS3. This result was true in both growing seasons. Furthermore, the lowest water productivity was found when maize preceded wheat in CS1.

Table 3: Spring wheat yield as affected by different crop sequences, percentage of yield increase (PI%) and water productivity (WP) in both growing seasons

	2013/14 growing season			2014/15 growing season		
Crop sequence	Wheat yield (ton ha^{-1})*	PI (%)	WP (kg m^{-3})	Wheat yield (ton ha^{-1})*	PI (%)	WP (kg m^{-3})
CS1	3.70^d	---	0.59	3.73^d	---	0.60
CS2	4.29^c	16	0.68	5.49^c	47	0.89
CS3	4.55^a	23	0.73	6.98^a	87	1.13
CS4	4.48^b	21	0.72	6.05^b	62	0.98

*Means with different letters indicated that it was significantly different

3.3 Effect of crop sequences on maize productivity

Maize yield was insignificantly lower in the CS2 compared to the CS1 in the first growing season. In CS2, maize yield increased by 7 % in the second growing season as a result of the residual effect of the legume crops from the first growing season (Table 4). The highest yield was obtained when cowpea was intercropped with maize in both growing seasons (CS4).

Intercropping cowpea with maize resulted in 3 and 13 % increase in maize yield in the first and second growing season, respectively. The results in Table 4 also revealed that in both growing seasons, there were insignificant differences between maize yield values in the studied crop sequences (*P <0.05*).

The highest water productivity for maize in both growing seasons were obtained when cowpea intercropped with maize (CS4), as a result of higher yield without any increase in the applied irrigation water for the intercropped system (Table 4).

Table 4: Maize yield as affected by different crop sequences in both growing seasons, percentage of yield increase (PI%) and water productivity (WP)

Crop sequence	2013/14 growing season			2014/15 growing season		
	Maize yield (t ha^{-1})*	PI (%)	WP (kg m^{-3})	Maize yield (t ha^{-1})*	PI (%)	WP (kg m^{-3})
CS1	5.43^{a}	--	0.64	5.40^{b}	--	0.60
CS2	5.39^{a}	-1	0.64	5.79^{ab}	+7	0.65
CS4	5.62^{a}	+3	0.67	6.12^{a}	+13	0.68

*Means with different letters indicated that it was significantly different

3.4 Effect of crop sequence on productivity of short season clover

Table 5 indicates that there were significant differences between short season clover productivity in the studied crop sequences (*P < 0.05*) in both growing seasons. The highest yield of short season clover was obtained when cowpea preceded it in both growing seasons (CS3), namely 35 and 37 % in the first and second season, respectively. However, when intercropping cowpea with maize preceded by short season clover (CS4), its yield was increased by 12 and 10 % only in the first and second growing season, respectively.

Furthermore, water productivity for short season clover increased when cowpea preceded it in CS3, compared to the other two crop sequences (Table 5).

Table 5: Short season clover dry yield as affected by different crop sequences in both growing seasons, percentage of yield increase (PI %) and water productivity (WP)

Crop sequence	2013/14 growing season			2014/15 growing season		
	Clover yield (t ha^{-1})*	PI (%)	WP (kg m^{-3})	Clover yield (t ha^{-1})*	PI (%)	WP (kg m^{-3})
CS2	4.30^{b}	--	0.78	5.10^{b}	--	0.93
CS3	5.82^{b}	35	1.06	6.97^{a}	37	1.27
CS4	4.79^{a}	12	0.87	5.62^{b}	10	1.02

*Means with different letters indicated that it was significantly different

3.5 Effect of crop sequence on cowpea productivity

Table 6 revealed that cowpea yield was significantly different in the studied crop sequences in both growing seasons (*P < 0.05*). Thus, cowpea yield was reduced by 45 and 35 % in the first and second season, respectively. Accordingly, water productivity followed the same trend as cowpea yield did in both crop sequences, where it was lower under intercropping with maize in both growing seasons (Table 6).

Table 6: Cowpea dry yield as affected by different crop sequences in both growing seasons, percentage of yield increase in its (PI %) and water productivity (WP) in both growing seasons

Crop sequence	2013/14 growing season			2014/15 growing season		
	Clover yield (t ha^{-1})*	PI (%)	WP (kg m^{-3})	Clover yield (tha^{-1})*	PI (%)	WP (kg m^{-3})
CS3	5.27^{a}	--	0.76	5.72^{a}	--	0.83
CS4	2.92^{b}	45	0.42	3.70^{b}	35	0.53

*Means with different letters indicated that it was significantly different

3.6 Total production of each crop sequence and its water productivity

The accumulated cereal units for each crop sequence are presented in Table 7. The results showed that the lowest value of accumulated cereal units were found for wheat followed by maize and it was higher in the second growing season. On the contrary, the highest values were obtained when cowpea preceded wheat and followed by short season clover in CS3 in both growing seasons. Furthermore, the highest percentage of increase in total yield of cereal units was found in CS3, namely 88 and 124 %, in the first and second season, respectively.

Table 7: Yield of crop sequences in cereal units (CU) in both growing seasons and percentage of increase (PI%)

Crop sequence	Total yield in 2013/14 (CU ha^{-1})	PI (%)	Total yield in 2014/15 (CU ha^{-1})	PI (%)
CS1	90.91	--	95.17	--
CS2	145.72	60	170.90	80
CS3	170.88	88	213.43	124
CS4	132.12	45	166.12	75

Table 8 reveals that CS3 attained the highest water productivity, compared to rest of crop sequences in both growing seasons.

Table 8: Water productivity (CU mm^{-1}) for each crop sequence in both growing seasons

	2013/14 season	2014/15 season
Maize/wheat (CS1)	0.062	0.063
Maize/clover/wheat (CS2)	0.073	0.082
Cowpea/ clover/wheat (CS3)	0.093	0.114
Cowpea with maize/clover then wheat (CS4)	0.066	0.080

4 DISCUSSION AND CONCLUSION

In this paper, four crop sequences included two major and important crops in Egypt were evaluated. The evaluation was done on the basis of its applied amount of irrigation water, on its total production calculated using cereal units method and on its water productivity.

Our results indicated that the applied amount of irrigation water for each crop in the four sequences was higher in the second year compared to the first year, except for wheat. Table 1 indicated that monthly ETo values in the first growing season of wheat (November-April) were lower than its counterpart in second season from January to April, which resulted in lower water requirements for wheat in the second growing season. Although the value of monthly ETo was lower in June and July in the second growing season of maize (May-September), it has a negligible effect of the applied water to maize and the amount was higher in the second season. In CS4, cowpea was intercropped maize, thus it obtained its water requirements from the applied amount to maize, which resulted in similar applied water to what was applied for CS4 (Table 2). Kamel et al., (2016) indicated that intercropping cowpea with maize did not require applying extra water to cowpea because it shared the applied water to maize.

Wheat cultivated in CS1 achieved the lowest productivity (Table 3), where it was planted after maize and both crops are exhausted to the soil, especially when its fertility is low. Hamd-Allah et al. (2015) indicated that low productivity of wheat was obtained when maize preceded it. Consequently, the lowest total production as expressed by cereal units was obtained for CS1. The increase in wheat yield in the second growing season can be explain by lower temperature in January and February in the second growing season, which could increase wheat tillering and positively affected grain yield. Porter and Gawith (1999) indicted that the optimum temperature for wheat shoot growth is 20.3 °C. Whereas, Hakim et al., (2012) stated that 20-25 °C is consider optimum for growth and development of spring wheat. Furthermore, wheat water productivity was the lowest in CS1in both growing seasons.

Furthermore, wheat yield was increased by inclusion of short season clover in CS2, or cultivation of cowpea and short season clover before it in CS3 in the first growing season. The second highest wheat yield value resulted from CS4 in the first growing season. In the second growing season, wheat yield was increased by higher values in CS2, CS3 and CS4 (Table 3). Accordingly, the highest value of wheat water productivity was obtained in CS3. This result attributed to the residual effect of the two legume crops (cowpea and short season clover) cultivated before wheat on increasing available nitrogen, which benefited wheat yield in the second growing season. This result is supported by the findings of Espinoza et al., (2015). The inclusion of legumes in a cropping sequence can also improve soil quality, porosity, and structure (McCallum et al., 2004) and influence specific microorganism populations in the rhizosphere (Osborne et al., 2010) for the benefit of following crops.

Regarding to maize in CS1, its yield was decreased in the second growing season, as a result of wheat cultivation before it in the first growing season (Table 4). Consequently, the lowest water productivity for maize existed in CS1 (Table 4), as well as the lowest total production (Table 7) and the lowest water productivity (Table 8) existed in the studied four crop sequences. The existence of legume crop (s) in the crop sequences CS2 and CS4 resulted in increasing maize yield and its water productivity (Table 4). This result can be attributed to the ability of legume crops to facilitate the absorption of P and K in the soil by cereal crops, in addition to its role in providing N through N-fixing rhizobium. Bado et al., (2006) stated that N2 -fixing legumes supply N to the subsequent crops through fallen senescent leaves and below ground parts, leading to an increase in succeeding crop yield. Hassan et al., (2010) indicated that legumes mobilize P in the soil during its growth, which increase P uptake of the following cereals. Ferguson et al., (2013) indicated that legumes have the ability to remove calcium and magnesium in the soil more than cereals and replace it with hydrogen, which results in removing OH^- ions and increases H^+ thus lowering the soil pH.

Regarding to maize, intercropping cowpea with it in CS4 increased its yield in the first growing season. Moreover, higher increase in maize yield was noticed in the second growing season, as a result of the residual effect of short season clover in CS4 (Table 4). Furthermore, the highest maize water productivity was attained in CS4 (Table 4).

Previous research on intercropping cowpea with maize in clay soil under surface irrigation indicated that maize yield was increased by 10 %, as a result of increased nitrogen content in the soil, reduction in the associated weeds competing with maize plants (Zohry, 2005a) and reduction in biological enemies that attack maize plants (Hamd-Alla, 2015) and it was also observed in our experiment. Inclusion of pure stand of cowpea in the crop sequence resulted in more positive effect on soil fertility, compared to its effect when it is intercropped with a soil exhausted crop like maize (Zohry, 2005a). The pure stand of cowpea produced higher yield compared to cowpea intercropped with maize as a result of lower plant density for cowpea, as well as inter-specific competition between cowpea and maize, where maize is the main crop in this system and cowpea is the secondary crop (Dahr et al., 2013). Gharnbari et al., (2010) indicated that cowpea intercropped with maize increased absorbed photosynthetically active radiation. This effect is shown in our experiment, where maize yield was increased, compared to sole maize planting. Furthermore, this intercropping system reduces water evaporation and improves conservation of soil moisture (Gharnbari et al., 2010). For that reason, in our experiment, the applied amount to sole maize was similar to what was applied to cowpea intercropped with maize. Kariaga (2004) concluded that this intercropping system reduced runoff through maintaining ground cover and also it reduced soil erosion.

Our results showed that there was superiority in water productivity for CS3 (only wheat was included), compared to CS1 (both maize and wheat were included) due to the high used amounts of water, which resulted in the highest yield values in both growing seasons. The two legume crops preceded wheat in this crop sequence resulted in higher wheat yield value. CS2 recorded the second with respect to the value of water productivity, where both maize and wheat were included, in addition to short season clover preceded wheat. However, the applied amount of water was the highest, with lower total yield in CU than what was obtained by CS3. In general, Najibnia et al., (2014) indicated that intercropping system was superior in water productivity, compared to sole planting. Thus, the best crop sequence for maize with respect to water productivity was CS4. Thus, it can be concluded that to attain higher yield and water productivity for wheat in new reclaimed soil in Upper Egypt, two legume crops should be cultivated before it. Similarly, to achieve higher yield and water productivity for maize in these types of soils, maize should be intercropped with legume crop, and another legume crop should follow it to benefit from its residual effect in the following growing season. The results of this experiment can be with great benefits to other countries with similar weather and soil conditions in the arid and semiarid regions.

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High salicylic acid concentration alters the electron flow associated with photosystem II in barley

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ABSTRACT

In this study, the effects of exogenously applied salicylic acid (0.5 and 5 mM SA) on the rates of photosystem II (PSII) activity was analysed in 4-week-old barley (*Hordeum vulgare*'Bahman') seedlings using chlorophyll (Chl) *a* fluorescence transient (*OJIP*) measurements. No evident changes in Chl and carotenoid contents as well as chlorophyll fluorescence transient curves were observed in either of the studied concentrations after 24 h of SA application. After 5 d, low SA concentration (0.5 mM) increased PSII activity, Chl *b* and carotenoid contents in barley seedlings. In contrary, 5 days after 5 mM SA treatment, the maximal quantum efficiency of PSII (F_v/F_m) and the Performance Index (PI_{ABS}), as an indicator of PSII structure and functioning, were significantly decreased. This lower F_v/F_m and PI_{ABS} coupled with lower levels of Chl *b* and carotenoids, and lower values of photosynthetic electron transport chain components including the electron transport flux (φE_o) and the inferred oxygen evolving complex activity (F_v/F_o). By monitoring the chlorophyll *a* fluorescence rise kinetics, from the initial "*O*" level to the "*P*" (the peak) level, a dramatic increase in "*OJ*" phase was detected, which coincides with an increased photo-reduction of Q_A as a result of blockage of electron flow. This study provided the evidence that the high concentration of SA induced damage to different sites of the PSII.

Key words: photosynthetic pigments; photosynthetic electron flow; *Hordeum vulgare* 'Bahman'; *OJIP* transient flourescence; salicylic acid

IZVLEČEK

VELIKA KONCENTRACIJA SALICILNE KISLINE SPREMINJA PRI JEČMENU FOTOSINTEZNI, S FOTOSISTEMOM II POVEZAN ELEKTRONSKI PRETOK

V raziskavi so bili preučevani učinki dodajanja salicilne kisline (0.5 in 5 mM) na aktivnost fotosistema II (PSII) pri 4-tedne starih kalicah ječmena (*Hordeum vulgare*'Bahman') z meritvami fluorescence (*OJIP*) klorofila a (Chla). Nobenih sprememb v vsebnosti klorofila in karotenoidov kot tudi ne sprememb v fluorescenci ni bilo opaznih po 24 urah dodajanja obeh koncentracij salicilne kisline. Po petih dneh so se v kalicah ječmena pri dodani manjši koncentraciji salicilne kisline (0.5 mM) povečali aktivnost PSII, vsebnost Chl b in karotenoidov. Nasprotno sta se pet dni po obravnavanju s 5 mM salicilno kislino značilno zmanjšala učinkovitost PSII (F_v/F_m) in PI_{ABS} indeks kot indikatorja zgradbe in delovanja PSII. Zmanjšanje F_v/F_m in PI_{ABS} je bilo povezano z zmanjšanjem vsebnosti klorofila b in karotenoidov ter z manjšimi vrednostimi komponent fotosintezne elektronske verige, vključno s elektronskim pretokom (φE_o) in z njim povezano aktivnostjo kompleksa, ki sprošča kisik (F_v/F_o). Pri spremljanju povečanja fluorescence klorofila a od začetne "*O*" na največjo vrednost "*P*" je bilo opazno njeno dramatično povečanje v fazi "*OJ*", kar je soupadalo s povečano fotoredukcijo Q_A kot posledica blokade fotosintezenega elektronskega pretoka. Raziskava dokazuje, da večja koncentracija salicilne kisline povzroči poškodbe na večih mestih PSII.

Ključne besede: fotosintezna barvila; fotosintezni elektronski pretok; *Hordeum vulgare* 'Bahman', *OJIP* prehodna fluorescenca; salicilna kislina

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1 INTRODUCTION

Salicylic acid (SA), as a common plant-produced phenolic compound, plays an important role in plant growth and development as well as in tolerance to biotic and abiotic stresses (Li et al., 2014; Khan et al., 2014; Janda and Ruelland, 2015). In recent years the involvement of SA in the plant growth and yield (Javaheri et al., 2012), and the regulation of some photosynthetic reactions (Arfan et al., 2007; Li et al., 2014) has widely been studied.

It has been suggested that the effects of SA on plant physiological and biochemical processes depends on the concentration of the applied SA (Miura and Tada, 2014). At low concentrations (0.1–0.5 mM for most plants), it enhances the efficiency of the antioxidant system and the efficiency of PSII photochemistry (Chen et al., 2016), whereas at higher concentrations (1-10 mM for most plants) it increases oxidative damage (Hara et al., 2012; Miura and Tada, 2014). Although negative effect of SA is probably correlated with an imbalance in antioxidant metabolism (Hasanuzzaman et al., 2013), the specific mechanisms of SA-mediated damages remain elusive. It is assumed that, plants respond to high SA depend on PSII response to this stress (Chen et al. 2016). To address this issue, the chlorophyll (Chl) *a* fluorescence transient (OJIP) measurements were used to study photosynthetic apparatus functioning in response to various SA concentrations and incubation times in this study.

Chl *a* fluorescence induction (*OJIP*, where *O* (or F_o) is the minimum fluorescence when all Q_A (the primary quinone acceptor of PSII) are in the oxidized state, *P* (or F_{max}) is the maximal fluorescence when all Q_A is in the reduced state (QA^-)) has been studied extensively in photosynthesis physiology research (Jee, 1995; Kalaji et al., 2011; Hamdani et al., 2015). The reduction of Q_A by PSII causes chlorophyll *a* fluorescence to rise from its minimal fluorescence level "*O*" to a "*J*" level (or F_J). Fluorescence rise from "*J*" level to the "*I*" level (or F_I) is related to the filling up of the plastoquinone pool. Finally, a traffic jam of electrons on the electron acceptor side photosystem I generates a fluorescence rise from the "*I*" level to the "*P*" level. The analysis of chlorophyll *a* fluorescence signals using '*JIP*-test', explores the information about the structure and function of the photosynthetic apparatus mostly related to PSII (Strasser et al., 2000; Bussotti et al., 2007) as well as some parameters due to energy fluxes for light absorption (ABS), trapping (TR) of excitation energy and electron transport (ET) per reaction center (RC) or per sample area called cross-section (CS) (Strasser et al., 2000).

As a noted above, the exact mechanisms by which SA affects photochemistry remain obscure. The present paper is the first report on the SA-mediated changes in specific chlorophyll fluorescence parameters. In order to improve our knowledge of barley photosynthetic apparatus in response to SA treatment, the *OJIP* fluorescence transient was measured in barley plants in responses to different concentrations of SA.

2 MATERIALS AND METHODS

2.1 Plant material and harvest

The randomly selected healthy seeds of barley (*Hordeum vulgare* 'Bahman') were sterilized with 5 % sodium hypo-chlorite solution for five minutes prior to sowing. Seeds were then sown on filter paper moistened with distilled water. Ten-day-old seedlings were transferred to modified Hoagland nutrient solution (Johnson et al. 1957) containing 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4$, 50 μM H_3BO_3, 2 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM H_2MoO_4 and 0.02 mM $FeSO_4$-EDTA for 15 days prior to the start of treatments. The pH of the nutrient medium was adjusted to 5.5–5.7. The seedlings were grown in a controlled growth room under a 16/8 light/dark cycle and a photosynthetically active radiation (PAR) of 200 ± 30 μmol m^{-2} s^{-1} and an average day/night temperature of 25 ± 1/18 ± 1 °C. Salicylic acid (SA) was dissolved in absolute ethanol then added drop wise to water (ethanol/water: 1/1000 v/v, pH was adjusted to 5.7) (Williams et al. 2003). At 25 days after germination, the foliar application of SA was carried out in the morning (between 08:00 and 10:00) with a compression sprayer of 1 L capacity. Non-SA applied plants were sprayed with ethanol/water (1/1000 v/v). At 1 and 5 days after treatment, the plants were harvested and the recent fully expanded and mature leaves were used for measurement of chlorophyll fluorescence and other analysis.

2.2 Chlorophyll *a* fluorescence measurements

Chlorophyll *a* fluorescence transients (*OJIP* transients) were measured with a Packet-PEA chlorophyll fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., King's Lynn, Norfolk, PE 32 1JL, England) in dark-adapted (for at least 20 min) leaves of barley. We used the *JIP*-test (Strasser and Strasser, 1995; Strasser et al., 2004) to analyse chlorophyll a fluorescence rises. The measured and calculated parameters are described in Tab 1. Specific parameters were calculated from energy fluxes for light absorption (ABS), trapping (TR) of excitation energy and electron transport (ETR) per reaction center (RC) or per sample area called cross-section (CS).

Table 1: Some groups of measured and calculated parameters using the *JIP*-test (Yusuf et al., 2010)

Nomenclature	Explanation
Data extracted from the recorded fluorescence transient OJIP	
Area	Total complementary area between F_o and F_m (reflecting the size of the plastoquinone pool)
F_J	Fluorescence intensity at the *J*-step (2 ms) of *OJIP*
F_I	Fluorescence intensity at the *I*-step (30 ms) of *OJIP*
Fluorescence parameters derived from the extracted data	
F_m or F_{max}	Maximal chlorophyll fluorescence intensity measured when all photosystem II (PSII) reaction centers are closed
F_o	Minimal fluorescence (all PSII RCs are assumed to be open)
F_v	Variable chlorophyll fluorescence (F_m-F_o)
V_j	Relative variable fluorescence at time *J* (relative variable fluorescence at phase *J* of the fluorescence induction curve
The specific energy fluxes (per reaction center, RC)	
ABS/RC	Light absorption flux (for PSII antenna chlorophylls) per RC
DI/RC	Dissipation energy flux per RC
ET/RC	Maximum electron transport flux (further than Q_A^-) per RC
TR/RC	Trapped (maximum) energy flux (leading to Q_A reduction) per RC
The phenomenological energy fluxes (per excited cross-section of leaf, CS)	
ABS/CS	Absorbed photon flux per cross section
TR/CS	Maximum trapped excitation flux per cross section
ET/CS	Electron transport flux from Q_A to Q_B per cross section
DI/CS	Dissipation energy flux per cross section
Quantum yields and efficiencies	
φE_o or phi (E_o)=ET_o/ABS	Quantum yield for electron transport (ET)
De-excitation rate constants of PSII antenna	
k_N	Non-photochemical de-excitation rate constant
k_P	Photochemical de-excitation rate constant
Performance index	
PI_{ABS}	The performance index that is calculated as: $(RC/ABS)\times(\varphi_{Po}/(1-\varphi_{Po}))\times(\psi_o/(1-\psi_o))$, where, RC is for reaction center; ABS is for absorption flux; φ_{Po} is for maximal quantum yield for primary photochemistry; and ψ_o is for the quantum yield for electron transport

2.3 Determination of total carotenoids and chlorophylls *a* and *b*

Leaf concentrations of chlorophylls and carotenoids were measured after extraction of pigments in the methanol according to Lichtenthaler and Wellburn (1985). The weighed samples were homogenized with homogenizer at 1000 rpm for one minute. The homogenate was filtered, and was centrifuged at 2500 rpm for 15 minutes. The supernatant was separated and the absorbance was read at 400-700 nm on spectrophotometer. Leaf concentrations of chlorophylls and carotenoids were calculated as:

$$\text{Chl } a = 15.65\ A_{666} - 7.340\ A_{653} \quad \text{(Eq.1)}$$

$$\text{Chl } b = 27.05\ A_{653} - 11.21\ A_{666} \quad \text{(Eq.2)}$$

$$\text{Total carotenoids} = 1000\ A_{470} - 2.860\ \text{Chl}a - 129.2\ \text{Chl}b/245 \quad \text{(Eq.3)}$$

2.4 Statistical analysis

Experiments were performed in complete randomized block design. All data satisfied the assumption for ANOVA for normal distribution and homogeneity of variance. Chlorophyll fluorescence were done on 20 plants from each treatment, and 3 replicates for each plant (n = 60) while the other measurements were performed on 4 plants from each treatment and we had one replicate for each plant (n = 4). Statistical analysis was carried out using Sigma Stat (3.5) with Tukey test ($P<0.05$). Correlation analysis using Spearman Rank Order Correlation in Sigma Stat was done to determine the relationship between PI_{ABS} and leaf carotenoids.

3 RESULTS AND DISCUSSION

We found that the low concentration of SA (0.5 mM) caused a significant increase in Chl *b* and carotenoid content compared with the control (Fig. 1). Our results are in line with the findings of Singh and Usha (2003), and Javaheri et al. (2012), who observed that the treatment with low SA increases photosynthetic pigment contents in some plants under normal or stress conditions. In the present study, treatment with higher concentration of SA (5 mM) resulted in a lower total Chl and carotenoid content as compared to the control. In agreement with our results, Chandra and Bhatt (1998), Moharekar et al. (2003) and Hayat et al. (2010) reported that high SA concentrations (1–5 mM) induced a reduction of chlorophyll contents in wheat and *Arabidopsis*.

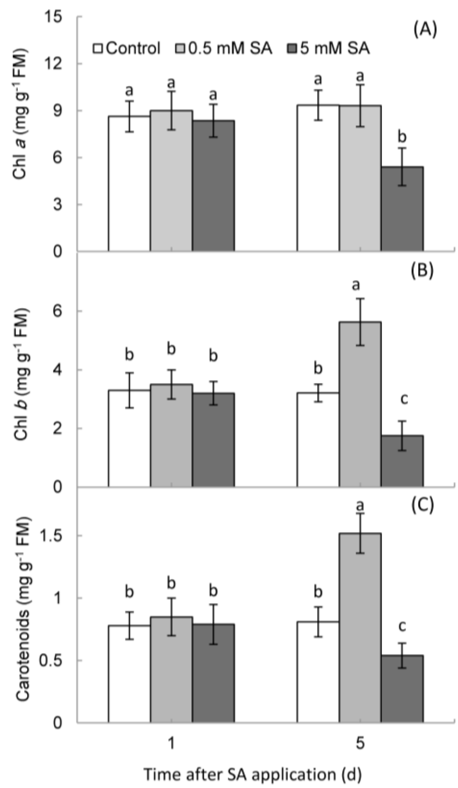

Figure 1: Effects of SA concentrations (0.5 and 5 mM) on the concentration of chlorophyll *a* (A), chlorophyll *b* (B) and total carotenoids (C) at different time intervals after treatment in barley plants. Bars indicated with the same letter are not significantly different ($p < 0.05$). Values are the mean ± SD ($n = 4$)

In present experiment, chlorophyll *a* fluorescence signals were measured by using the '*JIP*-test' (Strasser et al., 2000, 2004), in order to analyse the responses of the photosynthetic apparatus and energy flow among PSII in response to SA treatment. After 24 h of SA treatment, a slight decrease in the *IP* phase was noticed (Fig. 2). 5 days after 0.5 mM SA treatment, a much slower fluorescence rise from level "*I*" to a "*P*" level (or F_m) was observed, which coincided with a large increase in F_o fluorescence (Fig. 3), due to the structural damage leading to decreased excitation energy transfer from the antenna to the reaction center (Kalaji et al., 2011). Under these conditions, an upregulation of *OJ* phase was detected. This higher *OJ* phase rise is closely related to the increased photo-reduction of Q_A in the active PSII centers (Stirbet and Jee, 2011, 2012), mainly because of a blockage of electron flow.

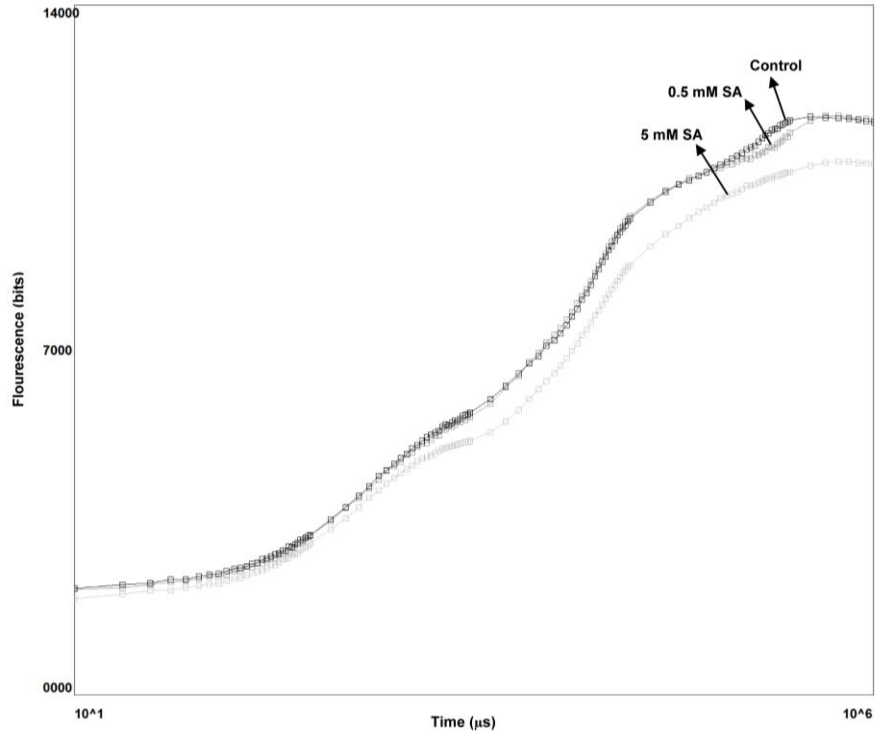

Figure 2: Chlorophyll *a* fluorescence induction curve of barley seedlings grown under 0 mM (Control), 0.5 mM and 5 mM SA for 24 h

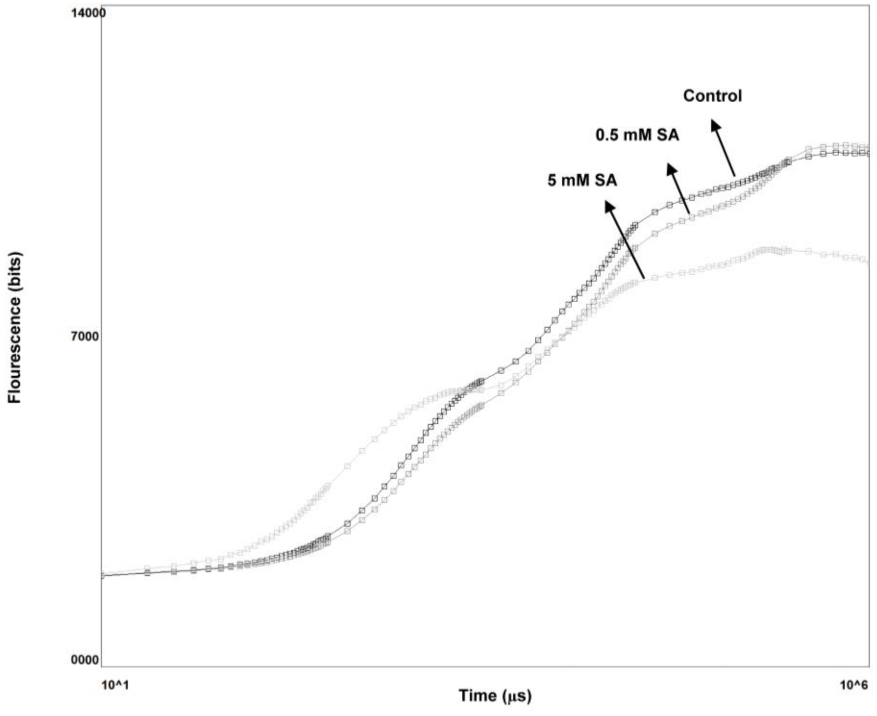

Figure 3: Chlorophyll *a* fluorescence induction curve of barley seedlings grown under 0 mM (Control), 0.5 mM and 5 mM SA for 5 days

After 24 h of SA treatment, the values of the maximal quantum efficiency of PSII (F_v/F_m) and the efficiency of the water-splitting complex on the donor side of PSII (as inferred from F_v/F_o) were similar to those of control plants (Fig. 4 and 5). But after 5 days of 0.5 mM SA treatment, an increase in the PSII function, as estimated by a large increase in performance index (PI_{ABS}), was obtained (Fig. 4 and 6). Carotenoids play an important role in photosynthesis and photoprotection (Cazzonelli and Pogson, 2010; Habibi and Ajory, 2015). Accordingly, we suggest that the increase in PI_{ABS}, after 5 days of treatment at 0.5 mM SA, was associated with the increased Chl *b* and carotenoid levels. Indeed, the accumulation of carotenoids by 0.5 mM SA foliar spray in barley plants; helped them to maintain higher rates of photosynthesis and photosystem II activity (Dong et al., 2013; Habibi and Ajory, 2015).

In contrary, the decreased F_v/F_m and PI_{ABS} in plants treated with 5 mM SA indicated that the high concentration of SA induced damage to photosynthesis, which is agreement with the findings of Chen et al. (2016) in wheat. This decrease in PI_{ABS} is coupled with lower levels of Chl *b* and carotenoids. In confirmation of this, there was a significant correlation (r = 0.84, p<0.01) between PI_{ABS} and carotenoid level in SA-supplied plants (Fig. 7). Additionally, this down-regulation of F_v/F_m and PI_{ABS} was associated with decreases in electron transport flux per chlorophyll (φE_o) and in efficiency of the water-splitting complex on the donor side of PSII (F_v/F_o) (Fig. 6), which might be related to the photosynthetic electron transport impairment (Pereira et al., 2000). In addition, we suggest that the increased accumulation of inactive reaction centers was due to the significantly higher values of the efficiency of non-photochemical de-excitation processes (K_N) (Kalaji et al., 2011). After 5 days of 5 mM SA application, the specific flux of energy (DIo/RC; dissipative energy flux per reaction center and ABS/RC; the absorption flux per reaction center) parameters were much higher than those determined in control plants (Fig. 6). Thus, the increase in ABS/RC might represent a compensatory mechanism (van Heerden et al., 2007) for maintaining electron transport flux per remaining active reaction centers. The specific rate of the electron transport from Q_A to Q_B, depends on the V_J (relative variable fluorescence at time *J*) value (Stirbet and Jee, 2011), and this parameter was significantly increased after 5 days of 5 mM SA application in the present study.

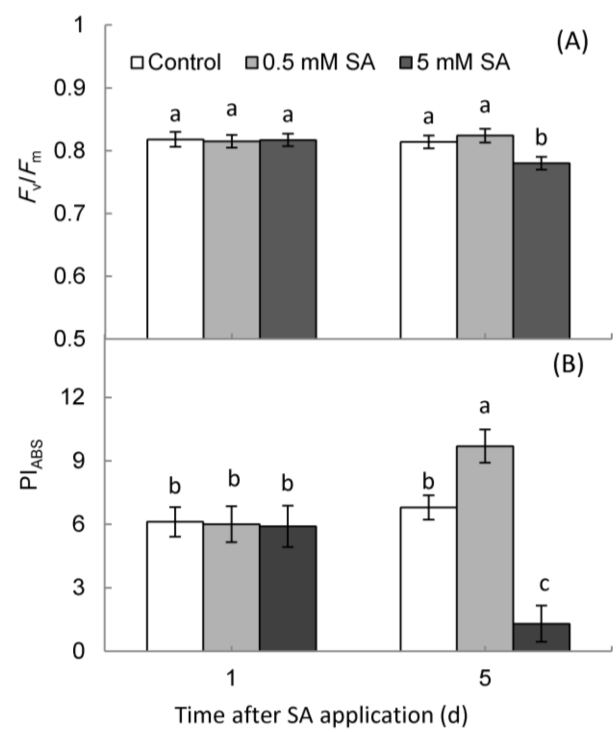

Figure 4: Effects of SA concentrations on the maximum quantum yield of PSII (F_v/F_m) (A) and the Performance Indexes (PI_{ABS}) (B) at different time intervals after treatment in barley plants. Bars indicated with the same letter are not significantly different ($p < 0.05$)

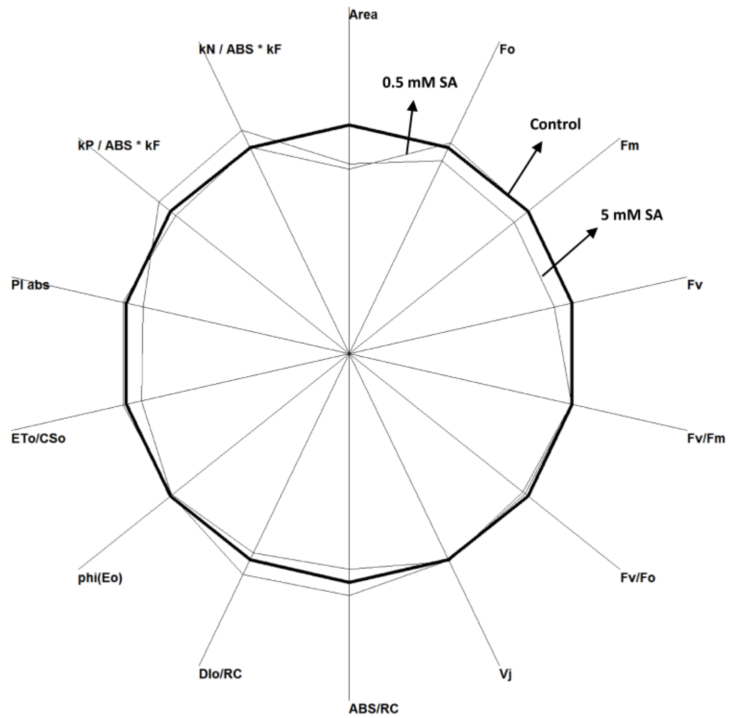

Figure 5: A 'spider plot' of selected parameters characterizing behavior of photosystem II of barley leaves exposed 24 h to 0 mM (Control), 0.5 mM and 5 mM SA (See Tab 1 for the meaning of the parameters)

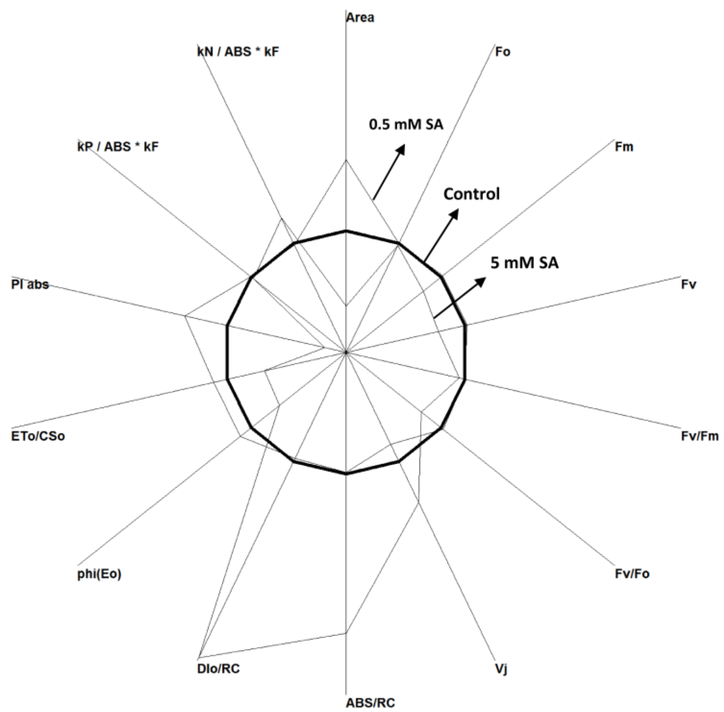

Figure 6: A 'spider plot' of selected parameters characterizing behavior of photosystem II of barley leaves exposed 5 days to 0 mM (Control), 0.5 mM and 5 mM SA (See Tab 1 for the meaning of the parameters)

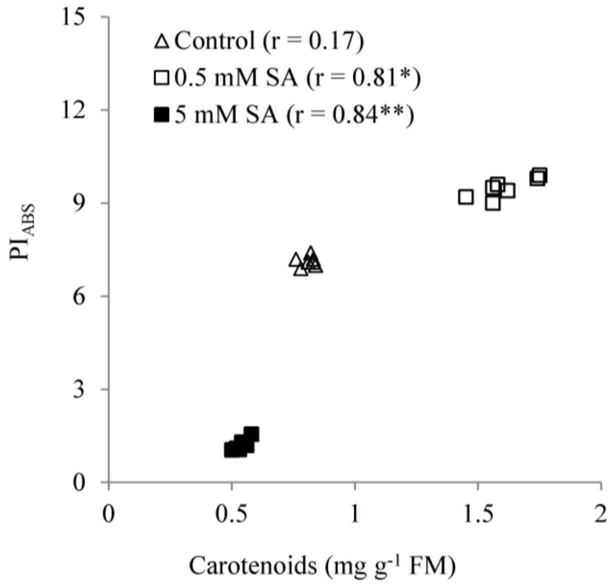

Figure 7: Correlations between the Performance Indexes (PI_{ABS}) and the leaf carotenoids levels in barley plants grown for 5 days under 0 mM (Control), 0.5 mM and 5 mM SA treatment: ns, *, and **: non-significant, significant at the 5 % and 1 % levels of probability, respectively

In conclusion, SA at low concentration improved the efficiency and the yield of energy transfer and primary photochemistry in barley seedlings as related to the higher levels of Chl *b* and carotenoids. In contrary, several parameters related to PSII activity (e.g., the time needed to reach the maximal chlorophyll fluorescence, the variable fluorescence, the inferred oxygen evolving complex activity, the electron transport flux, and the calculated Performance Index) were significantly decreased by SA application at high concentration indicating that the high concentration of SA induced damage to photosynthesis. On the other hand, increasing the photosynthetic activity of barley plants at low SA concentration can help for crop research and practical applications in order to improve crop productivity and increase plant nutritional value for a growing world population.

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Evaluation of genetic diversity and traits relations in wheat cultivars under drought stress using advanced statistical methods

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ABSTRACT

In order to study of genetic diversity and classify physio-agronomic characters under normal irrigation and drought stress in wheat cultivars, 15 cultivars were evaluated in the research farm of University of Mahabad, Iran. According to stepwise regression some of traits entered to final model that as far to correlation coefficients and path analysis regarding, the biggest part of correlation coefficient and direct effect was achieved for number of grains per spike, number spikes per plant with grain yield under two conditions. These traits had the highest indirect effect on the grain yield mutually. So, screening for high value for these traits can bring increase in wheat grain yield under two conditions. Factor analysis detected three and four factors which explained 91.23 and 92.43 percent of the total variation in non-drought stress and drought stress conditions, respectively. In drought stress condition the first factor, second factor, third factor and fourth factors were named as yield component, physiological, biomass and growth, and yield factor respectively. Cluster analysis based on the three and four factors grouped cultivars into the two groups under normal and three groups under drought stress conditions. Generally, tolerant cultivars can be used for direct culture or as parents for create of variation in breeding programs.

Key words: correlation; drought stress; factor analysis; path analysis; physio-agronomic traits; wheat

IZVLEČEK

OVREDNOTENJE GENETSKE RAZNOLIKOSTI IN RAZMERIJ MED LASTNOSTMI PRI SORTAH KRUŠNE PŠENICE V RAZMERAH SUŠNEGA STRESA S STATISTIČNIMI METODAMI

Z namenom preučevanja genetske raznolikosti in razvrščanja fizioloških in agronomskih lastnosti je bilo v razmerah sušnega stresa in normalnega namakanja ovrednotenih 15 sort krušne pšenice na raziskovalnem polju University of Mahabad, Iran. S postopno regresijo so nekatere lastnosti vključili v končni model na osnovi koeficientov korelacije in standardiziranih koeficientov multiple regresije in ugotovili, da so imele največji neposredni učinek v obeh razmerah poskusa lastnosti kot so število zrn na klas, število klasov na rastlino in pridelek zrnja. Te lastnosti so imele hkrati tudi največji neposrednik učinek na pridelek zrnja. Iskanje sort z velimi vrednostmi teh lastnosti lahko poveča pridelek pšenice v obeh razmerah poskusa. Faktorska analiza je ugotovila tri, oziroma štiri faktorje, s katerimi lahko razložimo 91.23 in 92.43 odstotkov celukupne variabilnosti v razmerah brez suše in ob sušnem stresu. V razmerah sušnega stresa so prvi, drugi, tretji in četrti faktor poimenovani kot komponento pridelka, fiziološki parametri, biomasa in rast ter pridelek. Klasterska analiza je na osnovi treh in štirih faktorjev uvrstila sorte v dve skupini v normalnih razmerah in v tri skupine v razmerah sušnega stresa. V splošnem bi odporne sorte lahko gojili neposredno v prozvodnji ali jih uporabili kot starše pri ustvarjanju raznolikosti v žlahtniteljskih programih.

Ključne besede: korelacija; sušni stres; faktorska analiza, multipla regresija; fiziološko-agronomske lastnosti; krušna pšenica

1 INTRODUCTION

Common wheat (*Triticum aestivum* L.) as the most important cereal crop is cultivated throughout the major agro-climatic zones of the world (Baik and Ullrich, 2008). World's wheat production was about 735.23 million tons in 2016 (FAO, 2016). Drought stress is the most important factor limiting crops production in

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agricultural systems in arid and semi-arid regions (Mollasadeghi et al., 2011). Drought stress is recognized as an important factor that affects the wheat growth and yield (Ashraf, 1998). Some morphological traits such as number of spike per m^2, number of grains per spike, number of fertile tillers per plant, 1000-grain mass, peduncle length, spike mass, stem mass and grain yield affect wheat tolerance to the moisture shortage in the soil (Plaut et al., 2004; Blum, 2005). Grain yield is a complex multi component character and is greatly influenced by various environmental conditions. Various morphological and physiological characters contribute to grain yield (Kahrizi et al., 2010). Also, environmental conditions and genotype interaction affect the relationships among plant characters. So, toward a clear understanding of the type of plant traits, correlation and path coefficient analysis are logical steps (Kashif and Khaliq, 2004). Path analysis is a tool that is available to the breeder for better understanding the cause involved in the associations between traits and to partition the existing correlation in to direct and indirect effects, through a main variables (Lorencetti et al., 2006). Generally, this method provides more information among variables than do correlation coefficients since this analysis provides the direct effects of specific yield components on yield, and indirect effects via other yield components (Garcia del Moral et al., 2003). Path analysis has been widely used in crop breeding to determine the nature of relationships between grain yield and its contributing components, and to identify those components with significant effect on yield for potential use as selection criteria (Board et al., 1997; Khalili et al., 2013; Naghavi et al., 2014). Different statistical techniques have been used in modeling crops yield, including correlation, regression, path analysis, factor analysis, factor components and cluster analysis (Mohamed, 1999). Factor analysis suggested by Walton (1972) has been widely used to identify growth and plant characters related to wheat (Moghaddam et al., 1998; Naghavi et al., 2015). This method basically reduces a large number of correlated variables to a small number of uncorrelated variables or factors. This method is a strong method that has been used to estimate the components of yield, to extract a subset of identical variables, to identify the basic concepts of multivariable data, to recognize applied and biological connections among the traits, to reduce a large number of correlative traits to a few number of factors and to explain the correlation among the variables (Bramel et al., 1984). Cluster analysis can be used to identify variables which can be classified into main groups and subgroups based on similarity and dissimilarity. This technique is useful for parental selection in breeding programs (El-Deeb and Mohamed, 1999) and crop modeling (Jaynes et al., 2003). Naghavi et al. (2015) showed a negative correlation between plant height and grain yield. They attributed that to the lower number of grains/spike with the tallest wheat plants. Kumbhar et al. (1983) and Mohamed (1999) had shown that grain mass/spike, biological yield and number of spikes/m^2 were closely related to grain yield g/m^2. The differential relations of yield components to grain yield may be attributed to environmental effects on plant growth (Asseng et al., 2002). Khayatnezhad et al. (2010) using factor analysis in his studies on durum wheat cultivars showed that the importance of factor coefficients characteristics of total and fertile tillers, main spike length, 1000-seed mass, and yield selected genotypes is desirable for dry conditions. Also, Naghavi, et al., (2015) used the factor analysis to identify growth and morphological traits relevant to yield in wheat and introduced four factors which included yield components, morphological traits, spike length and the number of grain per plant.

Our objective was to determine the relationship between grain yield and related characters under normal irrigation and drought stress. Also, one of another goals in this study was founding the direct and indirect effects of morphological and agronomic traits on grain yield under two conditions. On the other hands, the another objectives of this investigation were evaluate the relations of different characters and also identifying effective factors in yield improvement in wheat cultivars and grouping of cultivars according to achieved factors under normal irrigation and drought stress.

2 MATERIALS AND METHODS

Fifteen cultivars of wheat such as Mahabad Landrace cultivar and Sardari, Zarin, Azar, Homa, Alamoot, Shahriyar, Mihan, Zare, Urum, Pishgam, Toos, Alvand, Navid, Sabalan were cultivated in a split plot basis of randomized complete block design with four replications under two different conditions (normal irrigation and no irrigation after booting stage) at Research Farm of University of Mahabad, Iran (latitude 36.46°N, longitude 45.43°E, Altitude 1385 m above sea level) during growing season of 2015-2016. The climate is characterized by mean annual precipitation of 330 mm; mean annual temperature of 12 °C. The experimental treatments consisted of irrigation levels as the main plot at second levels: irrigation after 70 mm evaporation from class A pan (without stress), irrigation after 150 mm evaporation from class A pan (water deficit stress) and fifteen cultivars of wheat as the sub plot were considered in this study. Each plot contained 4 rows with 25 cm apart and 1m in length. All plots were irrigated after sowing and subsequent irrigations in the beginning of stem elongation. Weeds were controlled by hand during crop growth and development.

Agronomic characteristics and physiological criteria including: plant height (cm), plant dry mass (g), specific leaf area (cm^2/g), relative water content (%), proline content, chlorophyll content (ChC) and osmotic potential (OP), spike length (cm), number of tillers per plant, number of fertile tillers per plant, number of spikes per plant, number of grains per spike, 1000-grains mass (g), grain yield (g), were measured after the physiological maturity in 10 selected plants of each experimental plot, randomly.

Physiological criteria were used for flag leaf measurement. Specific leaf area was calculated on the basis of this formula: special leaf area (cm^2g^{-1}) = (leaf area)/(leaf dry mass) (Arias, 2007). Moreover, relative water content (%) was determined according to method of Turner (1986). Also, proline contents ($mg.g^{-1}FM$) were measured by acid hydrin method. The chlorophyll content was determined using a chlorophyll meter (SPAD-502, Japan). Osmotic potential was measured by osmometer (Martinez et al. 2004); mode: Osmomat 010, Genotel. Morphological and growth traits such as the plant height (cm), plant dry mass (g), spike length (cm), number of tillers per plant, number of fertile tillers per plant, number of spikes per plant, number of grain in spike, 1000 grain mass (g) and grain yield (g) were measured at the end of growth stage. Finally, mean of data used for analysis and simple linear correlation coefficients were computed and these coefficients were subjected to path analysis as described by Dewey and Lu (1959) using SPSS software. Also, mean of data used for analysis and simple linear correlation coefficients were computed then factor analysis on the base of major factors analysis and varimax rotations was done on the data. The factors which had a root bigger than one were selected and were used to form factorial coefficients matrix (Sharma, 1985; Tadesse and Bekele, 2001). Also eigen values, percent variance, variance, and cumulative percentage share of each of the extracted factors were calculated. Finally cluster analysis was performed according to values for cultivars basis of factors. Analysis of data and drawing of dendrogram were performed with SPSS software.

3 RESULTS AND DISCUSSION

3.1 Analysis of variance

The results of analysis of variance (Table 1) showed high significant differences ($P<0.01$) between of cultivars for all traits, except SLA and OP which was significant in probability level $P<0.05$. Also difference between normal irrigation and drought stress was significant for all of traits. This indicates that the magnitude of differences in cultivars was sufficient to select them against drought. Also, results indicated that there is a high variation for all traits which revealed the presence of genetic diversity for these attributes in the materials. Therefore, these traits have good potential for selection of the most tolerant and most sensitive cultivars for using in cross together and create genetically variation or using of direct culture for tolerant cultivars.

At the study of Garavandi and Kahrizi (2010), in which 20 bread wheat genotypes were evaluated, grain yield, spike number per square meter, number of seed per spike, spike density and awn length had the heights genetic diversity in compare with other traits. Kutlu and Kinaci, (2010) reported similar results for agro-morphological traits and grain yield in both stress and non-stress conditions. Also, Farshadfar (2012) showed significant difference among wheat genotypes in term of physiological traits under stress and non-stress.

Table 1: Analysis of variance for yield and other traits in wheat cultivars under different irrigation treatment

Source of Variation	df	Mean of Squares						
		†PH	PDM	SLA	RWC	PC	ChC	OP
Replication (R)	3	0.956	0.004	35.906	0.430	0.163	0.298	0.055
Stress (S)	1	421.98**	236.982**	702.76**	45.83**	3.739**	19.837**	0.629**
Error a	3	0.409	0.008	246.317	0.003	0.104	0.049	0.053
Genotype (G)	14	98.764**	5.278**	184.91**	69.724**	3.089**	13.656**	0.873**
G×S	14	24.891	1.073	310.189*	11.897	1.932*	0.985	5.167**
Error b	84	30.670	1.853	118.872	13.096	0.458	1.005	0.198
CV (%)		11.58	8.46	11.76	9.98	12.09	11.65	13.96

†PH, PDM, SLA, RWC, PC, ChI and OP indicate plant height, plant dry mass, specific leaf area, relative water content, proline content, chlorophyll content and osmotic potential respectively. Also, * and ** were significant at 5 % and 1 % probability levels.

Table 1: continued

Source of Variation	df	Mean of Squares						
		†SpL	NT	NFT	NSp	NGSp	1000-GM	GY
Replication (R)	3	0.061	0.550	0.499	0.329	43.871*	23.987	0.873
Stress (S)	1	353.894**	451.159**	490.461**	543.134**	556.092**	3984.930**	2196.561**
Error a	3	0.054	2.129	1.128	2.094	18.186	28.44	4.457
Genotype (G)	14	11.873**	15.905**	16.047**	18.947**	110.857**	65.192**	18.903**
G×S	14	0.436	1.198	1.209	1.762	11.940	8.093	2.320
Error b	84	0.936	1.122	1.432	1.875	12.945	9.670	2.406
CV (%)		10.55	13.08	17.49	18.21	13.44	10.55	18.98

†SpL, NT, NFT, NSp, NGSp, 1000-GM and GY indicate spike length, number of tillers per plant, number of fertile tillers, number of spikes per plant, number of grains per spike, 1000 grain mass and grain yield respectively. Also, * and ** were significant at 5 % and 1 % probability levels.

3.2 Correlation analysis

According to the results of the correlation, significant positive correlation was found between grain yield and spike length, number of tillers per plant, number of fertile tillers, under both conditions (Table 2). Moreover of these traits plant dry mass, relative water content, proline content, chlorophyll content, number of spikes per plant and number of grains per spike had significant positive correlation with grain yield under drought stress (Table 2). Also, significant negative correlation was found between grain yield and plant height, specific leaf area and osmotic potential under drought stress condition (Table 2).

Maximum of amount of correlation coefficients between studied traits with grain yield was achieved for number of grains per plant and number of spikes per plant under normal irrigation and drought stress. On the other hands, a negative significant correlation was found between 1000-seed mass and number of grains per spike under two irrigation conditions. Plant height was positively correlated with plant dry mass under both conditions. Also, correlation analysis showed physiological traits values were positive significantly correlated together under two conditions. Further, number of tillers with number of fertile tillers and number of spikes per plant had significant positive correlation under normal irrigation and drought stress. Also, spike length showed positive and significant correlation with number of grains per spike under normal irrigation and drought stress.

The analysis of correlation of different traits with grain yield can help to make decision about the relative importance of these traits and their merits as selection criteria (Dokuyucu and Akkaya, 1999). Various studies show that grain yield of wheat is significantly correlated with 1000-grain mass, the number of fertile tillers or spikes per plant and the number of spikelets per spike (Mohiuddin and Cory, 1980; Shanahan et al., 1985). Moghaddam et al. (1998) reported that yield, 1000-grain mass, and number of spikes per plant were correlated. In most of the previous studies, similar have been reported between yield and related characters such as, number of spikes, number of spikelets and 1000-grain mass (Sharma and Rao, 1989; Subhani and Khaliq, 1994). In the studies conducted by Sinha and Sharma (1979) and Belay et al. (1993), yield was positively correlated with yield components, with either positive or negative correlation between yield and plant height. Moghaddam et al. (1997) reported negative correlation between number of grains per spike and 1000-grain mass. Further, Passioura (1997) and Leilah and Al-Khateeb (2005) reported that grain yield of wheat has a positive correlation with number of spikes/m^2, 1000-grain mass, harvest index and biomass. Also, Fatemi Rika et al. (2013) reported significant correlation among grain yield, fertile tillers number, thousand grain mass, straw yield, plant biomass and harvest index under two conditions. Some of researches showed positive significant correlation between grain yield and number of spikes per plant (Kahrizi et al., 2010, Naghavi et al. 2015).

Table 2: Coefficient correlation between studied traits with grain yield under normal irrigation (under main diagonal) and under drought stress (above main diagonal)

	†PH	PDM	SLA	RWC	PC	ChC	OP	SpL	NT	NFT	NSp	NGSp	1000-GM	GY
PH	1	0.96**	0.40	-0.33	-0.34	-0.40	-0.41	0.32	-0.39	0.36	0.23	0.43	-0.41	-0.51*
PDM	0.90**	1	-0.52*	0.32	0.60**	0.69**	-0.29	-0.28	0.39	0.32	0.24	0.77**	-0.67**	0.60**
SLA	0.37	-0.28	1	-0.17	-0.40	-0.41	0.40	-0.40	0.30	0.20	0.37	-0.68**	0.60**	-0.73**
RWC	-0.11	0.29	-0.09	1	0.66**	0.60**	-0.30	0.79**	0.80**	0.82**	0.84**	0.32	0.53*	0.94**
PC	-0.20	0.26	-0.20	.58**	1	0.75**	-0.61**	0.76**	0.61**	0.76**	0.61**	-0.23	0.31	0.84**
ChC	-0.19	0.28	-0.28	0.38	0.65**	1	-0.63**	0.67**	0.76**	0.90**	0.74**	-0.80**	0.83**	0.71**
OP	-0.23	-0.08	0.26	-0.37	-0.64**	-0.40	1	-0.53*	-0.30	-0.43	-0.61**	-0.32	0.41	-0.65**
SpL	0.25	-0.10	-0.19	0.60**	0.66**	0.39	-0.33	1	0.76**	0.77**	0.87**	0.88**	0.75**	0.73**
NT	-0.20	0.15	0.32	0.63**	0.63**	0.36	-0.35	0.64**	1	0.88**	0.80**	-0.60**	0.60**	0.79**
NFT	0.28	0.12	0.29	0.61**	0.59**	0.31	-0.23	0.60**	0.60**	1	0.84**	-0.54*	0.65**	0.95**
NSp	0.10	0.08	0.28	0.62**	0.42	0.35	-0.31	0.60**	0.61**	0.63**	1	0.52*	0.69**	0.93**
NGSp	0.40	0.21	-0.40	-0.40	-0.30	-0.19	-0.23	0.53*	-0.28	-0.26	-0.22	1	-0.69**	0.98**
1000-GM	-0.39	-0.30	0.38	0.23	0.28	0.44	0.27	0.53*	0.30	0.25	0.29	-0.52*	1	-0.19
GY	-0.30	-0.12	-0.16	0.31	0.36	0.48	-0.11	0.59**	0.74**	0.54*	0.42	0.41	-0.21	1

† PH, PDM, SLA, RWC, PC, ChC, OP, SpL, NT, NFT, NSp, NGSp, 1000-GM and GY indicate plant height, plant dry mass, specific leaf area, relative water content, proline content, chlorophyll content, osmotic potential, spike length, number of tillers per plant, number fertile tillers, number of spikes per plant, number of grains per spike, 1000 grain mass and grain yield respectively. Also, * and ** were significant at 5 % and 1 % probability levels.

3.3 Path analysis

Path analysis was used to describe correlation to identify direct and indirect effects for entered traits into regression model. Path coefficient analysis was conducted by considering yield-related traits as predictor variables and grain yield as the response variable. In the control condition, comparing the direct and indirect effects between grain yield and some related traits were calculated (Table 3, 4). In this state, grain yield was positively correlated with chlorophyll content, number spikes per plant, number of grains per spike and negative correlation with 1000 grain mass and amount of correlation coefficient for 1000 grain mass was less rather than other traits (Table 4). According to this results and as regards to amounts of direct effects traits under normal irrigation the best of traits for selection of plant with high grain yield were chlorophyll content, number spikes per plant and number of grains per spike, because these traits had high direct effect and high correlation coefficient with grain yield under normal irrigation (Table 3, 4).

On the other hands, under drought stress condition, number of grains per spike, relative water content, number of spikes per plant, proline content and 1000 grain mass were entered to final regression model (Table 3). All of these traits showed a positive significant correlation with grain yield except of 1000 grain mass (Table 5). Under drought stress, traits such as number of grains per spike, number of spikes per plant and relative water content showed average direct effect on grain yield and these results showed that these traits act via other traits, cumulative effects with high positive correlations on grain yield was expressed. On the other hands, 1000 grain mass showed good direct effect on grain yield under drought stress but their effect via indirect effect of other traits decreased their correlation with grain yield (Table 5). The biggest part of correlation and direct effect on grain yield under drought stress was achieved for number of grains per spike, proline content and number of spikes per plant.

Generally, number of grains per spike and number of spikes per plant were the best criterion for improving grain yield in wheat under normal irrigation and drought stress conditions. So, screening for high amount for these traits can bring increase in wheat grain yield under two conditions. Naghavi et al., (2014), using path analysis in wheat found that the number of spikes per plant and number of fertile tillers had significant positive, direct effects on grain yield under drought stress conditions, as well as well-watered conditions. On the other hands, Baranwal et al., (2012) revealed that number of grains per spike, spike length and 1000-grain mass exhibited the maximum positive direct effect on grain yield. Also, Sheron et al., (1986) observed that yield components such as number of grains per spike and number of spikes per plant with plant height and spike length were directly related to grain yield.

Table 3: Results of stepwise regression analysis for grain yield as the response to other characters as predictors in non-stress and water deficit stress conditions

Stress conditions	Model	t-values	Unstandardized Coefficients		Standardized Coefficients	R^2	Adjusted R^2
			β	Std. E.	*β*		
Non-stress	Constant (α)	-0.955	-4.343	0.855			
	Number of Grains per Spike	0.112	0.064	0.009	**0.434**		
	Chlorophyll Content	0.094	0.070	0.008	**0.576**	0.598	0.583
	1000 Grain Mass	-0.112	-0.062	0.012	**-0.367**		
	Number of Spikes per plant	0.128	0.063	0.012	**0.432**		
Water stress deficit	Constant (α)	-0.124	-1.932	0.543			
	Number of Spikes per plant	0.118	0.063	0.014	**0.387**		
	Number of Grains per Spike	0.099	0.065	0.006	**0.437**		
	1000 Grain Mass	-0.139	-0.073	0.013	**-0.543**	0.634	0.622
	Relative water content	0.102	0.061	0.014	**0.365**		
	Proline Content	0.125	0.074	0.003	**0.643**		

† NGSp, ChC, 1000GM and NSp indicate number of grains per spike, chlorophyll content, 1000 grain mass and number of spikes per plant, respectively. Also, values in main diagonal are direct effects.

Table 4: Path analysis of grain yield with related traits in cultivars of wheat under irrigation conditions

variables added to the model	indirect effect via				
	†NGSp	ChC	1000GM	NSp	Correlation coefficient with grain yield
NGSp	**0.434**	-0.111	0.189	-0.096	0.412
ChC	-0.083	**0.576**	-0.162	0.149	0.477
1000GW	-0.224	0.254	**-0.367**	0.124	-0.210
NSp	-0.097	0.199	-0.105	**0.432**	0.423

† NGSp, ChC, 1000GM and NSp indicate number of grains per spike, chlorophyll content, 1000 grain mass and number of spikes per plant, respectively. Also, values in main diagonal are direct effects.

Table 5: Path analysis of grain yield with related traits in cultivars of wheat under drought stress

variables added to the model	indirect effect via					Correlation coefficient with grain yield
	†NSp	NGSp	1000-GM	RWC	PC	
NSp	**0.387**	0.229	-0.373	0.307	0.393	0.931
NGSp	0.202	**0.437**	0.374	0.118	-0.149	0.978
1000-GM	0.266	-0.301	**-0.543**	0.195	0.201	-0.193
RWC	0.326	0.141	-0.290	**0.365**	0.421	0.942
PC	0.237	-0.101	-0.169	0.239	**0.643**	0.840

† NSp, NGSp, 1000-GM, RWC and PC indicate number of spikes per plant, number of grains per spike, 1000 grain mass, relative water content and proline content, respectively. Also, values in main diagonal are direct effects.

3.4 Factor analysis

Since coefficients of correlation may singly not provide thorough information about the relations of different traits and given the various advantages of multivariate statistical analyses for deep understanding of data structure, factor analysis was used in the current study. By means of varimax rotation which maximizes the variance among the factors, the factors which justify more percentage of variations among the characters have had more importance and must be studied. So, the effective characters on each factor are identified and the factors are named according to the most effective characters (Tadesse and Bekele, 2001). In factor analysis by means of major factors analysis and on base of specific numbers larger than 1, under normal and stress conditions three factors were identified under normal irrigation and four factors were identified under drought stress and they all together justify 91.23 and 92.43 percent of existent variation among the characters, respectively (Table 6 and 7).

Under normal condition the first factor which made 50.43 % of the total variation was composed of the spike length, number of tillers per plant, number of fertile tillers per plant, number of spikes per plant, number of grains per spike, 1000 grain mass and grain yield. So, first factor was named as grain yield and yield components factor. Factor 2, which accounted 25.67 % of the total variation, was composed of plant height and plant dry mass and thus this factor was called as biomass factor. Factor 3, which accounted 15.13 % of the total variation, included specific leaf area, relative water content, proline content, chlorophyll content and osmotic potential. Because these traits were related to physiology so, this factor was named as physiological factor. On the other hand, under drought stress condition the first factor justified 36.23 % of total variation which included number of spikes per plant, number of grains per spike, 1000 grain mass. Therefore, this factor was identified as yield components factor. The second factor was composed of specific leaf area, relative water content, proline content, chlorophyll content and osmotic potential explained 24.18 % of total variation. Thus this factor was called as physiological factor. Factor 3, which accounted 18.26 % of the total variation, included plant height and plant dry mass. So, this factor was named as biomass factor. Factor 4, which accounted 13.76 % of the total variation was composed of spike length, number of tillers per plant, number of fertile tillers per plant and grain yield and thus this factor was called as growth and grain yield factor. These results showed that cultivars with the highest values of these factors had the highest values for associated traits to those factors. In general, factor analysis showed which from the factors under normal and drought stress condition, yield components factor with description of high amount from total variation was common that it showed importance of related traits to it.

Naghavi et al. (2015) used factor analysis to reduce variables in wheat cultivars and they reported four factors (growth and grain yield, grain traits, biomass and root) and two factors (grain yield and biomass) under normal and drought stress, respectively. Khayatnezhad et al. (2010) on durum wheat cultivars showed that the importance of factor coefficients characteristics of growth traits (fertile tillers and main spike length), 1000-seed mass and yield selected genotypes is desirable under drought stress. Also, Gholamin et al. (2010) showed the importance of factor coefficients related to biomass and yield components for selection of desirable genotypes under dry conditions. In other studies on bread wheat cultivars, Dawari and Luthra (1991) revealed that number of grains per spike, spike length and harvest index were the main yield components and that the selection in terms of them could improve the yield.

Table 6: Factor analysis for agro-morphological traits in wheat cultivars under normal irrigation

Traits	1	2	3	Communalities
†PH	-0.159	0.608	-0.015	0.877
PDM	0.395	0.642	0.204	0.901
SLA	-0.412	-0.003	0.508	0.887
RWC	0.305	0.199	0.621	0.931
PC	0.278	0.251	0.748	0.922
ChC	0.290	0.198	0.814	0.909
OP	-0.343	-0.078	0.567	0.912
SpL	0.902	0.204	0.312	0.941
NT	0.936	0.229	0.235	0.949
NFT	0.909	0.301	0.109	0.907
NSp	0.907	0.278	0.205	0.919
NGSp	0.831	0.290	-0.346	0.859
1000-GM	0.649	0.389	0.223	0.894
GY	0.908	0.335	0.309	0.885
Eigen values	9.71	4.98	2.33	
Proportional variance	50.43	25.67	15.13	
Cumulative variance	50.43	76.10	91.23	

† PH, PDM, SLA, RWC, PC, ChC, OP, SpL, NT, NFT, NSp, NGSp, 1000-GM and GY indicate plant height, plant dry mass, specific leaf area, relative water content, proline content, chlorophyll content, osmotic potential, spike length, number of tillers per plant, number of fertile tillers, number of spikes per plant, number of grains per spike, 1000 grain mass and grain yield respectively.

Table 7: Factor analysis for agro-morphological traits in wheat cultivars under drought stress

Traits	1	2	3	4	Communalities
†PH	-0.034	-0.173	0.798	0.309	0.893
PDM	0.128	0.109	0.856	0.356	0.904
SLA	-0.325	0.656	-0.105	-0.267	0.899
RWC	0.304	0.890	0.187	0.197	0.889
PC	0.250	0.776	0.298	0.258	0.910
ChC	0.318	0.809	0.167	0.102	0.875
OP	-0.201	0.656	0.095	-0.219	0.876
SpL	0.373	0.232	0.184	0.687	0.924
NT	0.315	0.207	0.390	0.898	0.897
NFT	0.309	0.203	0.167	0.783	0.901
NSp	0.898	0.307	0.249	0.401	0.899
NGSp	0.786	0.150	0.193	0.308	0.932
1000-GM	0.843	0.209	0.295	0.411	0.902
GY	0.256	0.247	0.145	0.913	0.882
Eigen values	4.53	4.13	2.46	2.02	
Proportional variance	36.23	24.18	18.26	13.76	
Cumulative variance	36.23	60.41	78.67	92.43	

† PH, PDM, SLA, RWC, PC, ChC, OP, SpL, NT, NFT, NSp, NGSp, 1000-GM and GY indicate plant height, plant dry mass, specific leaf area, relative water content, proline content, chlorophyll content, osmotic potential, spike length, number of tillers per plant, number of fertile tillers, number of spikes per plant, number of grains per spike, 1000 grain mass and grain yield respectively.

3.5 Cluster analysis based on extracted factors

According to the importance of all the studied traits and regarding to the correlation among traits that was effective in different factors, cultivars grouped on the basis of all factors under normal irrigation (Figure 1) and drought stress (Figure 2). According to the impact of factor coefficients cultivars were grouped according to tolerance and sensitivity under drought stress (Table 8 and 9). Cluster analysis with cutting of discriminant analysis based on the three and four factors under two conditions, cultivars grouped into the two and three groups under normal irrigation and drought stress respectively (Figure 1 and 2).

Under normal irrigation, 9 cultivars such as Urum, Sabalan, Zarin, Sardari, Alvand, Azar, Homa, Pishgam and Mahabad landrace were classified in the first cluster, forming group1. Cultivars in this cluster are linked with the highest rate to first to third factors (Table 8, Figure 1). So, these cultivars have high values for physiological traits, biomass traits and grain yield and yield components. Second group comprises 6 cultivars such as Toos, Mihan, Alamoot, Navid, Shahriyar and Zare. Cultivars of this cluster showed the lowest values for first to third factor. So, these cultivars have the lowest values for grain yield and yield components and other traits (Table 8).

On the other hands, under drought stress in the first group were placed 6 cultivars such as Pishgam, Toos, Alamoot, Homa, Mahabad landrace and Mihan. These cultivars showed average values of all factors and so cultivars of this group were named semi tolerant (semi-sensitive) (Table 9, Figure 2). Also, the second group was comprised of 3 cultivars such as Navid, Zare and Shahriyar which showed the lowest values for factor 1 to 4, so these cultivars had the lowest values of physiological traits, biomass, yield components and grain yield (Table 9). Further, third group included Sabalan, Urum, Azar, Sardari, Zarin and Alvand cultivars with the highest values for all of the traits according to factor 1 to 4 (Table 9). Generally cluster 3 and 2 were the most tolerant and the most sensitive cultivars under drought stress (Figure 2). So, cultivars of these clusters with inter-cross can be used to increase grain yield in breeding programs.

Table 8: The average of traits for achieved groups from cluster analysis based on factor analysis in 15 wheat cultivars under normal irrigation

Clusters	Factor 1	Factor 2	Factor 3
1	125.549	126.749	129.729
2	113.738	108.396	118.639

Table 9: The average of traits for achieved groups from cluster analysis based on factor analysis in 15 wheat cultivars under drought stress

Clusters	Factor 1	Factor 2	Factor 3	Factor 4
1	45.745	47.498	9.094	42.375
2	38.709	42.439	5.984	37.630
3	48.230	56.264	13.395	44.629

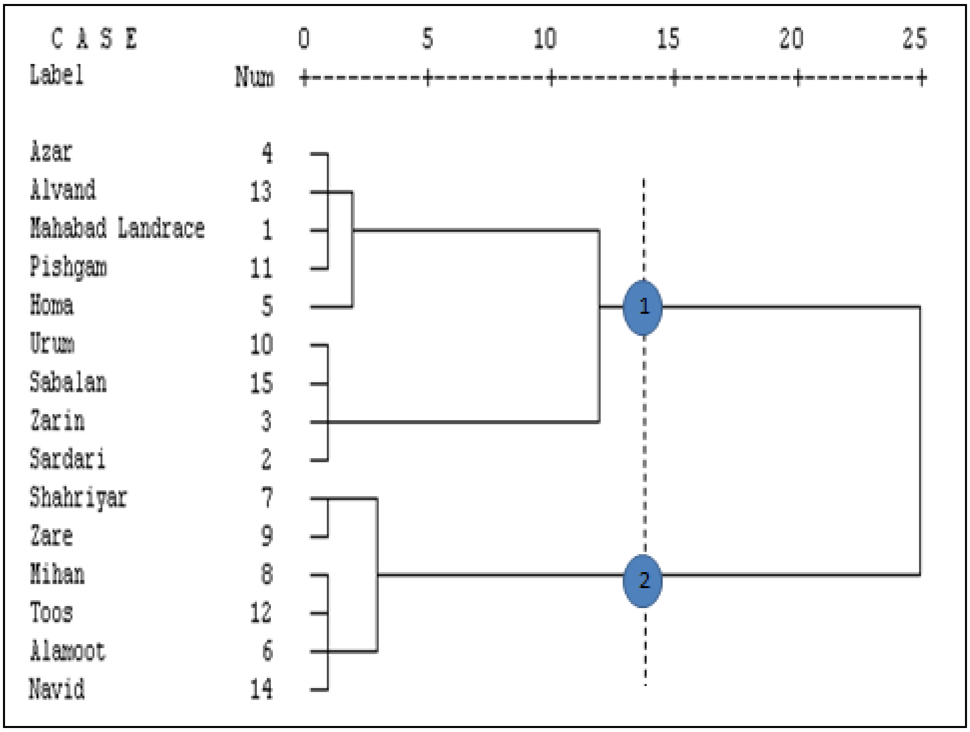

Figure1: Dendrogram for factors coefficient and cutting of discriminant analysis under normal irrigation

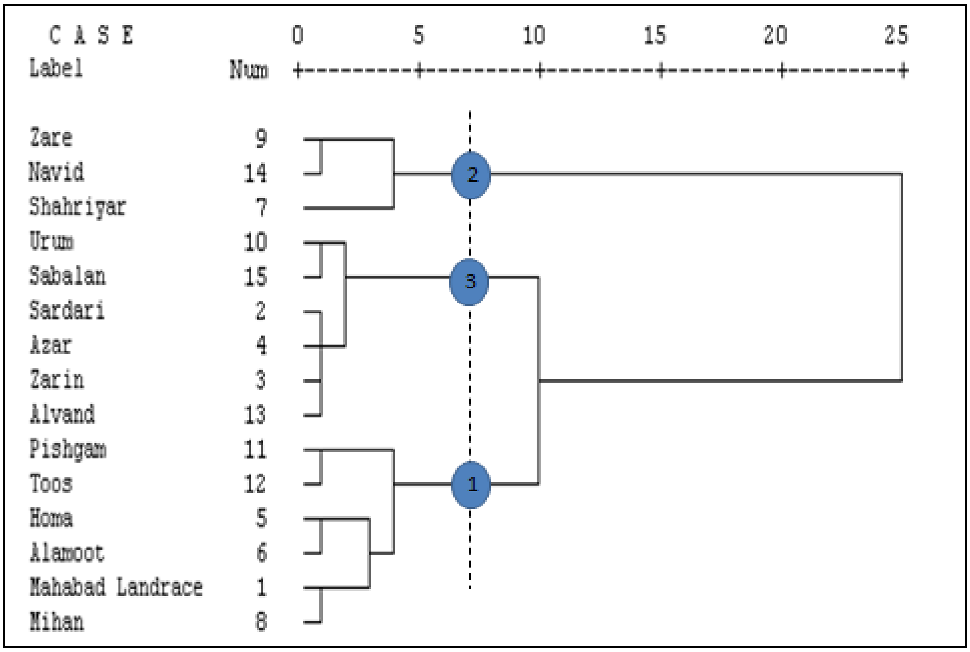

Figure 2: Dendrogram for factors coefficient and cutting of discriminant analysis under drought stress

4 CONCLUSIONS

Considering to our results, number of grains per spike and number of spikes per plant were the best criterion for improving grain yield in wheat under normal irrigation and drought stress conditions. So, screening for high values of these traits can bring increase in wheat grain yield under two conditions. Factor analysis detected three and four factors which explained 91.23 and 92.43 percent of the total variation in non-drought stress and drought stress conditions, respectively. In normal condition the first, second and third factor were identified as yield and yield components, biomass and physiological factors, respectively. While, under drought stress condition the first factor, second, third and fourth factors were named as yield components, physiological, biomass factor, growth and yield factor. Generally by cluster analysis with factors values was known "Sabalan, Urum, Azar, Sardari, Zarin, Alvand "and "Navid, Zare, Shahriyar" cultivars as the most tolerant and sensitive cultivars, respectively. Also, for further selection and breeding, parents may be selected from those clusters which had significant genetic distance for crossing in order to obtain genetic recombination and transgressed segregation in the subsequent generations. Also, it is suggested that in arid and semi-arid regions tolerant cultivars used directly.

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Effect of partial root-zone irrigation system on seed quality changes of Persian clover (*Trifolium resupinatum* L.) during seed development and maturation

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ABSTRACT

A field experiment was conducted in Aleshtar, Iran during 2015-16 growing season to evaluate the effect of limited water supply during seed filling period on seed quality changes of Persian clover. The experiment was carried out as a 2×6 factorial with three replications. Irrigation systems including conventional (all root system was irrigated) and partial root-zone irrigation (half of root system exposed to dry soil and the other half was watered) and harvest time (initiated from 10 days after flowering (DAA) and continued on six occasions at 5-day intervals) were used as treatments. Germination percentage, seedling dry mass, electrical conductivity and 1000-seed mass were used as seed quality traits. Seed quality attributes were significantly affected by harvest time and irrigation system, but the interaction of irrigation system × harvest time was not significant. While seed mass and electrical conductivity were not significantly affected by irrigation system, seedling dry mass and germination percentage were reduced under deficit irrigation. Partial root-zone irrigation reduced seed quality, Persian clover needs to experience no water deficit during seed formation and maturation period to produce high quality and quantity seeds.

Key words: persian clover; deficit irrigation; germination performance; seed production

IZVLEČEK

VPLIV DELNEGA NAMAKANJA KORENIN NA SPREMEMBE V KAKOVOSTI SEMENA PERZIJSKE DETELJE (*Trifolium resupinatum* L.) MED NJEGOVIM RAZVOJEM IN ZORENJEM

Za ovrednotenje vpliva omejene preskrbe z vodo v fazi polnjenja semen perzijske detelje na spremembe njihove kakovosti je bil v Aleshtarju, Iran, v rastni sezoni 2015-16 izveden poljski poskus. Poskus je bil 2×6 faktorski poskus s tremi ponovitvami. Obravnavanja so obsegala običajno namakanje (namakan je bil celoten korerninski sistem), delno namakanje (polovica koreninskega sistema je bila namakana, druga je ostala suha) in čas pobiranja semen (začetek 10 dni po cvetenju (DAF) in nato šestkrat v 5-dnevnih intervalih). Odstotek kalitve, suha masa kalic, električna prevodnost vode, v kateri je bilo namočeno vzorčeno seme in masa 1000 semen so bili uporabljeni kot znaki kakovosti semena. Na znake kakovosti semena sta značilno vplivala čas pobiranja semena in način namakanja posevka medtem, ko njuna interakcija ni imela značilnega vpliva. Na maso semen in električno prevodnost načina namakanja nista značilno vplivala, suha masa kalic in odstotek kalitve sta se ob pomanjkanju namakanja zmanjšala. Delno namakanje korenin je zmanjšalo kakovost semena, kar kaže, da potrebuje perzijska detelja za uspešno tvorbo in zorenje kakovostnega semena razmere brez pomanjkanja vode.

Ključne besede: perzijska detelja; deficitarno namakanje; kalitev; tvorba semena

1 INTRODUCTION

Persian clover (*Trifolium resupinatum* L.), originated from central Asia, is an important legume fodder crop in West-Asia, especially Iran. However, there is a serious shortage of high quality seed in the region, resulting from less attention of farmers to seed production of Persian clover. Furthermore, clover seed production also depends on the environmental conditions such as water limitation, prevailing during seed formation and maturation (Bakhiet et al., 2012).

Adequate water supply is necessary for optimum growth and seed production of crops. However, available water

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resources for agriculture have been decreasing in recent years, emphasizing on necessity of the efficient use of the limited water resources (Kang and Zhang, 2004). Water deficit conditions not only reduce seed yield of crops (Ghassemi-Golezani et al., 1997) but also negatively affect seed quality. In this case, Zehtab-Salmasi et al (2006) showed that limited water supply during seed development and maturation led to significant reduction in seed quality of dill. However, some researches revealed that seed quality was not significantly affected by water limitation during seed filling period (Eskandari et al., 2015; Vieira et al., 1992). These results show that different crops have different reaction to water shortage during seed filling period. Thus, the response of seed quality to low water supply during seed formation and maturation should be evaluated separately in each crop. Partial root-zone irrigation (PRI) is a new water-saving technique for substantial saving of irrigated water. Although the beneficial effects of this limited irrigation system has already investigated on several crops (Davies et al., 2002; Dasgan et al., 2009), its effects on seed quality need to be more documented.

Although it has been reported that irrigation scheduling affects the seed production process of clover (Singh and Kang, 2004), the knowledge about the influence of water deficit on seed quality of Persian clover is inconsistent and limited. Therefore, the current research was conducted to evaluate the reaction of seed quality of Persian clover to water shortage (induced by partial root-zone irrigation system) during seed development and maturation and respond the following questions: 1. What is the best developmental stage of Persian clover under well and limited irrigation at which maximum seed quality is attained? 2. In some crops such as lentil (Dabbagh-Mohammadi Nasab et al., 2001) maximum seed quality was achieved earlier under limited water supply. Does such trend also occur in Persian clover?

2 MATERIALS AND METHODS

The experiment was conducted in a research field in Aleshtar, Iran (33°51′N, 48°15′E) during 2015-2016 growing season. The effects of two factors including irrigation regime and harvest time were evaluated on the quality of Persian clover seed during seed formation and maturation. A 2×5 factorial based on randomized complete block design (RCBD) with three replications was employed to compare the treatments. The two irrigation regimes were conventional irrigation in which all root system parts were irrigated and fixed partial root-zone irrigation (PRI) in which half of root system was irrigated and half of it was exposed to dry soil. For partial root-zone irrigation system, cultivation furrows were watered every other furrows.

Persian clover seeds cultivar Aleshter, which is commonly cultivated in the region, were sown on 13 November 2015. All plots were fertilized with the same amount of fertilizer, containing 75 kg ha^{-1} P_2O_5, 75 kg ha^{-1} and 20 kg ha^{-1} N (as a starter fertilizer). At the end of March 2016, when seasonal precipitation ended and irrigation was essential, treatments of the experiment were started coinciding with the end of flowering and onset of seed filling period. Since Persian clover was cultivated for seed production, no forage cutting was applied during growing season. After flowering (a plot was considered as having flowered, when a minimum of 60 % of its plants flowered) plants from an area of 0.5 m^2 from each plot were harvested on six occasions, at 5-day intervals and 1000-seed mass, germination percentage, electrical conductivity of seed lot and seedling dry mass were determined.

The 1000-seed mass was determined by using the mean mass of 200 seeds in three replicates, using proportion. Two moist filter papers were placed in sterilized Petri dishes. Petri dishes had a diameter and a depth of 10 cm and 2 cm, respectively. Then, four replicates of 50 dried seeds (seeds were oven-dried at 75 ºC for 48 hours) from each sample were placed in sterilized Petri dishes and incubated at 18 °C for 10 days and germination percentage was determined. Both seminal roots and shoots of germinated seeds were cut and dried in an oven at 75±2 ºC for 24 hours. The dried seminal roots and shoots were weighed as seedling dry mass. Electrical conductivity of seed leachates was determined using two replicates of 50 fresh seeds from each sample. Seeds were weighed (SM_1 and SM_2) and each replicate immersed in 250 ml deionized water in a container at 20 ºC for 24 hours. The seed-soak water was then gently decanted and electrical conductivity (EC) was measured (EC_1 and EC_2) using EC meter instrument. The following equation was applied to calculate conductivity (Powell et al., 1984):

$$EC\ (\mu s\ cm^{-1}\ g^{-1}) = [(EC_1/SM_1) + (EC_2/SM_2)]/2$$

The data were tested for normal distribution. The data of germination percentage were transformed through inverse square root. However, for better comparison, the data were introduced as percentage in figures. ANOVA and the comparison of the means were carried out; using MSTATC statistical software (Khanlou, 2004) and Duncan’s multiple range test, respectively.

3 RESULTS AND DISCUSSION

Effects of harvest time and irrigation system were significant (*P≤0.05*) on seedling dry mass and germination percentage of clover seeds. Electrical conductivity and 1000-seed mass were significantly influenced (*P≤0.05*) by harvest time, but irrigation system had no significant effect on these traits. The interaction of irrigation system × harvest time was not significant on all traits (Table 1).

Table 1: The analysis of variance for the effect of irrigation method and harvesting time on qualitative indices of Persian clover

Source of Variance	df	Mean Square			
		seedling dry mass	germination	1000-grain mass	Electrical Conductivity
Replication	2	4.19^{ns}	4.08^{ns}	0.003^{ns}	267.22^{ns}
Irrigation (I)	1	148.03^{*}	182.25^{**}	0.039^{*}	252.18^{ns}
Harvest time (H)	5	175.56^{**}	4108.05^{**}	0.479^{**}	583.08^{*}
I×H	5	7.96^{ns}	8.89^{ns}	0.003^{ns}	76.43^{ns}
error	22	19.34	6.75	0.015	0.836
CV (%)		18.48	4.80	13.73	11.35

ns, * and ** indicate not significant, significant at P≤0.05 and P≤0.01, respectively

Low water supply, induced by partial root-zone irrigation system, reduced seedling dry mass and germination percentage (Table 1). Although low watering did not significantly changed seed mass of Persian clover, this does not mean that no seed yield reduction was occurred. Seed yield is depended on seed size (mass) and number. However, seed yield of Persian clover was significantly (*P≤0.01*) reduced by limited irrigation (the seed yield of Persian clover under conventional and partial root-zone irrigation systems was 472 kg ha^{-1} and 287 kg ha^{-1}, respectively) showing that seed number per plant experienced reduction under water deficit condition. Low seedling dry mass, resulted from low germination percentage, and lower seed yield revealed that Persian clover need to be well watered during seed formation and maturation to produce appropriate seed in terms of quality and quantity.

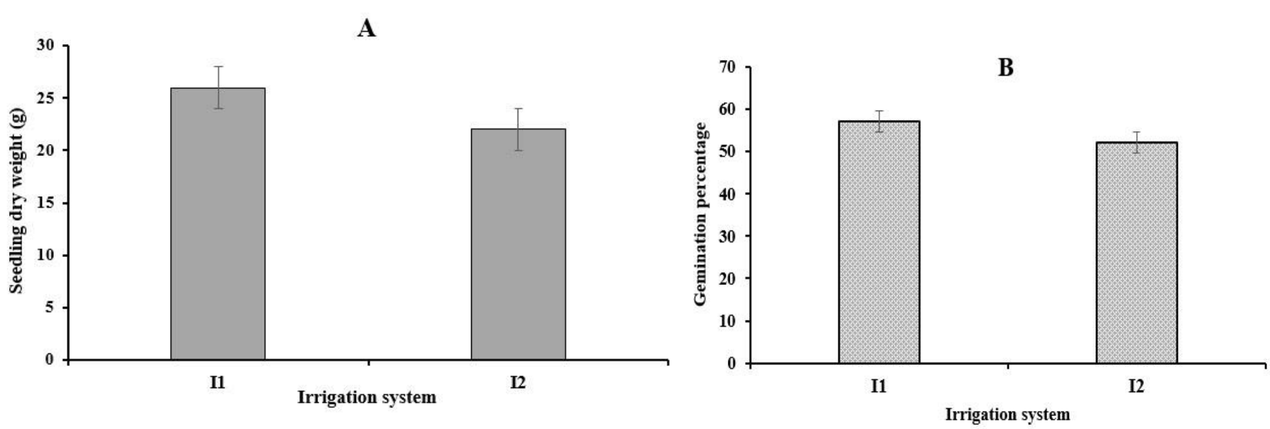

Figure 1: Effect of irrigation system on seedling dry mass (A) and germination percentage (B) of Persian clover. I_1: conventional irrigation; I_2: Partial root-zone irrigation

With increasing maturity, seed mass of Persian clover were increased. The highest seed mass was attained at 35 days after flowering (DAF). However, seed mass at this date was not significantly (P≤0.01) different with the dates of 25 DAF and 30 DAF. Therefore, it can be declared that Persian clover reached the highest seed mass at 25 DAF (Figure 2) .Completing the necessary structures of seed during maturation and increasing the number of endospermic cells have been reported to be two main reasons for increasing seed dry mass during seed filling period (Madandoust, 2006). However, it has been observed that at the end of seed filling period, increasing in seed cells respiration may lead to reduction of seed dry mass (Eskandari et al, 2013). However, such a seed dry mass reduction was not recorded in this research.

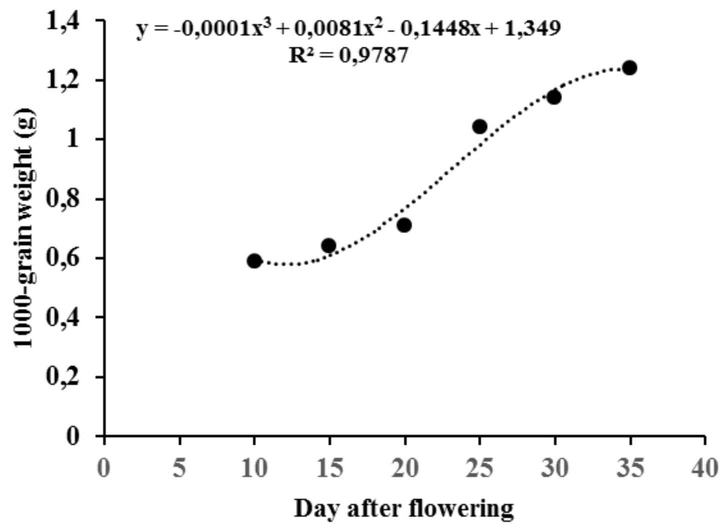

Figure 2: 1000-seed mass of Persian clover during seed development and maturation

Seedling dry mass has an increasing trend (Figure 2) reaching to its highest value at 30 DAF. The highest seedling dry mass was achieved (30 DAF) five days after reaching the highest seed mass (25 DAF). Thus, achieving the maximum mass of seed and seedling dry mass were not coincided (Figure 2 and Figure 3).

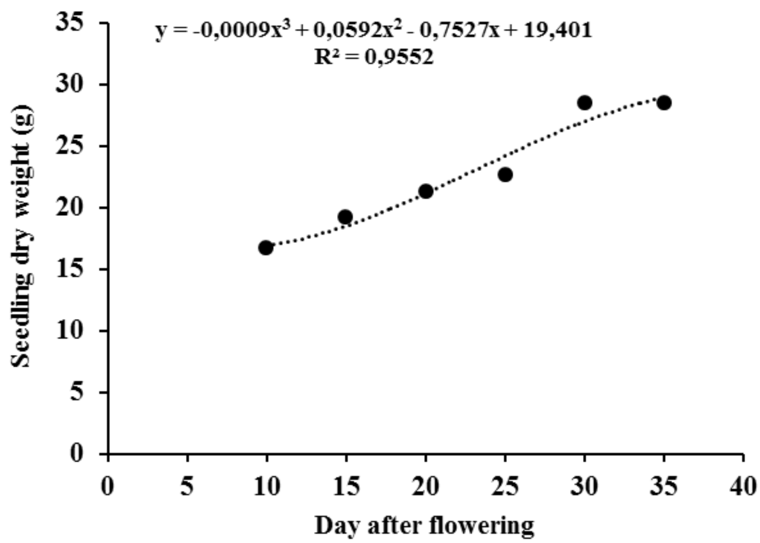

Figure 3: The variation of seedling dry mass of Persian clover during seed development and maturation

Germination percentage of Persian clover was increased with increasing in harvest time. The highest germination percentage was recorded at the last harvest stage (35 DAF). In the other word, the time of reaching maximum germination was not coincided with the highest seed mass. It has been declared that low germination, seed mass and seedling dry mass at early stages of seed growth and development is related to seed immaturity (Ghassemi-Golezani et al., 1996). In the early stages of seed growth and development, seeds essential structures are not fully formed (Rasyad et al., 1990) resulting in low seed germination performance.

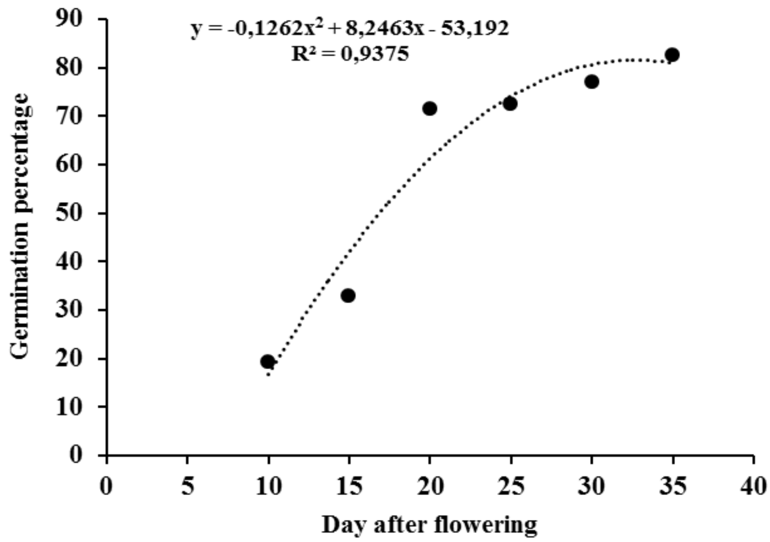

Figure 4: The variation of germination percentage of Persian clover during seed development and maturation

Electrical conductivity of Persian clover seed was lowered as harvest time increased. The lowest electrical conductivity was observed at 35 DAF which was not significantly ($P \leq 0.01$) difference with harvest time of 25 DAF (Figure 4). In fact, the time of reaching minimum electrical conductivity was not coincided with the highest value of seed mass. Higher electrical conductivity shows immaturity of seed. In early stages of seed growth and development, internal materials of seed leak out, resulting in higher electrical conductivity. However, with the increasing in seed growth and development and formation and consolidation of necessary structures, seed cell leaking decreases (Ghassemi-Golezani et al., 1997) resulting in the reduction of electrical conductivity.

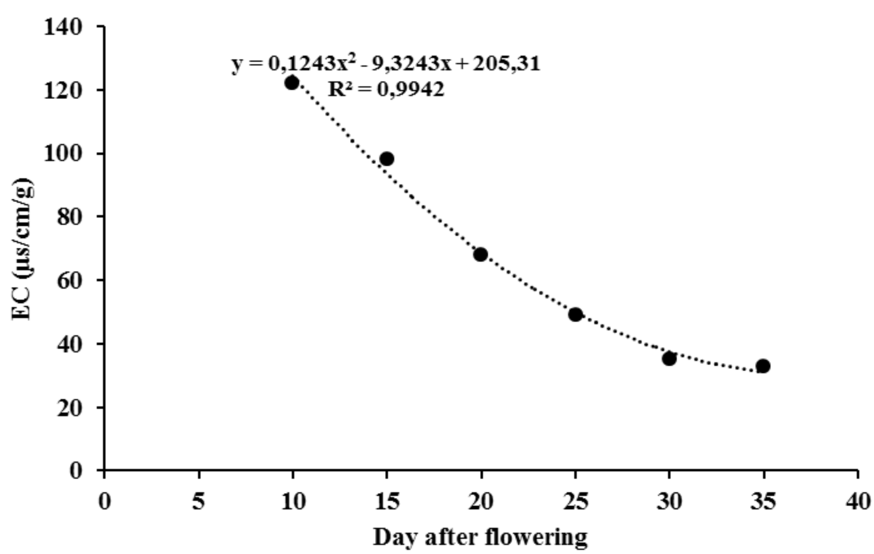

Figure 5: The variation of electrical conductivity of Persian clover during seed development and maturation

According to Harrington (1972), maximum quality of seeds is attained at the end of the seed filling period, thereafter seeds quality declines because they then begin to age. This stage was termed physiological maturity (Sanhewe and Ellis, 1996). Although Harrington's (1972) hypothesis has been shown to be occurred in some crops such as triticale (Bishnoi, 1974), wheat (Rasyad et al. 1990) and maize (Tekrony and Hunter, 1995), it has been also observed that maximum seed quality was only attained sometime after the end of the seed filling period, contradicting Harrington's hypothesis (Demir and Smith, 2001, Demir et al. 2002).

In the current experiment, while the highest seed mass attained at 25 days after anthesis (DAA), seed germination, seedling dry mass and electrical conductivity were improved up to 30 DAA. Thus, the results of this experiment revealed that physiological maturity of Persian clover is occurred sometimes after mass maturity. In other words, this experiment does not support Harington’s (1972) hypothesis. In conclusion, Persian clover reached to highest seed quality 30 DAA and needs to experience no water deficit during seed formation and maturation period to produce high quality and quantity seeds.

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The use of the model for determining potato (*Solanum tuberosum* L.) tuber distribution in the soil

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ABSTRACT

The paper focuses on the testing of a model for determining the distribution of potato tubers in the soil. Analytical testing of the model was performed at the laboratory of the Biotechnological Faculty (University in Ljubljana) in 2015 and in the same year, the model was tested in practice on a field owned by the company Zeleni Hit d.o.o. in Ljubljana. After the laboratory testing, the results were analyzed and additional steps were taken to expedite field measurements. To optimize the determination of the distribution of potato tubers in the soil, the program was upgraded to include three-dimensional data acquisition. This allows accurate determination of the horizontal, vertical and longitudinal spans of the distribution of tubers in the soil. Specifically, the program calculates the shape of the tubers, vertical cover of tubers with soil and their minimum distance from the left and right edges of the ridge. The program also locates the center of the tubers, which is a key parameter (along with tuber mass) for determining the area of the tuber cluster. The laboratory testing of the model revealed successful data processing of the program and adequate precision analytics. The testing of the model in the field on Arizona potato variety revealed that the model includes all the data necessary for further processing. Based on the calculated data, it can be assumed with great certainty that the model enables the acquisition of all necessary data and accurately determines the distribution of potato tubers in the soil, ideal shape of the ridge and the minimum necessary depth and distance for the planting of Arizona seed potatoes.

Key words: potato; potato tubers; distribution in the soil; mathematical model; ridge

IZVLEČEK

UPORABA MODELA ZA UGOTAVLJANJE RAZPOREDITVE GOMOLJEV KROMPIRJA (*Solanum tuberosum* L.) V TLEH

V letu 2015 smo na laboratoriju Biotehniške fakultete Univerze v Ljubljani preizkusili model za določanje razporeditve gomoljev krompirja v tleh. Praktično vrednost modela pa smo istega leta preizkusili na polju podjetja Zeleni Hit d.o.o. v Ljubljani. Pri preizkusu modela v laboratoriju smo določili korake za opravljanje meritev in opravili analizo rezultatov. Za optimalno določitev razporeditve gomoljev krompirja v tleh smo nadgradili program, ki sedaj omogoča 3D zajem podatkov. S tem lahko natančno ugotovimo horizontalni, vertikalni in vzdolžni razpon razporeditve gomoljev v tleh. S pomočjo programa za preračun oblike gomoljev lahko natančno določimo tudi vertikalno pokritost gomoljev ter minimalno oddaljenost od levega ter desnega roba lehe. Določimo lahko tudi center gomoljev, ki je skupaj z maso gomoljev ključen parameter za izračun ploščine ogrinjače. Ob preizkusu modela v laboratoriju smo ugotovili, da je s programi mogoče natančno zajeti podatke ter jih v programih tudi primerno obdelati. Tudi pri preizkusu modela na poskusnem polju smo na sorti krompirja Arizona spoznali, da je mogoče zajeti vse podatke, ki so potrebni za nadaljnjo obdelavo. Na podlagi izračunanih podatkov lahko z veliko gotovostjo trdimo, da model omogoča pridobivanje vseh potrebnih podatkov, s katerimi lahko merimo in zanesljivo napovemo razporeditev gomoljev krompirja v tleh, optimalno obliko lehe ter minimalno potrebno globino in razdaljo sajenja semenskega gomolja krompirja pri sorti Arizona.

Ključne besede: krompir; gomolji; razporeditev gomoljev v tleh; matematični model; leha

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[5] The program was developed at the Biotechnical Faculty, Department of Agronomy in cooperation with Microsoft programmers within Microsoft .NET initiative in 2002 in object-oriented programming language C# (C-Sharp).

1 INTRODUCTION

Optimal shape of the potato ridge and adequate soil coverage of potato tubers are crucial technological parameters in potato cultivation. Producers are faced with various damages and deformations of potato crop, which are in direct connection with suboptimal formation of the ridges, shallow planting of seed tubers etc (Kouwenhoven, 1967; Kouwenhoven 1970). Inadequate size of potato ridges may lead to potato greening, fungal diseases and other faults on marketable crop (Vučajnk, 2006). Horizontal and vertical distribution of potato tubers differs greatly among potato varieties. Vučajnk (2009) demonstrated the importance of vertical soil cover in his experiments. This parameter is linked to the cross-sectional area of the ridge, planting depth and the time of mechanical soil covering (Vučajnk, 2009). The amount of soil in the ridge also affects soil temperature. The better the vertical soil cover the lower the temperature of the soil surrounding the seed potato. The temperature rises towards the surface of the ridge, which is particularly problematic in summer months. Shallow planting and undersized ridges cause higher soil temperatures, which promote secondary growth of potato tubers and higher percentages of non-marketable potato crop per production area. Similarly, the amount of soil water is reduced towards the surface of the ridge. The lack of moisture (Dolničar et al., 2004) in the soil during seed tuber germination can be a significant problem in potato cultivation. Moreover, smaller vertical soil cover increases the likelihood for development of green tubers and infection with potato blight (Phytophthora infestans (Mont.) de Bary). Contrary, mechanical potato harvesting is facilitated in fields with optimally-formed ridges (Godeša, 2002) and fewer tubers get damaged during the harvest. Knowing the position of tubers in the ridge is therefore extremely important for the development of mechanization, particularly ridgers and harvesters (Hawkins, 1957). Optimal soil cover of potato tubers varies from 5 to 10 cm (Kouwenhoven et al., 2003). Vučajnk (2006) determined the optimal cross-section area of the ridge in 90 cm inter-row spacing in range of 1211-1320 cm^2. Kouwehoven et al. (2003) proposed minimal cross-sectional ridge area of 900 cm^2 for varieties with longer tubers and larger horizontal tuber span. Bugarčič (2000) also stressed the importance of trapezoid cross-section of the ridge, ridge height of 20-25 cm and central location of seed tubers in the potato ridge. The determination of vertical, horizontal and longitudinal span of the ridge is crucial for assessing tuber distribution in the soil. Bernik et al. (2015) demonstrated significant variability in horizontal and vertical tuber distribution among different potato varieties. No measurements on longitudinal distribution have been performed until now. In our research, the existing two-dimensional method of determining the position of the potato tuber in the ridge was upgraded to a three-dimensional method. This way, we obtain accurate information about the expansion of tubers, in the vertical, horizontal and transverse direction. The three-dimensional determination also shows the extreme points of potato expansion, by which we can calculate precisely the complete growth area of the potato tubes in the ridge.

2 MATERIALS AND METHODS

A one-year experiment was carried out on the surfaces of the company GREEN HIT d.o.o. in Ljubljana (Slovenia). The soils were defined as silty clay loam. In order to test the practical applicability of the method, the experiment was processed according to the integrated method, which is most present in the farmer's fields. Zeleni Hit produced potatoes in four-year crop rotation. Potatoes were planted in rows of 68 m long. The distance between rows was 75 cm. The planting distance in the row was 25 cm. The width of the entire plot was 12 m. The experimental field was fertilized according to the Zeleni hit fertilization plan, which is also used in their potato production. Before planting, we used 1000 kg/ha of fertilizer Entec 14-7-7. At hilling, we used 150 kg of KAN (27 % N) and 300 kg of potassium sulfate (Table 1).

Table 1: Used fertilizers
Tabela 1: Uporabljena gnojila

Fertilizer plan	N (kg / ha)	P_2O_5 (kg / ha)	K_2O (kg / ha)	MgO (kg / ha)
Recommended quantities	100 - 200	120 - 150	200 - 320	60 - 160
1000 kg ENTEC 14-7-17 + 2MgO + ME 150 kg KAN 27 % 300 kg POTASSIUM SULPHATE 0-0-51	180.5	70	323	23

In the experimental field we used mechanical weed control. We did not use any plant protection products against weeds, pests and pathogens.

In the experiment, we used potato variety Arizona. Arizona is a medium early variety with a yellow skin and light yellow flesh.

The nearest meteorological station is the Ljubljana Bežigrad. The duration of the experiment was from April to August 2015, therefore we use information on average, maximum and minimum temperature and precipitation for the duration of the experiment.

The period from 1.4.2015 to 31.8.2015 was on average about 3 ^{o}C warmer than the long-standing average (Table 2). The biggest deviation was in July, which was just 4.4 ^{o}C warmer than the long-standing average. The smallest difference was in April, when the average temperature was 1.9 ^{o}C higher than the long-term average. In the period from 1.4.2015 to 31.8.2015, the rainfall was 127.9 mm less than the long-term average (Table 3). The biggest deviation was in April, when 63.2 mm of rainfall was less than Long-term average. The smallest difference was in July, when there was less than 4 mm precipitation, which is a long-term average.

From this we can conclude that the spring was not in favor of a good emergence of potatoes, as the moisture in the soil was lacking. Therefore, it was especially important that the soil was well prepared and the planting was carried out optimally. Multiple shortages of precipitation and then their abundance is a major stress for potato growth. This can result in tuber cracking and secondary growth. Strong plates or floods also have a strong impact on the shape of the ridge. In July and August, a period of drought occurred again

Table 2: The average monthly air temperature in 2015, compared to the long-lasting average of the period 1961-1990 in Ljubljana Bežigrad (^{o}C) (meteo portal, 2015).
Tabela 2: povprečne mesečne temperature zraka v letu 2015 v primerjavi z dolgoletnim povprečjem 1961-1990 za Ljubljano Bežigrad (^{o}C) (Meteo portal, 2015).

Month	Year 2015	Year 1961-1990
	Average Temperature (° C)	Average Temperature (° C)
April	11.8	9.9
May	17	14.6
June	20.6	17.8
July	24.3	19.9
August	22.3	19,1
Average	19.2	16.26

Table 3: The average monthly rainfall in the year 2015, compared to the long-lasting average of the period 1961-1990 in Ljubljana Bežigrad (mm) (Meteo portal, 2015).
Tabela 3: Povprečna količina padavin v letu 2015 v primerjavi z dolgoletnim povprečjem 1961-1990 za Ljubljano Bežigrad (mm) (Meteo portal, 2015).

Month	Year 2015	Period 1961-1990
	Precipitation (mm)	Precipitation (mm)
April	46.8	110
May	114.9	122
June	150.4	155
July	117.6	122
August	96.4	145
Sum	526.1	654

At the beginning we determined individual steps for calculating the distribution of tubers in soil. The steps of the model were as follows:

1: Measurements were taken at several points in the ridge with a measuring device recording three-dimensional coordinates (Figure 1.). The device was specifically developed at the Biotechnical Faculty, Department of Agronomy. It measures absolute and relative distances in transverse direction in range of 1000 mm, in longitudinal direction in range of 450 mm and in vertical direction in range of 600 mm. The accuracy of the device is +/- 0.5 mm in all directions.

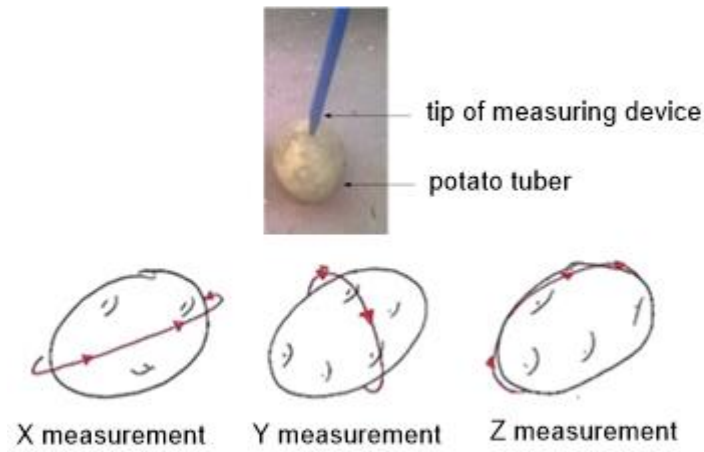

Figure 1: Three-dimensional measurements of the potato tuber
Slika 1: Tri dimenzionalno merjenje gomoljev krompirja

2: The acquired data were elaborated with the use of the program for performing 3D measurements (Figure 2.). The program was developed at the Biotechnical Faculty, Department of Agronomy and is primarily used for measuring the shape of the ridge and potato tubers in the field (Gospodarič and Fajdiga, 2016).

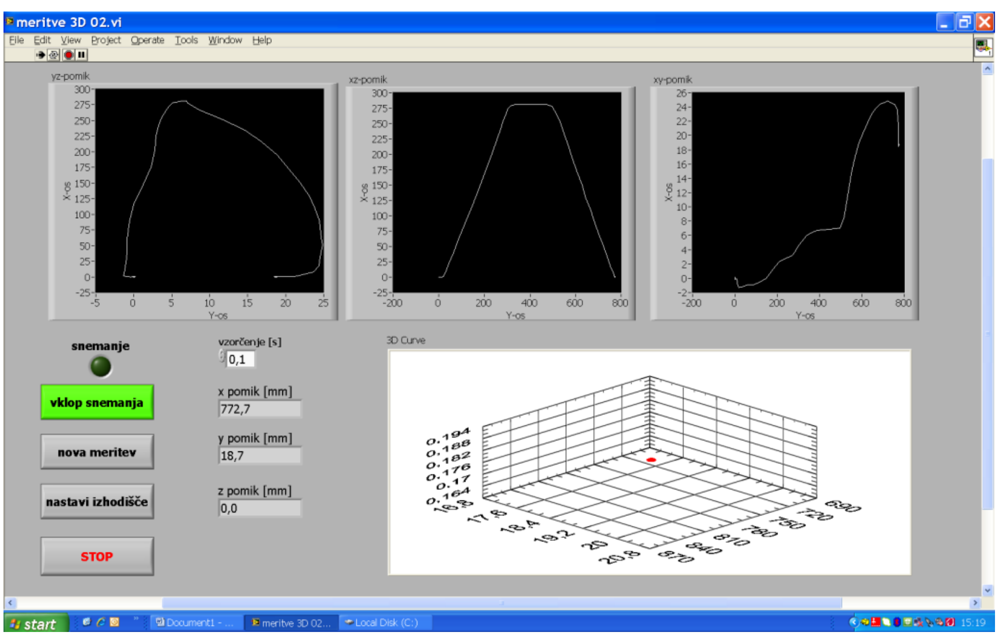

Figure 2: Pop-up window of the 3D measuring program
Slika 2: Grafični vmesnik programa 3D merilni program

3: Data were processed in Microsoft Excel and the following parameters were obtained:

- Vertical span of an individual tuber in the soil.
- Horizontal span of an individual tuber in the soil.
- Longitudinal span of an individual tuber in the soil.
- Vertical span defines the maximum distance from the lower to the upper point of the potato tuber. When combining the data of vertical span for all tubers the

program denotes the span from the lowest point of the lowest tuber to the highest point of the topmost tuber in relation to the height of the ridge. Vertical span of potato tubers is presented on the Y axis.

- Horizontal span defines the maximum distance from the right to the left edge of a potato tuber. When combining data of horizontal span for all tubers the program denotes the span of tubers situated at the extreme right to the extreme left point in the ridge – perpendicular to the ridge. Horizontal span of potato tubers is presented on the X axis.
- Longitudinal span defines the maximum distance from the front to the back edge of the potato tuber. When combining the data of longitudinal span for all tubers the program denotes the span of tubers situated at the extreme front to the extreme back in the ridge – longitudinal to the ridge. Longitudinal span of potato tubers is presented on the Z axis.
- Transformation of data for processing in the 'Calculation of the form and distribution of potato tubers' program.

4: Data processing in the 'Calculation of the form and distribution of potato tubers' program. The program was developed at the Biotechnical Faculty, Department of Agronomy in cooperation with Microsoft programmers within Microsoft .NET initiative in 2002 in object-oriented programming language C# (C-Sharp). The program requires ».Net» platform version 3.5 because it is written in C# version 3.0. After completion of data processing the following output data were acquired:

- Minimum vertical distance of the potato tuber from the edge of the ridge, which corresponds to minimum vertical soil cover of potato tubers.
- Minimum distance of the potato tuber from the left edge of the ridge.
- Minimum distance of the potato tuber from the right edge of the ridge.
- Minimum distance of the potato tuber from the ridge, irrespective of the position.
- Ridge cross-sectional area.
- Transformation of data for processing in a mathematical model enabling calculation of the area surrounding individual tubers (the central point of the tuber is depicted with x;y coordinates)

5: Calculation of the area surrounding the tuber, which corresponds to the growth space of the potato tuber. The calculation was made with the use of MatLAB mathematical model. Previously employed methods proved insufficiently accurate and therefore, the model was improved in 2012 at the Department of Agronomy, Chair of Phytomedicine, Agricultural Engineering, Crop Production. A compact mathematical model was developed in MatLAB software, which enables the calculation of cross-sectional area of tuber distribution in the ridge (Potrpin and Bernik, 2015). The cross-sectional area was termed tuber cluster. The method for tuber cluster assessment is based on a minimum convex polygon (Burgman et al., 2013) and represents the convex area around the potato tuber (Figure 5). The mathematical model also incorporates the volume of potato tubers, which is calculated based on potato mass and density (Cedilnik, 2012). Potrpin and Bernik (2014) performed an experiment for assessing average density of potato tubers and reported insignificant differences in potato density among the studied varieties (1.11 g/cm^3). The value was used in mathematical function along with the mass of individual tubers. First, the position of the tuber in the ridge was recorded and then the tubers were cleaned and weighted. Mathematical model for assessing tuber cluster assumes that the existing volume of the potato is equal to the equivalent volume of the sphere. A corrective parameter may be used to reduce the statistical error as potato tubers are not uniform in shape.

3 RESULTS AND DISCUSSION

Individual steps of the 3D mathematical model for calculation of the distribution of tubers in the ridge were first tested in the laboratory of the Department of Agronomy at the Biotechnical Faculty in Ljubljana. A metal ridge model was prepared based on an ideal shape of the ridge and three potato tubers were placed in the model to test the suitability of the mathematical model.

Three potato tubers were placed across the width of the model ridge for horizontal measurements. For vertical measurements, the potato tubers were placed at different heights in the ridge and for longitudinal measurements the three tubers were placed lengthwise in the model ridge (Figure 3.). Polystyrene foam was placed between the tubers if necessary and only served as a tool, which kept the tubers in the desired position. Positions of individual tubers were recorded and data were processed in specially developed programs.

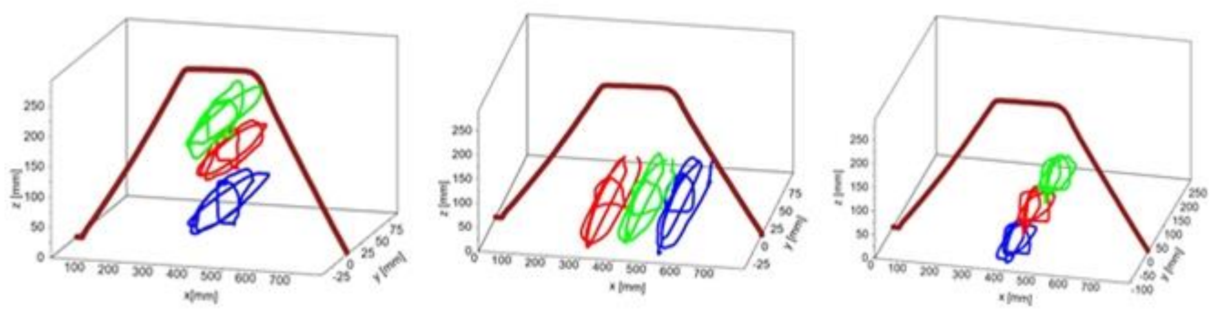

Figure 3: The initial display of the data readings in 3D space
Slika 3: Preliminarne meritve v laboratoriju

The analysis of the results confirmed operational suitability of the device for three-dimensional data acquisition. Recorded data provide all necessary information for determining the distribution of potato tubers in the soil and calculation of tuber cluster area regardless of the position of tubers in the soil (horizontally, vertically or longitudinally to the ridge).

After laboratory measurements a field trial was conducted at ZELENI HIT d.o.o. potato fields located in Ljubljana, Slovenia in order to test the suitability of the method and model in practice. Measurements of potato tuber distribution were recorded before the harvest and all measuring sites were marked with poles. The poles were not moved throughout the experiment and were regarded as reference points (coordinate origin) for all additional measurements at the location. The device was positioned above the site and adjusted to the horizontal position in all directions with the aid of screw spindles. The measuring tip was adjusted to the reference pole and computer readings were recorded. The measuring tip was moved by hand along the surface of the ridge until reaching the opposite side of the ridge. At that point the device was disconnected and a diagram depicting the shape of the ridge appeared on the computer screen. Additionally, the program calculated cross sectional area of the ridge (cm^2). The distribution of potato tubers in the ridge was also determined with the same measuring device. Average cross-sectional area of the ridge (442.11 cm^2) was estimated from the minimum (289.48 cm^2) and maximum recorded values (572.41 cm^2).

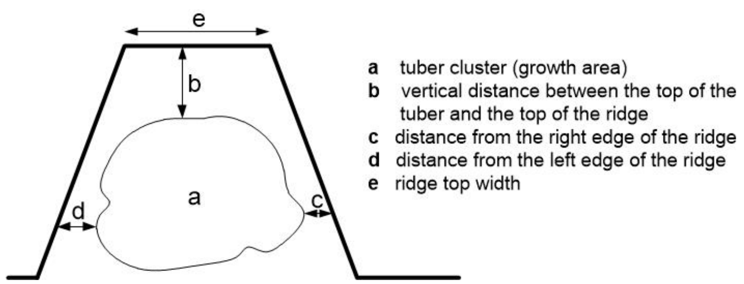

Figure 4: Ridge
Slika 4: Greben (leha)

Vertical distance denotes the distance between the top of the highest potato tuber and the top of the ridge (Figure 4). Negative values in the tables were ascribed to tubers which were not fully covered by the soil and were subjected to potato greening. Average vertical distance was -5.3 mm, suggesting that most potato tubers were partly located at the ridge surface. Typically, tubers should be covered with soil at least 5 cm thick.

Additionally, horizontal, vertical and longitudinal span of potato tubers in the ridge were determined. Average horizontal span of the tubers was 272.67 mm, average vertical span was 119.54 mm and average longitudinal span in the ridge was 247.77 mm. Based on maximum recorded longitudinal span of 'Arizona' potato tubers it can be concluded that this variety should be planted at higher planting distances of at least 368 mm. Moreover, somewhat higher ridges should be formed for this variety as the maximum recorded vertical span of potato tubers in field experiments was 184 mm. If we add the recommended 50 mm of soil for optimal potato cover the height of the ridge should be at least 234 mm. The results are in accordance with data reported by Bugarič (2000), who recommended ridge height between 200 and 250 in standard potato production. Optimal ridge top width is in direct connection with suitable soil cover of the tubers and is defined based on the horizontal span of potatoes in the ridge. Ridge top width for Arizona variety should be 28 cm (Table 1), which corresponds to previous reports of Vučajnk (2009). The mathematical model also enabled the calculation of tuber cluster area in the experimental ridge in the field (Table 1). Tuber cluster area is directly linked to cross-sectional area of the ridge. The larger the tuber cluster area the larger the cross-sectional area of the ridge. Recommended cross-sectional area of the ridge for Arizona potatoes was calculated based on maximum tuber cluster area and should be at least 794 cm^2. According to the report by Arends and Kus (1999) this is a medium-sized ridge and therefore, at least 75 cm inter-row spacing should be maintained (Table 4).

Optimal ridge form for the Arizona potato variety was sketched based on the recordings and is presented in Figure 5 and Table 4.

Table 4: Optimal ridge form for the Arizona potato variety
Preglednica 4: Oblika optimalnega grebena za sorto Arizona

Recommended inter-row distance (a)	75 cm
Recommended height of the ridge (v)	23.4 cm
Recommended ridge top width (c)	28 cm
Recommended planting distance in the row	37 cm

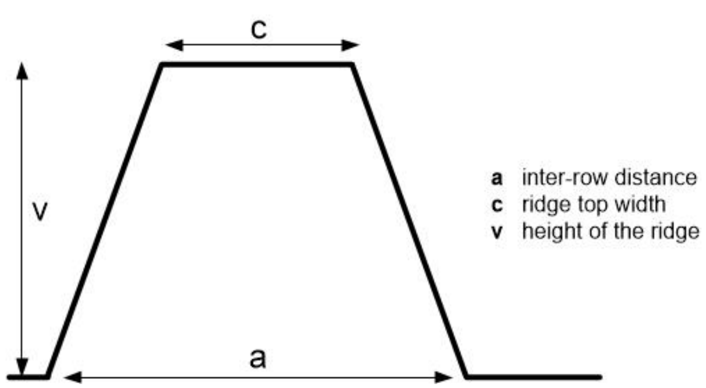

Figure 5: Basic dimensions of the ridge (Benec, 2015)
Slika 5: Greben in razponi (Benec, 2015)

4 CONCLUSION

The aim of the experiment was to evaluate the applicability of the mathematical model for determining potato tuber distribution in the soil at field conditions. A coordinate-measuring device was used, a 3D program was upgraded and mathematical model was developed in order to assess the dispersal of tubers in the soil. Most problems, which afflict potato producers derive from inadequate soil cover of potato tubers. Green tubers are formed as a result of light exposure. These represent non-marketable potato crop and consequently, a loss in field production capacity and farm economy. 3D model enables detailed determination of potatoes in the soil. Input data are then used for calculation of horizontal, vertical and longitudinal span of tubers in the soil (Figure 6). Data are available in .txt form and thus suitable for further processing.

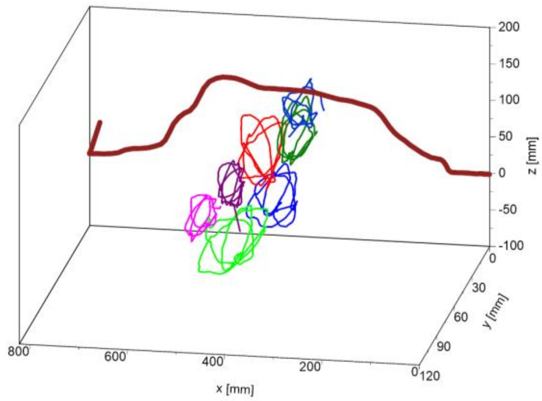

Figure 6: 3D graphics of one of the measuring sites
Slika 6: 3D grafični prikaz enega od merilnih mest

Further data processing in the 'CALCULATION OF THE FORM AND DISTRIBUTION OF POTATO TUBERS' program[5] determines the minimum vertical distance from the top of the highest tuber to the top of the ridge, tuber distance from the left and right edge of the ridge and its minimal distance from the edge of the ridge. This information is needed for determining optimal ridge form of a specific variety. A sketch of an ideal ridge for Arizona variety is presented in Figure 5. The model also enables recommendation on suitable planting depth and distance, inter-row spacing and other parameters crucial for optimally formed ridges. The data are useful for correct setting of mechanization for specific potato varieties as the distribution of potato tubers can be predicted with adequate certainty. Up to now, no research has been conducted on the effect of different planting techniques (inter-row spacing and planting distance) and weather conditions on potato tuber distribution and the shape of tuber cluster. Therefore, our further studies will be aimed to determine the connections among these factors.

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Vrednotenje pridelka koruze za sonarave oblike kmetovanja

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IZVLEČEK

Sodobne hibridne sorte koruze že več desetletij prevladujejo tako v Sloveniji kot drugod po svetu. V Sloveniji je v pridelavi ohranjenih le malo starih populacijskih sort, 614 pa jih vzdržujemo v sklopu programa Slovenska rastlinska genska banka na Biotehniški fakulteti Univerze v Ljubljani. Izboljšanje populacijskih sort z rekurentno selekcijo je mogoče in ima lahko v primeru posebnih lastnosti svoje mesto v tržni pridelavi. V sortnih poskusih v osrednji Sloveniji in v Beli krajini smo proučevali izbrane stare populacije koruze. Pridelki koruze so bili na obeh lokacijah in v obeh letih močno odvisni od genotipa. Populacije 'Rdeča bohinjka' in delno 'Rumena bohinjka', podobno pa tudi 'LJ 180' so imele majhne pridelke, bile pa so med najbolj ranimi, medtem ko sta imeli sodobni hibridni sorti, 'P 9074' in 'Ronaldinio KWS', pričakovano največji pridelek. Pridelek populacije 'P 9074' je bil na laboratorijskem polju Biotehniške fakultete skoraj dvakrat večji od druge najboljše populacije poltrdinke 'Metliška Plut'. Prav ta populacija pa se je v vseh štirih poskusih pokazala kot zelo primerna za nadaljno vzdrževanje in prijavo sorte, saj bi lahko v manj intenzivni pridelavi pomenila ustrezno alternativo hibridnim sortam.

Ključne besede: pridelek koruze; starejše akcesije; genska banka; populacijske sorte

ABSTRACT

EVALUATION OF MAIZE CROPS FOR SUSTAINABLE FARMING

Modern hybrid maize varieties dominated for several decades both in Slovenia and elsewhere in the world. The production is maintained only of few landrace populations, but some are maintained at Slovenian Gene Bank. Improvement of local varieties is possible by recurrent selection and may in the case of their specific characteristics deserve their cultivation for specific purposes. Presented experiments have been conducted to characterize selected old Slovenian accessions in two regions - in central Slovenia and in Bela Krajina. Yields of corn at both locations and both years were significantly depending on the genotype. 'Rdeča Bohinjka' and partially 'Rumena Bohinjka' and, similarly, the 'LJ 180' had a predominantly low yields but were also the earliest, while modern hybrid variety 'P 9074' and 'Ronaldinio KWS ' had the expected highest yield. Yield of 'P 9074' grown at the Laboratory field of the Biotechnical Faculty was almost twice higher than the second best accession 'Metliška Plut'. However it is this semi-flint accessions 'Metliška Plut' that proved very suitable at all four experiments and is recommended for further maintenance and varietal testing as it can serve as appropriate alternative in a less intensive production.

Key words: maize yield; older accessions; gene bank; cross pollinated varieties

1 UVOD

Pridelovanje koruze ima dominantno vlogo v slovenskem kmetijstvu že več desetletij. Kmalu po drugi svetovni vojni so se pričele pospešeno pridelovati hibridne sorte koruze, s tem pa se je genofond lokalnih populacij pričel hitro zmanjševati. Zato je profesor Franc Mikuž že v 50. letih dvajsetega stoletja pričel z zbiranjem lokalnih populacij, ki jih zdaj hranimo v slovenski rastlinski genski banki (Rozman 2012). Zbran material je bil tudi osnova žlahtnjenja domačih hibridnih sort koruze. Iz tega sklopa so tri med njimi, 'LJ-180', 'LJ 220W' in 'LJ-275t' na seznamu avtohtonih in tradicionalnih sort kmetijskih rastlin (Uradni list RS, št. 33/04 in 110/04).

Sodobne hibridne sorte koruze po pridelkih zvečine močno prekašajo starejše populacijske sorte (Kutka,

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2011). Vendar avtor meni, da je izboljšanje populacijskih sort z rekurentno selekcijo mogoče in ima lahko v primeru posebnih lastnosti svoje mesto v tržni pridelavi. Več avtorjev pa poudarja pomen lokalnih populacij kot virov določenih lastnosti primernih za uporabo v žlahtnjenju sodobnih sort. Tako denimo Rebilla in sod. (1998) in Shelton in Tracy (2015) poudarjajo uporabnost populacijskih sort sladke koruze za žlahtnjenje in ki bi bile primerne za pridelavo v severnih območjih Evrope in ZDA. Muntean in sod. (2012) pa poudarjajo uporabo inbridiranih linij pridobljenih iz lokalnih populacij za pridelovanje sintetičnih kultivarjev koruze prilagojenih lokalnim rastnim razmeram.

Izhodišče naše raziskave je bilo ugotoviti, ali je mogoče identificirati lokalne populacije koruze primerne za ekstenzivni način kmetovanja oziroma možnost njihove prijave kot sorte z dovoljenjem za trženje.

2 MATERIALI IN METODE

2.1 Poskus v letu 2015

Setev koruze je v letu 2015 potekala na dveh lokacijah, v Jabljah na Infrastrukturnem centru Kmetijskega inštituta Slovenije (kjer pridelava koruze poteka na konvencionalen način) in na ekološkem posestvu v Krasincu. Na obeh lokacijah smo zasnovali poskus z 9 različnimi populacijami koruze, in sicer 'Lj-180', 'LJ-275t', 'Rdeča bohinjka', 'Rumena bohinjka', 'Metliška Plut', 'Štajerska bela', 'Metliška rdeča', 'Prekmurska dolga', 'Belokranjska trdinka'. Poskus je potekal v treh blokih, znotraj katerih smo naključno razporedili različne populacije koruze. V Jablah smo koruzo sejali 8. maja, v Krasincu pa 30. aprila 2015.

Tabela 1: Seznam vključenih akcesij
Table 1: List of tested acessions

Akcesija	Izvor
'Rdeča bohinjka'	populacija SRGB 3299
'Rumena bohinjka'	populacija SRGB 3299
'Metliška Plut'	populacija SRGB 7081
'Štajerska bela'	populacija SRGB 3315
'Metliška rdeča'	populacija SRGB 3307
'Prekmurska dolga'	populacija SRGB 3252
'Belokranjska trdinka'	populacija. SRGB 3309
'LJ 180'	F1 hibrid Biotehniška fakulteta
'Lj 275t'	F1 hibrid Biotehniška fakulteta
'Ronaldinio KWS'	F1 hibrid KWS
'P 9074'	F1 hibrid DuPont Pioneer

Ocenjevali smo naslednje parametre: čas do metličenja, vlago ob spravilu koruze in pridelek koruze (ob 14 % vlagi) v t/ha. Spravilo koruze je v Jabljah potekalo 22. oktobra, v Krasincu pa 28. oktobra.

2.2 Poskus v letu 2016

Devet različnih populacij koruze smo v letu 2016 posejali na dveh različnih lokacijah, in sicer na Laboratorijskem polju Biotehniške fakultete Univerze v Ljubljani ter na ekološkem posestvu v Krasincu. V poskus smo vključili sorti 'LJ-180', 'LJ-275t' in populacije 'Rdeča bohinjka', 'Rumena bohinjka', 'Metliška Plut', 'Štajerska bela', 'Metliška rdeča', 'Prekmurska dolga', 'Belokranjska trdinka'. Poleg prej omenjenih smo za primerjavo posadili še hibrid 'P074 F1' na Laboratorijskem polju Biotehniške fakultete, in hibrid 'Ronaldinio' na ekološkem posestvu v Krasincu (Tabela 1).

V letu 2016 smo poleg pridelka ocenjevali še višino rastlin (v cm) in poleganje koruze. Na obeh lokacijah smo ocenjevali okuženost rastlin s koruzno progavostjo (*Setosphaeria turcica* (Luttr.) K.J. Leonard & Suggs). Uporabili smo ocenjevalno lestvico po Fullerton-u (1982), kjer ocena 4 predstavlja močno okužbo, ocena 3 pa zmerno okužbo. Poleganje rastlin smo ocenili z indeksom poleglosti, izraženim z vrednostjo od 0-4, kjer je bil indeks 0 nepoleglo, indeks 1 do 33 % poleglo, indeks 2 34 do 66 % poleglo, indeks 3 67 do 99 % poleglo in indeks 4 povsem poleglo glede na vizualno oceno.

Poskus je bil zasnovan v treh blokih, znotraj katerih smo naključno razporedili obravnavan populacije koruze. Na Laboratorijskem polju smo koruzo sadili 4. maja 2016; na ekološkem posestvu v Krasincu pa 26. aprila 2016.

2.3 Statistična analiza podatkov

Rezultate poskusa smo statistično ovrednotili s programom Statgraphics Centurion XVI (Statgraphics Centurion, 2009). Razlike v pridelku med posameznimi populacijami smo ovrednotili z analizo variance (ANOVA) in Duncanovim ter Student Newman Keulsovim testom mnogoterih primerjav (P≤0,05). Razlike v obsegu okužbe s koruzno progavostjo med populacijami koruze smo ovrednotili z analizo variance (ANOVA) in Student Newman Keulsovim testom mnogoterih primerjav (P≤0,05).

3 REZULTATI

3.1 Poskus v letu 2015

Akcesije so se med seboj razlikovale po ranosti, kar je razvidno iz ocene pojava metličenja (Jablje). Najzgodnejši je bil hibrid 'LJ 180', najpoznejša pa populacija 'Belokranjska trdinka'. Podobno so se na tej lokaciji akcesije razlikovale v vsebnosti vlage v zrnju ob spravilu, kjer je imel hibrid 'LJ 180' najmanjšo, 'Belokranjska trdinka' pa največjo (Tabela 2).

V Jabljah sta imeli značilno najmanjši pridelek populaciji 'Rdeča bohinjka' (3,87 t/ha) in 'Rumena bohinjka' (3,95 t/ha). Ob spravilu smo pri populaciji 'Rdeča bohinjka' zabeležili 26,3 % vlage v zrnju, pri populaciji 'Rumena bohinjka' pa 26,6 %. Signifikatno največji pridelek je imela populacija 'Metliška Plut' (7,7 t/ha). Vlaga zrnja ob spravilu je znašala 27,2 % (Tabela 2).

Table 2: Čas metličenja, vlaga in pridelek po posameznih akcesijah koruze na lokaciji v Jablah (2015)
Table 2: Days to tasseling, seed moisture and yield according to accessions on location Jable (2015)

Akcesija	Dnevi do metličenja	Vlaga ob spravilu (%)	Pridelek ob 14 % vlagi t/ha
'LJ 180'	49	27,8	6,35 bc*
'Rdeča bohinjka'	51	26,3	3,87 a
'Lj 275t'	52	29,1	6,86 cd
'Rumena bohinjka'	52	26,6	3,95 a
'Metliška Plut'	56	27,2	7,70 d
'Štajerska bela'	57	30,9	5,56 b
'Metliška rdeča'	59	29,9	5,56 b
'Prekmurska dolga'	59	29,9	5,85 bc
'Belokranjska trdinka'	61	32,8	6,84 cd

* različne črke v stolpcu pomenijo statistično značilno razliko po Duncanu (P≤0,05)

V Krasincu je imela značilno najmanjši pridelek populacija 'Rdeča bohinjka' (2,60 t/ha). Vlaga ob spravilu je znašala 28,4 %. Značilno največji pridelek je imela populacija 'Metliška Plut' (4,12 t/ha in 29,6 % vlage v zrnju) (Tabela 3).

Tabela 3: Delež vlage v zrnju ob spravilu ter pridelek po posameznih akcesijah koruze na lokaciji v Krasincu (2015)
Table 3: Seed moisture content and yield of maize populations evaluated on location Krasinec (2015)

Akcesija	Vlaga ob spravilu	Pridelek ob 14 % vlagi t/ha
'LJ-180'	30,9	3,27 bc*
'Rdeča bohinjka'	28,4	2,60 ab
'LJ -275t'	27,6	3,61 bc
'Rumena bohinjka'	26,2	2,13 a
'Metliška Plut'	29,6	4,12 c
'Štajerska bela'	29,5	3,10 abc
'Metliška rdeča'	29,8	3,60 bc
'Prekmurska dolga'	29,0	3,29 bc
'Belokranjska trdinka'	32,3	3,62 bc

* različne črke v stolpcu pomenijo statistično značilno razliko po Duncanu ($P \leq 0{,}05$)

3.2 Poskus v letu 2016

Na Laboratorijskem polju Biotehniške fakultete je imela značilno najmanjši pridelek zrnja populacija 'Rdeča bohinjka' (1,4 t/ha). Rastline omenjene populacije so bile poleg rastlin populacije 'Rumena bohinjka' (169 cm) značilno najnižje, s povprečno višino 176 cm (Tabela 4).

Pridelek zrnja je bil značilno največji pri populacijah 'Metliška Plut' (3,52 t/ha) in 'P 9074' (6,5 t/ha). Rastline populacije 'Metliška Plut' so bile v povprečju visoke 265 cm, rastline hibrida 'P 9074' pa 234 cm. Sorti z najvišjim pridelkom sta dosegli indeks poleganja koruze 0,4. 29,5 % vlage v zrnju smo namerili ob spravilu pri populaciji 'Metliška Plut'; 26,3 % vlaga pa je bila zabeležena pri hibridu 'P 9074'(Tabela 4).

Tabela 4: Višina rastlin, indeks poleglosti, vlaga ob spravilu in pridelek po posameznih akcesijah koruze na njivi na Laboratorijskem polju Biotehniške fakultete Ljubljana (UL) (2016)
Table 4: Plant height, lodging index, seed water content moisture and yield according to accessions on location at Laboratory field of Biotechnical Faculty Ljubljana (2016)

Akcesija	Višina rastlin (cm)	Indeks poleglosti (0-4)	Vlažnost zrnja ob spravilu	Pridelek ob 14 % vlagi kg/ha
'Rdeča bohinjka'	176 a*	1,7	25,1	1,47 a
'Rumena bohinjka'	169 a	3,0	22,6	1,62 a
'Prekmurska dolga'	214 bcd	1,7	27,6	2,06 ab
'Štajerska bela'	187 ab	1,7	30,1	2,4 abc
'Belokranjska trdinka'	258 e	0,4	34,8	2,84 bc
'LJ 180'	209 bc	2,0	22,5	2,95 bc
'Metliška rdeča'	250 e	0,4	31,1	3,03 bc
'LJ 275t	246 de	2,0	28,3	3,30 bc
'Metliška Plut'	265 e	0,4	29,5	3,52 c
'P 9074'	234 cde	0,4	26,3	6,50 d

* različne črke v stolpcu pomenijo statistično značilno razliko po Duncanu ($P \leq 0{,}05$)

Največji povprečni pridelek v Krasincu je imel hibrid 'Ronaldinio KWS' (7,28 t/ha), medtem ko je imel značilno najmanjšega zgodnji hibrid 'Lj 180' (1,78 t/ha).

Pridelek zrnja populacije 'Metliška Plut' je znašal 5,49 t/ha. Rastline slednje so bile v povprečju visoke 2,84 m (Tabela 5).

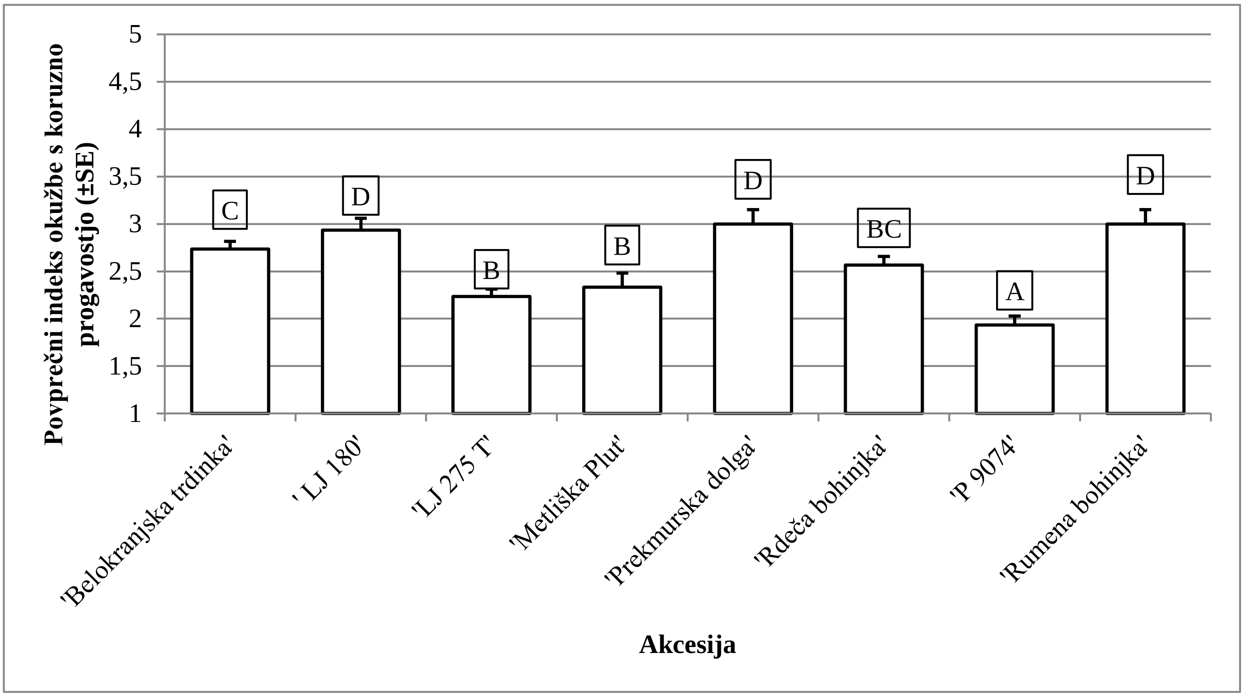
Tabela 5: Višina rastlin, indeks poleglosti, vlaga zrnja ob spravilu in pridelek zrnja po posameznih akcesijah koruze na njivi v Krasincu (2016)

Table 5: Plant height, lodging, seed moisture and grain yield according to accessions on location in Krasinec (2016)

Akcesija	Višina rastlin (cm*)*	Indeks poleglosti (0-4)	Vlažnost zrnja ob spravilu	Pridelek ob 14 % vlagi t/ha
'Rdeča bohinjka'	215 a	0,33	24,1	2,09a
'Rumena bohinjka'	238 ab	0,33	23,5	5,48bc
'Prekmurska dolga'	300 d	0,33	26,6	6,70bc
'Štajerska bela'	245 ab	0,33	28,1	5,69bc
'Belokranjska trdinka'	298 d	0,33	30,3	6,69c
'LJ 180'	216 a	0,33	23,1	1,78a
'Metliška rdeča'	298 d	0,33	26,0	6,43bc
'LJ 275t	257 bc	0,33	23,7	4,58b
'Metliška Plut'	284 cd	0,33	24,7	5,49bc
'Ronaldinio KWS'	252 b	0,33	22,9	7,28c

* različne črke v stolpcu pomenijo statistično značilno razliko po Duncanu (P≤0,05)

V letu 2016 smo na obeh lokacijah ocenjevali tudi obseg okužbe s koruzno progavostjo (*S. turcica*). Na Laboratorijskem polju Biotehniške fakultete je bila povprečna ocena značilno največja pri populaciji 'Prekmurska dolga' (3,00±0,15) in hibridu 'LJ 180' (2,93±0,13). Povprečni obseg okužbe je bil značilno najmanjši pri hibridu 'P 9074' (1,93±0,09), za populacijo 'Metliška Plut' pa je znašal 2,33±0,14 (Slika 1).

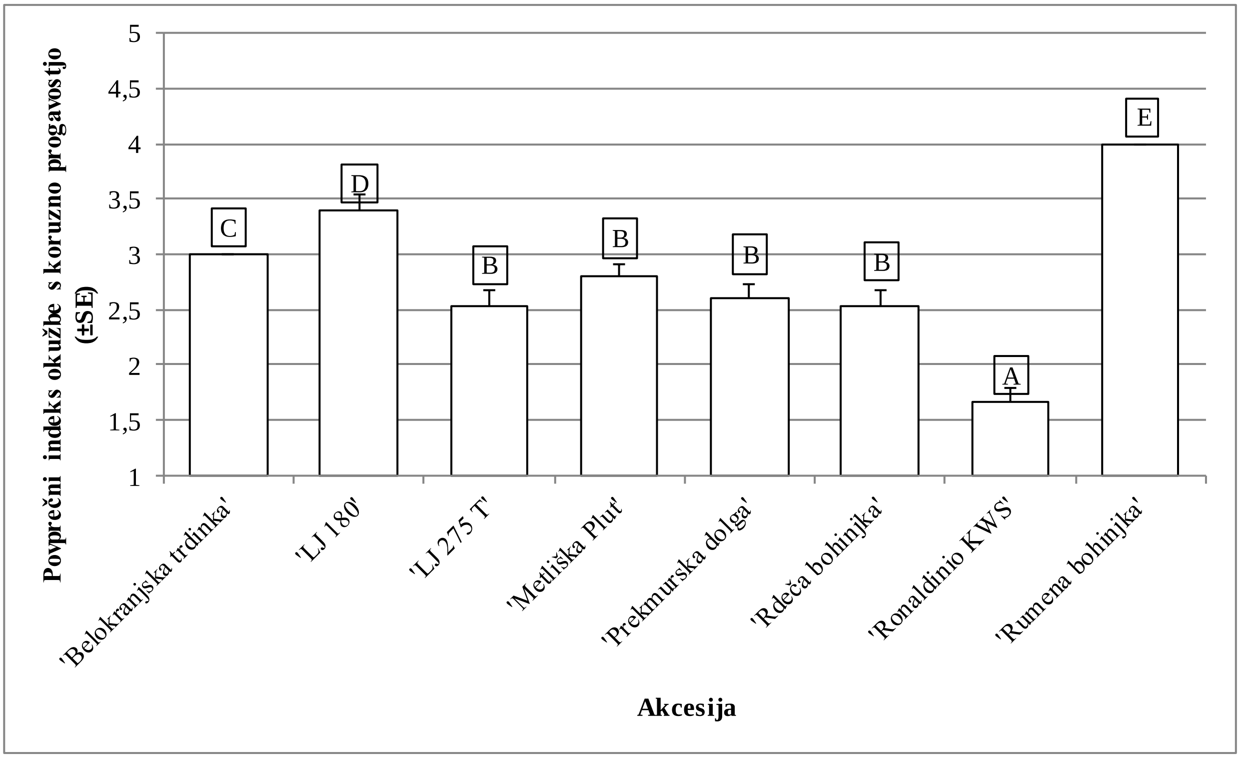


Slika 1: Povprečni indeks okužbe s koruzno progavostjo (*Setosphaeria turcica* (Luttr.) K.J. Leonard & Suggs) (±SE) na Laboratorijskem polju Biotehniške fakultete Ljubljana (2016)

Figure 1: Average index of infection by northern leaf blight (*Setosphaeria turcica* (Luttr.) K.J. Leonard & Suggs,) (±SE) at Laboratory field of Biotechnical Faculty Ljubljana (2016)

* različne črke pomenijo statistično značilno razliko po Duncanu (P≤0,05)

V Krasincu je bil povprečni obseg okužbe signifikantno največji pri populaciji 'Rumena bohinjka' (4,0±0,0). Hibrid 'LJ 180' je imel povprečni indeks okuženosti 3,4 ± 0,13; populacija 'Metliška Plut' pa 2,8 ± 0,10. Najmanjši povprečni indeks okuženosti je imel hibrid 'Ronaldinio KWS' (1,66 ± 0,12). (Slika 2).



Slika 2: Povprečni indeks okužbe s koruzno progavostjo (*Setosphaeria turcica* (Luttr.) K.J. Leonard & Suggs) (±SE) v Krasincu (2016)
Figure 2: Average index of infection by northern leaf blight (*Setosphaeria turcica* (Luttr.) K.J. Leonard & Suggs) (±SE) at Krasinec (2016)
* različne črke pomenijo statistično značilno razliko po Duncanu (P≤0,05)

4 RAZPRAVA

Pridelki koruze so bili na obeh lokacijah in v obeh letih močno odvisni od genotipa. 'Rdeča bohinjka' in delno 'Rumena bohinjka', podobno pa tudi 'LJ 180' so imele pretežno majhne pridelke, so pa bile tudi med najbolj ranimi, torej je bil manjši pridelek pričakovan. Sodobni hibridni sorti 'P 9074' in 'Ronaldinio KWS' sta imeli pričakovano največji pridelek. Pridelek populacije 'P 9074' je bil na Laboratorijskem polju Biotehniške fakultete skoraj dvakrat večji od populacije 'Metliška Plut'. Prav slednja je pokazala zadovoljive rezultate na vseh poskusih in je zato lahko primerna za lokalno pridelavo tudi v ekološkem načinu pridelave. 'Metliška Plut'je raznolika glede na dolžino in obarvanost storžev (Slika 3), kar pa bi lahko z nadaljnjo selekcijo izboljšali.

Slika 3: Variabilnost akcesije 'Metliška Plut' pri vzdrževalcu (Aleš Plut, Cerovec 17, Semič) (pridelek 2016).
Figure 3: Variability of accession 'Metliška Plut' at maintainers site (Aleš Plut, Cerovec 17, Semič) (yield 2016)

Rezultati naše raziskave kažejo, da se lokalne populacije različno odzivajo na abiotične in biotične dejavnike. Andjelković in sod. (2014) denimo navajajo, da so bile različne akcesije koruze različno odporne na sušo.

Ugotovili smo, da obstajajo razlike med posameznimi populacijami koruze glede na pridelovalne sisteme. V Osrednji Sloveniji smo koruzo gojili na konvencionalen način, medtem ko smo v jugovzhodni Sloveniji poskus z različnimi genotipi koruze zasnovali na ekološki kmetiji. V obeh letih poskusa je po pridelku zrnja med populacijami izstopala 'Metliška Plut'. V drugem letu poskusa je bil njen pridelek primerljiv s hibridnimi sortami v obeh letih poskusa. V 2016 smo raziskavo nadgradili, saj smo poleg pridelka spremljali tudi indeks okuženosti s koruzno progavostjo. Hibridni sorti sta bili na obeh lokacijah najmanj dovzetni na širjenje koruzne progavosti. Da so hibridne sorte koruze manj dovzetne za napad škodljivih organizmov, v svojemu delu navajajo Beaver in sod. (2011).

Indeks okuženosti s koruzno progavostjo je bil manjši na poskusu v osrednji Sloveniji, kar med drugim lahko pripišemo dobri kmetijski praksi.

5 ZAKLJUČEK

Poskusi uporabne vrednosti slovenskih populacij in sort koruze v dveh različnih regijah so pokazali, da lokalne populacije po pridelku in odpornostjo proti boleznim niso neposredno konkurenčne sodobnim hibridnim sortam. Vsekakor pa je potrebno poudariti, da zlasti populacija poltrdinke 'Metliška Plut' odstopa od ostalih in je primerna za pridelavo na izbranih lokacijah. Akcesijo že razmnožujemo in pripravljamo za vpis na sortno listo.

6 ZAHVALA

Raziskava je bila izvedena v sklopu projekta V4-1313 'Vzpostavitev sistema vzdrževalne selekcije in pridelave semenskega materiala kmetijskih rastlin za sonaravne oblike kmetovanja'. Zahvaljujemo se izr. prof. dr. Ludviku Rozmanu za aktivnost pri štartu projekta in dolgoletnemu delu na genskih virih koruze.

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The effects of silicon and titanium on safflower (*Carthamus tinctorius* L.) growth under moisture deficit condition

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ABSTRACT

Safflower is one of important crop in semi-arid regions of the world, where the precipitations are limited. In order to investigate the effect of foliar spray of nano-silicon dioxide (10 and 20 mM) and nano titanium dioxide (25 and 50 mM) and water-deficit stress (irrigation after 110 mm evaporation) on growth parameters and yield components of spring safflower a field experiment was carried out at the highland semi-arid region, in, North West of Iran. Water deficit stress significantly reduced morpho-physiological traits such as ground cover, canopy width, leaf fresh mass, leaf are and plant height) as well as yield components (e.g. capitulum diameter, seed mass and seed number per capitulum). However, the plants grown under water deficit condition showed the higher harvest index than well irrigated plants. Comparison of the foliar treatments showed that the both nano-particles (silicon and titanium) improved the plant growth and yield components over the control. However, the effect of nano-silicon was more prominent than titanium. The highest amount of seed oil was recorded under well irrigated condition (irrigation after 60 mm evaporation) with foliar application of nano-titanium. The percentage of palmitic acid, arachidic acid and myristic acid in seed increased by nano-titanium application. Altogether, principal component analysis indicated that spray of 10 mM nano silicon dioxide was best foliar treatments under all moisture regimes.

Key words: safflower; agronomic traits; foliar spraying; nano-particles; principal component analysis; semi-arid region

IZVLEČEK

UČINKI SILICIJA IN TITANA NA RAST ŽAFRANIKE (*Carthamus tinctorius* L.) V RAZMERAH POMANKANJA VODE

Žafranika je pomembna poljščina v semiaridnih območjih, kjer so padavine omejene. Z namenom ugotavljanja učinkov pršenja listov z nano-silicijevim (10 in 20 mM) in nano titanovim dioksidom (25 in 50 mM) ter stresa zaradi vodnega deficita (namakanje po 110 mm evaporacije) na rastne parametre in komponente pridelka pomladanske žafranike je bil izveden poljski poskus na višinskem semiaridnem območju severno zahodnega Irana. Vodni deficit je značilno zmanjšal morfološko-fiziološke lastnosti (pokritost tal, širino krošnje, svežo maso listov, listno površino in višino rastlin) kot tudi komponente pridelka (premer koška, maso semen in število semen na košek). Kljub temu so imele rastline, ki so rastle v razmerah pomanjkanja vode večji žetveni indeks kot dobro namakane. Primerjava foliarnih obravnavanj je pokazala, da so oboji nano delci (silicijevi in titanovi) izboljšali rast rastlin in komponente pridelka v primerjavi s kontrolo. Učinek silicijevih nano delcev je bil bolj izražen kot titanovih. Največja vsebnost olja v semenih je bila v dobro namakanih rastlinah (namakanje po 60 mm evaporacije) s foliarno dodajanimi nano delci titana. Odstotki palmitinske, arahidonske in miristične kisline v semenih so se povečali po uporabi nano titanovih delcev. Analiza glavnih komponent je pokazala, da je bilo pršenje z 10 mM nano silicijeva dioksida najboljše foliarno obravnavanje pri vseh vodnih režimih.

Ključne besede: žafranika; agronomske lastnosti; foliarno pršenje; nano-delci; analiza glavnih komponent; semiaridna območja

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1 INTRODUCTION

The world's population is growing rapidly, so that by 2050, it is predicted to reach 9.1 billion, 34 %more than today (UN, 2013). Indeed, majority of this population growth is expected to occur in developing countries. These trends refer to the imperative note that, the food security is (and will increasingly continue to be) faced with a major challenge, necessitating anincrease in food production in these areas. However, limited remaining arable lands and restricted water resources bolds the significance of improvingcrop management and implementing strategies to increase drought toleranceto achieve the required massive increase in global food production. Additionally, climate change is one of the main factors restricting the yield, performance and stability of crop production. However, in terms of climate, most of developing countries are located in semi-arid areas which are characterized by highly variable and unpredictable rainfall and total precipitations below potential evapotranspiration, making them frequently engaged with drought stress. However, even at mild intensity, drought stress can inhibit photosynthesis and stomatal conductance significantly, resulting in considerable yield reductions (Shahrokhnia & Sepaskhah, 2017).

Safflower (*Carthamus tinctorius* L.) is one of the oldest cultivated crops which is partially adapted to water-deficit zones. Being a highly branched, herbaceous, thistle-like annual plant, it is commercially cultivated for extracting vegetable oil from its seeds. Besides, safflower is grown for flowers used for coloring, flavoring foods, dyes, medicinal properties, and forage. Safflower can be considered as a promising substitute crop in dryland agro-ecosystems due to its unique agronomic characteristics (Hussain et al., 2016). Safflower is aboriginal to semi-arid environments, and its adaptation to water-deficit conditions is largely resulted from its extended roots which can penetrate to a depth of down to 2 meters to absorb nutrients (HaghighatiMalek & Ferri, 2014). Nutrient requirements of safflower are similar to those of winter cereal such as wheat and barley, but compared to other annual plants, safflower has a longer growth period. Although the deep rootsystem of safflower allows the plant to utilize nutrients and water from lower depths, its long, dry spell without effective precipitation can negatively affect the seed yield. High evapotranspiration, limited water resources and other parameters in semi-arid regions has invoked the interests for studying the effect of water-deficit stress. Indeed, it is necessary to find or develop appropriate techniques for producing crop under water limited condition (Asadzade et al., 2015). This can be partially reached through growing drought-tolerant varieties while setting forth more rational strategies for exploiting landscape. The use of fertilizers and improving the soil water holding capacity represent other options (Murungweni et al., 2016). Additionally, application of innovative nanotechnologies in agriculture (including the advantageous nano-particles) has been shown to be a promising approach to improve crop production considerably (Lal, 2008; Liu & Lal, 2015). Ingredients with particle sizes smaller than 100 nm in at least one dimension are generally classified as nano-materials. A number of engineered nano-materials have been investigated for use in agricultural scope to increase crop productivity and enhance crop protection (Khot et al., 2012). In this regard, foliar application of beneficial nano-particles has opened a new avenue for makingnanotechnology a feasible option in the field.

Among the wide range of inorganic nano-materials introduced recently, silicon (Si) and titanium (Ti) nanoparticles have gained the largest deals of focus. Although silicon is the second most abundant element in the earth's crust (following oxygen), biological role of Si in plants is yet to be thoroughly studied for specific plants. Being absorbed by plants in the form of silicic acid, siliconis the only nutrient element that is not toxic at high concentrations (Ma et al., 2001; Ma, 2004). Beneficial roles of Si in inducing defense mechanisms under various biotic and abiotic stresses have been reported (Van Bockhaven et al., 2013; Zhu & Gong, 2014). More interestingly, alleviative effects of Si under water-deficit stress have been observed (Shiet et al., 2016).

Likewise, titanium is the ninth most abundant element in the earth's crust; it is introduced as a beneficial element for plant growth. Nano-titanium dioxide ($nTiO_2$) has been reported as an effective photo-catalyst under ultraviolet radiation (Gupta & Tripathi, 2011). Therefore, leaf-spray of nano titanium dioxide may improve the efficiency of plant photosynthesis and related physiological activities, thereby improvingthe plant growth. Foliar spray of $nTiO_2$solution on barley under supplemental irrigation conditions may positively affect some morphophysiological characteristics like days to anthesis, chlorophyll content and straw yield (Janmohammadi et al., 2016b). However, there have been reportson possible adverse effects of $nTiO_2$ suspension on corn leaf development and transpiration (Asli & Neumann, 2009). Also, these authors reposted that, titanium has an inhibitory effect on hydraulic conductivity of roots. Jaberzadeh et al. (2013) showed that, foliar application of low concentrations of $nTiO_2$on wheat seedling increased the plant biomass and seed yields under water-deficit stress. These studies supported several earlier studies showing that the response to a nano-material is significantly dependent on environmental conditions and plant species (Choi et al., 2003; Zheng et al., 2005; Choi et al., 2010; Mastronardi et al., 2015). Although comparative studies have been carried out on the effects of nano-particles, most studies have been done on cultured samples underartificial conditions, with their applicability to plant responses under natural water-deficit condition remained unclear. The objectives of the present research were: (1) to evaluate foliar application of $nTiO_2$ and nano-silicon dioxide ($nSiO_2$) on agronomic traits of safflower in a semi-arid, high-elevation Mediterranean area, (2) to assesschanges in fatty acids under water-deficit stress, and (3) to identify optimum nano-material and its concentration for safflower.

2 MATERIAL AND METHODS

Field experiments were conducted during 2015/16 growing season at the Maragheh Agricultural Research Institute located in a semi-arid area in northwestern Iran. Geographically, the study area was located at 46°16'Eand 37°23'N (altitude = 1485 m from mean sea level). Based on Koppen's classification, this area has a semi-arid and cold temperate climate with annual precipitation of 375 mm, consisting of 73 % rain and 27 % snow. Total rainfall during the growing season was measured at 97.8 mm. The area is located within Sahand Mountain highs in northwestern Iran, providing itwith very cold winters with minimum air temperatures falling below -15 °C and more than 100 days with freezing temperatures. Average maximum and minimum temperatures during the growing season were 21°C and 8°C, respectively.Local soil was clay loam in typeand low in organic carbon (0.43 %) with a pH value of 6.85 and total nitrogen and $CaCO_3$ contents of 0.17 % and 19 %, respectively. Electrical conductivity (EC) and iron, manganese, copper, zinc, and potassium contents of the soil were measured at 0.84 ds m^{-1}, 1.62 ppm, 6.37 ppm, 0.49 ppm, 0.73 ppm, and 627 ppm, respectively.

The field was left as fallow for a year before the cultivation. The previous cultivated crop in experimental site was bread wheat. The experimental field was ploughed once in early fall and harrowed twice to bring the soil to fine tilth one week before planting. The recommended dose of fertilizer (100 kg of N and 70 kg of P_2O_5 per hectare) was applied in the form of urea and triple superphosphate at the time of seed bed preparation. The experimental design was factorial on the basis of randomized complete block in three replicates.

The experiments were arranged as split-plot, based on the randomized complete block design with three replications. Two irrigation treatments, namelywell irrigated (W: irrigation after 60 mm evaporation from class A pan), and water-deficit conditions (S: irrigation after 110 mm evaporation from class A pan) were assigned to the main plots and suspensions of nanoparticlesat different concentrations were allocated to sub plots. Under water-deficit condition,irrigation gravimetric water content (θg) of soil was measured before the irrigation, indicating16 % to 27 % water content at a depth of 30 cm.The treatments were subjected to foliar application at 5 levels including control (spray of distilled water), $nSiO_2$(at 10 and 20 mM) and $nTiO_2$ (at 25 and 50 mM). Nanoparticles were purchased from the Pishgaman Nano Co., Iran. According to the manufacturer, particle sizes of the purchased SiO_2 ranged within 20-100 nm. Synthesized nanoparticles were characterized morphologically by transmission electron microscopy (Figure 1). According to the results, specific surface area of the nano-sized particles was 180-600 m^2 g^{-1}at 99.7 % purity.

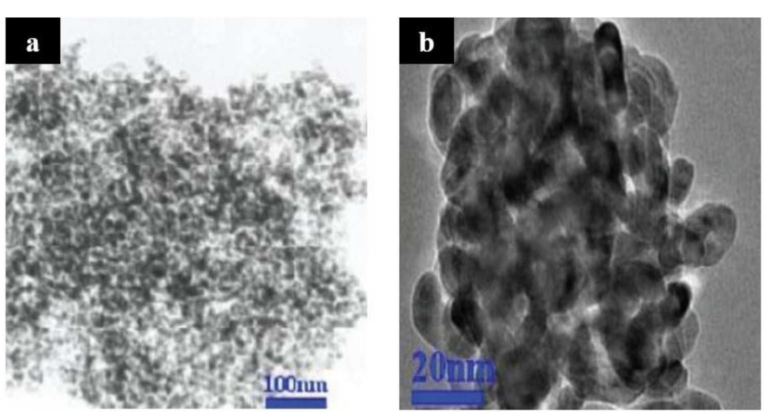

Figure 1: Transmission electron microscopy (TEM) micrograph of synthesized nano-silicon dioxide (a) nano-titanium dioxide (b)

A productive and thorn-less variant of safflower, locally referred to as 'Golestan', was used in the present study. Before the start of the experiment seed were propagated in isolated fields under full irrigated condition, according to Sabaghnia et al. (2015), in northwest of Iran. Seeds were treated with a mixture of carboxin (5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxamide) and actellic (a.i. pirimiphos methyl) at (2:1) to minimize the probability of seed- and soil-borne diseases. The seedswere sown at 30-cm row spacing in 2.5 × 6 m plots (15 m^2) using an experimental seed drill, on March 28^{th}. All plots were irrigated twice after sowing and subsequent irrigations were applied according to the treatments (W and S) by drip irrigation system. During the irrigations, the plots were irrigated to up to 70 % of field capacity.

All necessary cultural practices and plant protection measures were taken uniformly for all plots during the entire period of experimentation. Weeds were controlled by hand weeding in spring. Nanoparticlesuspensions were applied using an atomizer sprayer. Foliar spray treatments were initiated 40 days after planting and repeated once each 10 days until grain filling stage. Relative water contents were evaluated at the beginning of capitulum formation stage (BBCH scale: 50). Five fresh leaves of the same size and age were collected from five plants from each treatment, and then weighted (F_M). Leaf segments were kept immersed in distilled water for 24 h at room temperature in the dark. The turgid mass (T_M) of the leaves was measured before having the leaves oven-dried at 80 °C for 72 h until constant mass and then reweighted (D_M). The fresh mass, turgidity, and dry weights of the leaf segments were used to determine hydration and relative water content according to Sangakkara et al. (1996).Accordingly, hydration was determined as H (%) = 100 – 100 (D_M / F_M) and relative water content (RWC) was determined as RWC (%) = [(F_M– D_M) / (T_M– D_M)] × 100. Leaf area was estimated at the end of flowering (BBCH scale: 69).

Non-contact surface temperature of the leaves was measured at early flowering stage (BBCH scale: 61) using an IR thermometer device (Testo830-T2). Measurements were done on 10 plants per plot. Chlorophyll index was measured on 10 fully expanded leafs of a plant at each plot using a portable chlorophyll meter (SPAD) at capitulum and fruit development stage (BBCH scale: 71). The ground cover was evaluated in terms of the amount of dead plant material covering the soil surface. Average canopy spread was measured as the average horizontal width of the plant canopy, taken from right to left as one moves around the plant. Ground cover and canopy spread were measured during the flowering stage (BBCH = 65; 50 % of florets open in flowers on the main shoot). For eliminating the border effects, lateral rows at both ends of each plot were excluded from the measurements. At maturity stage, the plants were cut at ground level from two middle rows and then oven-dried at 80 °C until a constant mass was reached. Seeds were separated from straw by crushing. The seed and straw (stem plus leaves) were weighted by a balance and yields were determined per unit area for different treatments. Total biomass was also calculated by summing upsafflower seed and straw. Evaluated agronomic traits were number of branches per plant, stem diameter (mm), capitulum diameter (mm), number of capitula per plant, number of achenes per capitulum, achene mass per capitulum, and thousand-achene mass. Harvest index was calculated as the ratio of achene yield to aboveground dry matter at maturity. Protein and oil contents (%) of the seeds were measured using a near-infrared seed analyzer (Zeltex). The oil contents of the samples were determined according to Darinkaboud and Gharibi (2016) using soxhlet extraction technique. For this purpose, the seeds were ground in a mill to a particle size of 0.5 mm. Then 500 mg of the seed meal was transferred into a weighed cellulose extraction thimble which was then sealed with cotton wool. The samples were dried in the thimbles (at 60 °C for 15 h) before reweighting the thimbles. The oil was extracted with petroleum ether in a 500-ml soxhlet instrument at 70 °C for 10 h. Oil contents of the samples were determined after drying and weighing the extracted samples with the thimbles.

Contents of palmiticacid, arachidic acid and myristic acid were evaluated by gas chromatography according to Rudolphi et al. (2012). For this purpose, 200 mg of the seed meal was mixed with 0.5 ml of sodium-methylate. The mixes were then incubated twice in a water bath at 20 °C for 10 min and mixed in between the incubation courses. After adding 300 µl of $NaHSO_4$ (5 %) and 300 µl of iso-octane to the mix, the sample was centrifuged at 2,000 U/min for 10 min. Subsequently, 200 µl of the upper liquid phase was analyzed by gas chromatographically (Chrompack CP 9001, equipped with a flame ionizing detector).The data was subjected to analysis of variance (ANOVA) using MSTATC statistical package. Differences were compared by Least Significant Difference Test (LSD) at alpha 0.05.

3 RESULTS

Results of ANOVA showed that, most of the morphophysiological traits were significantly affected by moisture regimes and foliar treatments (Table 1). The main effects of moisture regime and foliar treatment as well as their interaction effects (moisture regime × foliar treatment) were found to be statistically significant ($p < 0.01$) for the ground cover percentages. Water-deficit conditions reduced this trait considerably. Assessments of ground cover between different combined treatments revealed that, the highest values were recorded for the plants grown under well irrigated condition and those subjected to foliar application with 25 mM Ti, 10 mM Si and 50 mM Ti, respectively (Figure 2). A parallel trend was also observed for canopy width, wherethe plant treated with the nano-particles at low concentration showed the widest canopy. The smallest canopy width was, however, recorded for the plants treated with distilled water (control) and 20 mM Si under water-deficit stress.

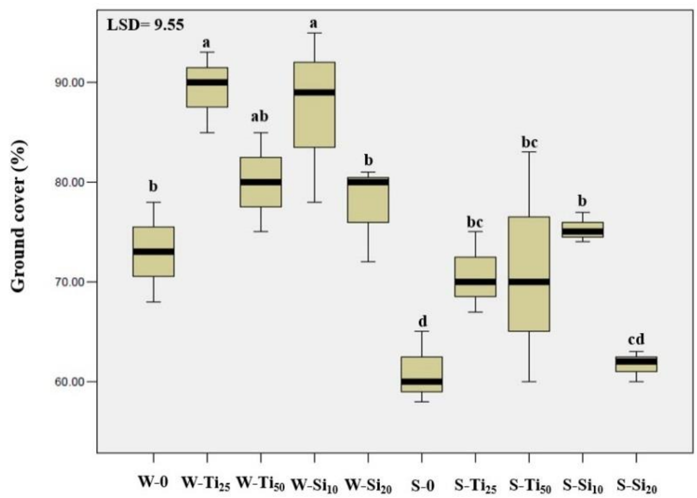

Figure 2: The effect of foliar application of nano-particles on ground cover percentage of safflower plants under different moisture condition. Ti: nano titanium dioxide, Si: nano silicon dioxide, W: well irrigated, S: water deficit condition. The numbers beside the nano-particles refers to the concentration of nanoparticles suspension. Number of zero refers to control conditions (spraying of distilled water). Vertical bars in each column are standard error. Between the columns with different names there are statistically significant differences.

Likewise, assessment of fresh leaf mass showed that this trait has been significantly affected by both moisture regimes and foliar treatments (Table 1). Water-deficit stress reduced the fresh leaf mass by 33 % in comparison with that under well irrigated condition. A comparison on average fresh leaf mass between the foliar treatments revealed that, the highest mass were those of the plants treated with nano-particle suspensions at low concentration (10 mM Si and 25 mM Ti). Water-deficit stress was seen to significantly increase the canopy temperature (by up to 2 °C), while the plants treated with titanium nano-particles had lower canopy temperatures (Table 1).

Table 1: Effect of different moisture deficit stress and foliar application of nano-practices on morpho-physiological traits of safflower (*Carthamus tinctorius* L.) plants

Treatment	GC	CW	FWL	CT	PH	RWC	CHL	LA	DCE
Well irrigated	81.46a	34.61a	14.56a	26.72b	58.96a	75.00a	49.11a	666.30a	72.13a
moisture deficit	67.93b	24.10b	10.88b	28.71a	52.42b	64.13b	49.53a	465.78b	64.06b
control	67.00c	23.38c	8.97d	28.01a	49.10b	65.33c	42.75b	464.91c	63.33c
Nano-TiO_2 25 mM	80.00a	34.51a	13.85ab	27.31ab	60.29a	72.00a	53.65a	557.82b	70.83ab
Nano-TiO_2 50 mM	75.50ab	27.73b	11.37b	26.97bc	54.35ab	70.50ab	48.14ab	537.09bc	68.00ab
Nano-SiO_2 10 mM	81.33a	34.67a	16.40a	27.55ab	60.06a	73.33a	52.56a	674.93a	72.16a
Nano-SiO_2 20 mM	69.66bc	26.47bc	13.02bc	28.75a	54.66ab	66.66bc	49.81ab	573.10b	66.16bc
Level of significance									
S	**	**	**	**	**	**	NS	**	**
N	**	**	**	*	*	**	*	**	*
S×N	**	**	NS	NS	NS	*	NS	*	NS

GC: ground cover percentage, CW: canopy width (cm), FWL: leaves fresh mass (g), CT: canopy temperature (°C), PH: plant height (cm), RWC: relative water content (%), CHL: chlorophyll content (SPAD unit), LA: leaf area (cm^2), DCE: number of day from sowing to capitulum emergence. Different letters within columns indicate statistically significant differences at the 5 % level of significance. S: moisture stress, N: nano-particles, S×N: the interaction effect of nano-particles and stress. NS = Not significant, * = Significant at 5 % level of probability, ** = Significant at 1 % level of probability.

Investigation of plant height indicated that, the water-deficit regime reduced this trait by 12 % compared with that of well irrigated plants. On the other hand, most of the plants treated with the nano-particles were longer than control plants (treated with distilled water). Investigation of relative water content (RWC) showed that, application of $nTiO_2$ and $nSiO_2$ at low concentration could improve leaf water status under both moisture regimes. However, the positive impact of 10 mM Si was more prominent than those of other treatments considered. Also, application of 20 mM $nSiO_2$ under water-deficit regimes failed to rectify leaf water statue significantly (Figure 3).

Evaluation of chlorophyll content (SPAD unit) revealed that, the application of nano-particles affected this trait ($p< 0.05$) significantly. Foliar spray of 25 mM Ti, 50 mM Ti, 10 mM Si and 20 mM Si could increase the chlorophyll content by 25 %, 12 %, 23 % and 16 %, respectively, in comparison with control plants (Table 1). Leaf area was seen to be significantly affected by both factors ($p< 0.01$). The largest leaf area was recorded for the plant grown under well irrigated condition with foliar application of 10 mM Si, while the lowest value was recorded for the plants grown under water-deficit condition without nano-particles application or those treated with 25 mM Ti.

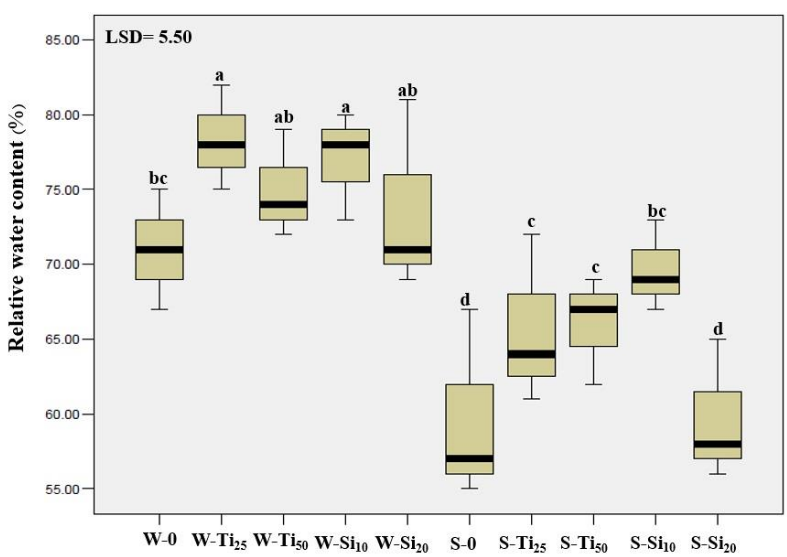

Figure 3: Mean comparison of relative water content of safflower leaves under different foliar treatment with nano-particles and moisture regimes

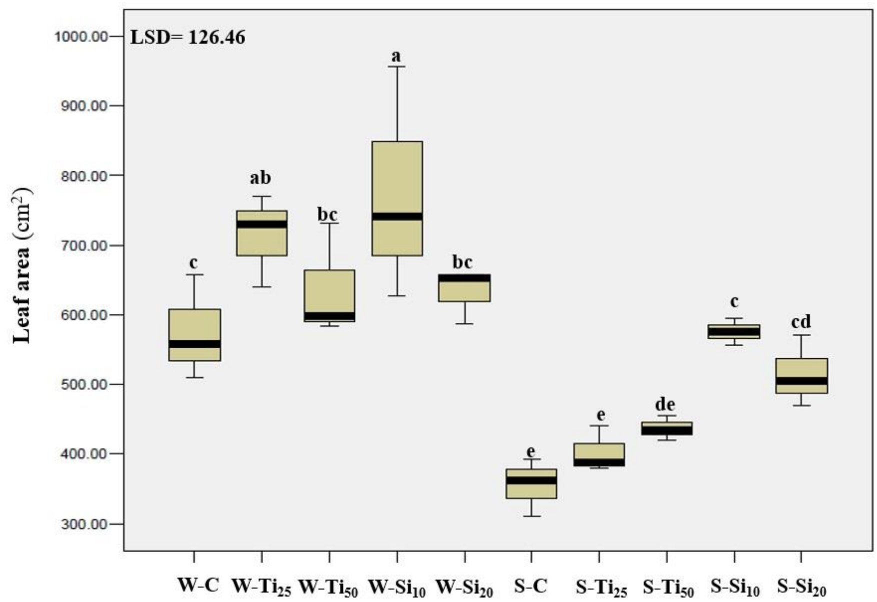

Figure 4: The impact of foliar spray of different nano-particles on leaf area of safflower plants under different moisture regimes

A glancing comparison between the two moisture regimes showed superior positive effects of n-TiO_2 under well irrigated condition (Figure 4). Phenological development was also seen to be affected by moisture regimes, so that the plant grown under water-deficit condition reached capitulum emergence stage way earlier. On the other hand, foliar application of the nano-particles delayed the developmental stage considerably, as compared to control plants (Table 1).

The effects of moisture regimes and foliar treatments on yield componentsare shown in Table 2. Investigation of mean capitulum diameter showed that, the water-deficit stress reduced this trait by 18 % in comparison with that under well irrigated condition. The largest capitulum was recorded for plants treated with 25 mM Ti and 10 mM Si, while the smallest one was of control plants and those treated with 20 mM Si. Number of the capitula per plant exhibited a significant decrease under water-deficit stress. Accordingly, this parameter responded to foliar spray positively, so that the application of Si and Ti suspensions at low concentration could increase this yield component slightly ($p< 0.01$).

Table 2: Mean comparison of yield and yield components of Safflower (*Carthamus tinctorius* L.) as affected by irrigation levels and nano-particles

Treatment	MCD	NCP	TSW	SNC	SY	BY	HI	PRO	OIL
Well irrigated	29.66a	7.46a	39.20a	29.53a	1268.53a	5011.00a	25.44b	13.90b	30.74a
moisture deficit	25.26b	6.88b	32.33b	23.17b	958.17b	3521.66b	27.26a	19.29a	26.31b
control	23.47c	6.13bc	34.05b	24.49c	1058.33c	4204.33ab	25.36b	18.82a	25.44c
Nano-TiO_2 25 mM	31.70a	8.00a	38.20a	28.99a	1156.66ab	4547.00a	25.72b	16.42b	32.32a
Nano -TiO_2 50 mM	25.98bc	6.80b	33.67b	25.76bc	1055.00c	4068.33a	26.39ab	14.38c	30.07a
Nano -SiO_2 10 mM	30.38ab	8.13a	38.04a	27.25ab	1224.06a	4289.33ab	28.66a	17.54a	26.81c
Nano -SiO_2 20 mM	25.78bc	6.82b	34.85b	25.26bc	1071.33c	4225.66ab	25.62b	15.82bc	28.01b
Level of significance									
S	*	*	**	**	**	**	*	**	**
N	*	*	**	*	**	**	*	**	*
S×N	NS	NS	*	NS	NS	**	NS	NS	*

MCD: mean capitulum diameter (mm), NCP: number of capitula per plant, TSW: thousand seeds mass (g), SNC: number of seed per capitulum, SY: seed yield (kg ha^{-1}), BY: biological yield (kg ha^{-1}), HI: harvest index (%), PRO: seed protein content (%), OIL: seed oil content (%; Soxhlet) .Different letters within columns indicate statistically significant differences at the 5 % level of significance. S: moisture stress, N: nano-particles, S×N: the interaction effect of nano-particles and stress. NS = Not significant, * = Significant at 5 % level of probability, ** = Significant at 1 % level of probability.

Evaluation of thousand-seed mass revealed that moisture regime-foliar treatmentinteraction effect was statistically significant at 95 % confidence level. Significantly lower seed mass (by 21 %) were observed under water-deficit stress conditions. The heaviest seeds were those of the plants grown under well irrigated condition and treated with Si and Ti suspensions at low concentration, while the smallest seeds were recorded for the plants grown under water-deficit stress and sprayed with 50 mM Ti and 20 mM Si (Figure 5).

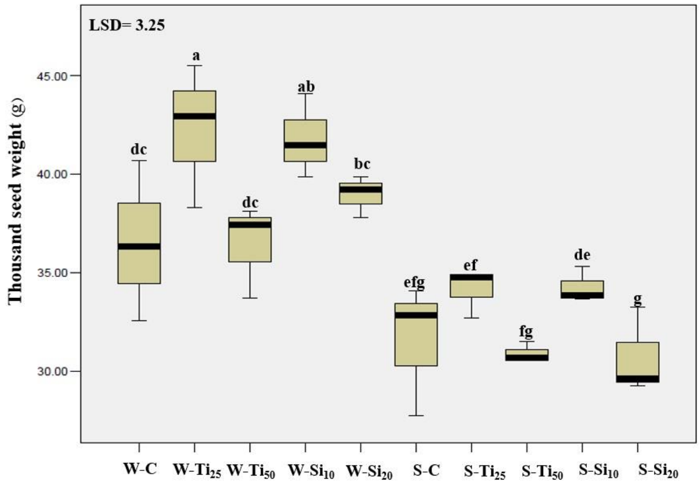

Figure 5: Mean comparison of thousand seed mass in safflower plants under different foliar treatment with nano-particles and moisture regimes

As one of the most important yield indicators, number of seeds per capitulum decreased noticeably under water shortage (by 27%). Furthermore, foliar treatment affected the number of seeds per capitulum at 95% confidence level ($p < 0.05$), so that the largest number of seeds was recorded for the plants treated with 25 mM Ti suspension.

Results showed that, water-deficit stress could reduce the seed yield by 32 %, as compared to that under well irrigated condition. Also, seed yield responded to foliar treatments significantly, so that the application of 10 mM Si increased the seed yield by 15 % over the control plants. All by all, the highest seed yield was obtained by applyingSi and Ti suspensions at low concentration. Furthermore, the results showed that, water scarcity tends to reduce the biological yield considerably (by 42 %). A comparison on average values for combined treatments showed that, the highest biological yield was related to the plant grown under well irrigated condition with foliar application of 25 mM Ti, while the lowest yield was recorded for the plant grown under water-deficit condition with foliar application of 50 mM Ti (Figure 6).

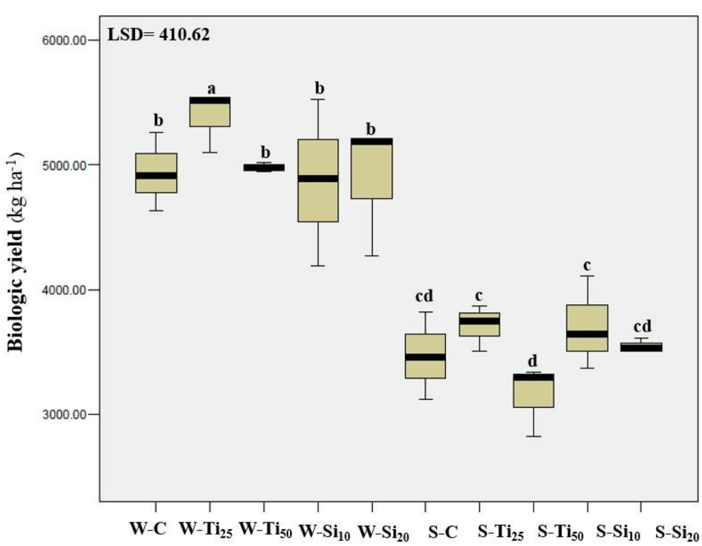

Figure 6: The impact of foliar treatment with different nano-particles on biological yield of safflower plants under different moisture regimes

Interestingly, water-deficit stress increased the harvest index by 7 % in comparison to that under well irrigated condition. Also foliar application of 10 mM Si increased the harvest index by 13 % over the control plants. The first component in principal component analysis (PCA) clearly separated moisture regimes, with the second component revealingthe statistical distinction of foliar application of 10 mM Si from other spray treatments under well irrigated condition (Figure 7). Similarly, PCA showed that, although there is no significant difference between foliar treatments under water-deficit stress, application of nano-particles at low concentration (10 mM Si and 25 mM Ti) tends to outperform other treatments.

Evaluation of quantitative traits also revealed that, even though most of these parameters were affected by both factors, the responseswere somewhat different for quantitative traits. Water scarcity resulted in a considerable increase in seed protein percentage (Table 2). On the other hand, evaluation of the effect of foliar treatments on seed protein content reveled that, all treatments, except for 10 mM Si, reduced this parameter significantly.

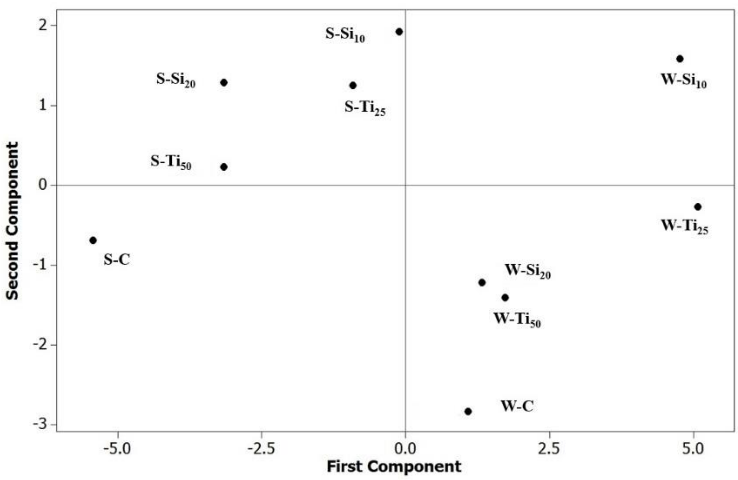

Figure 7: Principal component analysis (PCA) of combined treatments (moisture regimes and nano-particles foliar application) in safflower plants. Ti: nano titanium dioxide, Si: nano silicon dioxide, W: well irrigated, S: water deficit condition. The numbers beside the nano-particles refers to the concentration of nano-particles suspension. Number of zero refers to control conditions (spraying of distilled water).

However, water-deficit stress reduced the seed oil content most intensively (Table 2). Although foliar spray improved the oil contentin general terms, the greatest increase was seen to be related to the application of Ti. Moreover, fatty acid profiles of safflower seed oils revealed that these component were significantly affected by moisture regimes and foliar treatments (Figure 8). Noticeably lower palmitic acid content was seen in water shortage. On the other hand, foliar application of $nTiO_2$, regardless of the concentration, under well irrigated condition resulted in the highest level of palmitic acid. Adverse effects of water shortage on arachidic acid and myristic acid contents were more prominent than that on palmitic acid content (Figure 8). However, the response of arachidic acid to foliar treatments was largely similar to that of palmitic acid, where the highest fat acid content was obtained by foliar application of 50 mM Ti. All by all, the effect of foliar treatment under well irrigated condition was more distinguished on arachidic acid and palmitic acid contents, rather than meristic acid content.

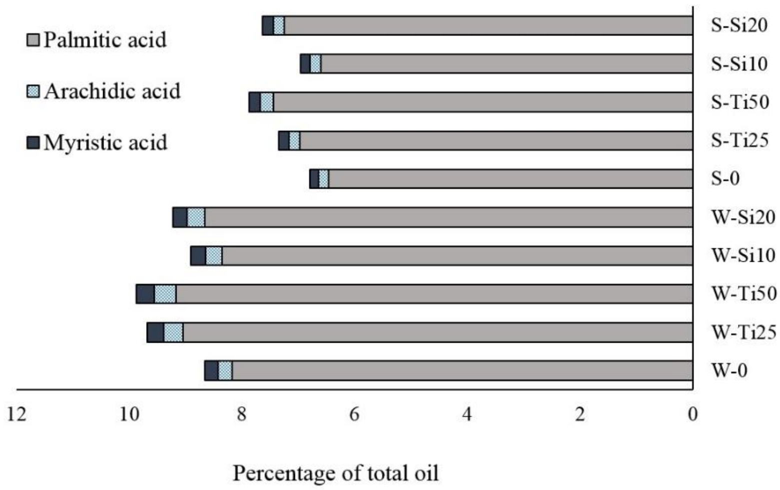

Figure 8: A stacked bar chart which display the change of fatty acids between the combined treatments. Ti: nano titanium dioxide, Si: nano silicon dioxide, W: well irrigated, S: water deficit condition. The numbers beside the nano-particles refers to the concentration of nano-particles suspension. Number of zero refers to control conditions (spraying of distilled water)

4 DISCUSSION

It was found that water-deficit stress in the semi-arid region reduced plant growth and seed yield considerably. On the other hand, foliar application of TiO_2 and SiO_2nano-particles could significantly affect the evaluated traits. Although the effects of nanoparticles were more obvious under well irrigated condition, low concentrations of SiO_2nano-particles could significantly improve some of crucial agronomic traits such as ground cover percentage. As a very essential attribute of spring crop in a semi-arid region, fast canopy closure may affect the economic yield extensively. In Mediterranean semi-arid areas, precipitation regime is anticipated to be dominated by low, erratic and unpredictable rainfalls. The moisture supplied to the soil from rain is mostly lost by evaporation. Fast canopy closure and a high percentage of ground cover under the Mediterranean conditions may provide numerous benefits. Extended canopy can improve the capturing and use of solar radiation for photo-assimilate synthesis throughout rainy spring months (Soleimanzadeh & Gooshchi, 2012). Also, quick ground covering can reduce the moisture loss through evaporation and increase competitive power of plant against weeds. This also accords with our earlier observations which showed that, canopy width and ground cover percentage are significantly affected by foliar application of SiO_2nano-particles (Janmohammadi et al., 2016b).

On the contrary, if the nanoparticles show a good translocation through the phloem, the application should be done via foliar spraying. In addition, the nano-materials moving along the phloem are likely to be accumulated in the plant organs which may act as sink, such as seeds. The beneficial effects of Si have been thought to be due to the precipitation of amorphous silica in plants, which acts as a protectant. As a physico-mechanical barrier, nano-silicon can protect plants against biotic and abiotic stresses (Ma, 2004). Nano-silicon can boost plant's defense mechanismsincluding the accumulation of lignin, phenolic compounds, and phytoalexins (Ma & Yamaji, 2006). Nano-silicon can also deposit on the walls of epidermis and vascular tissues of the stem and leaf surface in most plants; it further controls physiological properties of plants. Also during the pathogenic attack, Si can induce rapid and extensive defense mechanisms (Fauteux et al., 2005). From a physiological standing point, silicon is able to increase the plasma membrane integrity by providing more stable lipids involved in cell membrane (Sahebi et al., 2015). Previous studies have suggested that, application of nano-silicon may alleviate the adverse effects of environmental stress on plants, increasingtheir water-use efficiency and photosynthesis rate (Ma, 2004). It has been suggested that, exogenous silicon may improve plant growth by enhancing antioxidant defense (Karimi and Mohsenzadeh, 2016). Nano-Si can also reduce grazing-resulted damages, such as that by insects, through changes in the tissue level to reduce palatability. Compared to control, treated plants with Si maintained higher stomatal conductance, relative water content, and higher water potential. Also, their leaves were larger and thicker, thereby limiting the loss of water through transpiration (Hattori et al., 2005) and reducing water consumption (Eneji et al., 2008). Si further affects the root structure and improves root resistance in dry soils (Hattori et al., 2005). It has been observed that, Si increased antioxidant defense capabilities and therefore maintained physiological processes such as photosynthesis (Pei et al., 2010).

The results demonstrated that, the low concentration of nano-particles could simultaneously increase the leaf area, chlorophyll concentration, and seed yield components. Within a plant, the source is the photosynthesizing tissue or the organ exportingcarbon skeletons, the sink is the one requiring carbon feed, the sink strength is the ability of a tissue or an organ to mobilize photo-assimilates, the sink size is the capacity of a tissue or organ to import and store further compounds from the source(s), and the sink activity is measured by the rate of respiration. Our findings revealed that, both of the moisture regimes and foliar treatments affected the source-sink relation considerably. Water scarcity was seen to reduce source and sink sizes significantly, while increasing the sink strength. Water stress increased the seed protein content noticeably. Seed protein was resulted from protein degradation in source tissue and amino acid remobilization for the seed. Available soil, water, temperature, nutrients, light, and CO_2 are indubitably important drivers of plant growth (Pessarakli, 2014). The former three (water, temperature, and nutrients) are, however, fundamentally different from the latter two (light and CO_2) because they can affect both sink and source activities, while light and CO_2 can only affect the source activity (Fatichi et al., 2014). It seems that, foliar application of nano-particles increases the plant growth and seed yield through improving the function of photosynthetic apparatus (source activities) and photo-assimilate translocation.These results are consistent with those of other studies and suggest thatthe application of Si may represent an approach to improve the growth of this crop and increase its production in arid or semi-arid areas where water is at a premium;this technique, however, would not fully substitute for an adequate water supply (Kaya et al., 2006).

However, in some of the evaluated traits, especially qualitative aspects and canopy temperature, the application of $nTiO_2$ended up with better results. The main reason for the improvements of these traits could have been the photo-sterilization and photo-generation of “active oxygen (e.g. superoxide) and hydroxide anions” by n-TiO_2 that could increase the plant stress resistance and promote efficiency of stomatal conductivity (Zheng et al., 2005). $nTiO_2$ increases plant growth by enhancing nitrogen metabolism (Yang et al., 2006) which improves the absorption of nitrate in spinach, and also by accelerating the conversion of inorganic nitrogen into organic nitrogen which increases the fresh and dry masses. Morteza et al. (2013) suggested that foliar utilization of $nTiO_2$ can improve plant growth and grain yield by improving the biosynthesis of pigments and conversionof light energy to chemical energy, thereby increasing photosynthetic efficiency. Effects of $nTiO_2$ on the content of light harvesting complex II (LHC II) on thylakoid membranes of spinach increases LHC II content (Hong et al., 2005; Lei et al., 2007). These promote energy transfer and oxygen evolution in photosystem II (PS II) of spinach (Lei et al., 2007). It was found that, nano-anatase TiO_2 promotes antioxidant stress by decreasing the accumulation of superoxide radicals, hydrogen peroxide, and malonyl dialdehyde content while enhancing the activities of superoxide dismutase, catalase (ascorbate peroxidase), and guaiacol oxidase, thereby increasing the evolution oxygen rate in spinach chloroplasts under UV-B radiation (Lei et al., 2008).. Khot et al. (2012) concluded that, the nano size of TiO_2 might have increased the absorption of inorganic nutrients, accelerated the breakdown of organic substances, and also caused quenching of oxygen free radicals formed during the photosynthetic process, hence increasing the photosynthetic rate. This finding supports our previous research which showed that foliar application of $nTiO_2$ on barley plants in a semi-arid region positively affected growth rate and seed yield (Janmohammadi et al., 2016a). It seems that, $nTiO_2$ plays a significant role in activatingdefense mechanism and modulating biosynthesis of phytohormones such as cytokinins and gibberellin (Mandeh et al., 2012). However, the accumulation of the nano-particles in plant tissue can be very important point from food safety perspective, therefore; their measurement in future experiments can be very valuable and provide precious information.

5 CONCLUSION

Our examination of the effects of SiO_2 andTiO2 nano-particles on safflower found that these nano-particles had differing effects on plant growth and quantitative and qualitative aspects of seed yield, and that the concentration of the nano-particles played largely contributed to these differences. Results revealed that, water-deficit stress drastically reduced the seed yield and seed oil content, while increasing the seed protein percentage. For almost all evaluated traits, the best performance was recorded under well irrigated condition with spraying low concentrations of nano-particles. Under water-deficit stress, however, the differences between foliar treatments were not significant.Our study suggests that, foliar application of SiO_2 suspension at low concentration can be a suitable agronomic monument for improving safflower performance.

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First report of nematodes *Parasitylenchus bifurcatus* Poinar & Steenberg, 2012 parasitizing multicolored Asian lady beetle *Harmonia axyridis* (Pallas, 1773) in Slovenia

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ABSTRACT

In years 2015 and 2016 around 200 specimens of ladybird species *Harmonia axyridis* were collected in Slovenia. Ladybirds were dissected and checked under stereomicroscope for presence of nematodes. Nematodes were discovered in samples of ladybirds *H. axyridis* collected at Brdo pri Lukovici and Trboje. The incidence of parasitized ladybirds was around 30 %. Nematodes were analysed morphometrically and the characteristic features of *Parasitylenchus bifurcatus* nematodes were observed: straight stylet lacking basal thickenings, a bursa and a forked tail tip in the vermiform females and juvenile males. The identity was confirmed with 18S rDNA region sequence (acc. no. LT629306 and LT629307) which showed high similarity (>99.9 % nucleotide identity) to the *P. bifurcatus* sequences in the public domain. This is a first report of *P. bifurcatus* nematode species found parasitizing ladybird *Harmonia axyridis* in Slovenia. The species compromises fecundity of its host and has therefore a potential to be used as a biological control agent to control high abundance of invasive *H. axyridis* ladybirds.

Key words: parasitic nematodes; ladybirds beetles; *Parasitylenchus bifurcatus; Harmonia axyridis*; harlequin ladybird; biological control

IZVLEČEK

PRVA NAJDBA OGORČIC *PARASITYLENCHUS BIFURCATUS* POINAR & STEENBERG, 2012, PARAZITOV PISANE POLONICE *HARMONIA AXYRIDIS* (PALLAS, 1773) V SLOVENIJI

V letih 2015 in 2016 smo v Sloveniji nabrali okrog 200 osebkov pisane polonice *Harmonia axyridis.* Pikapolonice smo secirali in pod lupo preverili prisotnost parazitskih ogorčic. Ogorčice so bile prisotne v pikapolonicah nabranih na Brdu pri Lukovici in v Trbojah. Stopnja parazitiranih pikapolonic je bila okrog 30 %. Morfometrična analiza je razkrila karakteristične znake ogorčic vrste *Parasitylenchus bifurcatus*: ravno bodalo brez zadebelitev pri osnovi, burso in razcepljeno konico repa pri črvastih samicah in ličinkah. Identiteto najdenih ogorčic smo potrdili z določitvijo nukleotidnega zaporedja odseka 18S rDNA (št. v javni bazi: LT629306 in LT629307), ki kaže veliko podobnost z zaporedji vrste *P. bifurcatus* dostopnimi v javnih bazah (več kot 99,9 odstotna enakost). Gre za prvo najdbo vrste *P. bifurcatus,* parazita pisane polonice *Harmonia axyridis,* v Sloveniji. Ogorčice vrste *P. bifurcatus* zmanjšajo rodnost svojega gostitelja in so kot take potencialni biotični agens za nadzor invazivne vrste pikapolonice *H. axyridis*.

Ključne besede: parazitske ogorčice; *Parasitylenchus bifurcatus*; pikapolonice; pisana polonica; *Harmonia axyridis*; biotično varstvo

1 INTRODUCTION

The multicolored Asian lady beetle, *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) originating from Asia has been applied as a biocontrol agent to control aphids and other harmful insects in orchards, vineyards, greenhouses, crop fields, and gardens (Koch 2003, Poinar and Steenberg 2012). It has been introduced to North America several times in the beginning of 20th century, to Eastern Europe in 1960s and become commercially available as a biological control agent in 1980s in the Western Europe. While its' ravenous appetite resulted in efficient biological control of harmful organisms, no one predicted that

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establishment of this introduced species in the natural environment will result in rapid spread and build-up of large populations endangering biodiversity of native insects as it feeds on numerous species of insects. It is also a fruit pest since it feeds and aggregates on apples, pears and grapes. It endangers wine production as wine produced from grape clusters containing adult beetles has an unpleasant flavour and odour. Further, it is a nuisance for humans since they try to overwinter in homes, release haemolymph from their legs when agitated (reflex bleed) leading to unpleasant odours and stains, and even occasionally bites humans and cause allergic reactions (Kovach, 2004).

Because of the above mentioned reasons the European and Mediterranean Plant Protection Organization (EPPO) has removed *Harmonia axyridis* from the list of recommended biological control agents in 2009 (EPPO, 2016). Multicolored Asian lady beetle has rapidly spread even in the countries where it has not been purposely introduced (e.g. Austria, Denmark, Great Britain) (Brown et al., 2008). The first observation of *H. axyridis* in natural environment in Slovenia dates back to 2007 (Polak, 2013). There is no known evidence that *H. axyridis* was used for biological control purposes in Slovenia. Its spread in Slovenia may result from (illegally) imported specimens escaped from greenhouses or gardens or it has spread naturally from the neighbouring countries, possibly from Austria. Since then, multicolored Asian lady beetle has become widely spread in Slovenia (Laznik et al., 2012, Polak, 2013).

The multicolored Asian lady beetle has a great reproductive and expansion potential (Polak, 2013). Furthermore, the successful invasion of this species can be attributed to extreme resistance of this species to diseases and parasites that attack other ladybirds. In Slovenia chemical control of multicolored Asian lady beetle is limited as there is no registered pesticide for controlling this species in agricultural production. However biocides can be used for the species control in household environments which are not intended for crop production. Therefore researchers have allocated their efforts in the search of natural enemies of multicolored Asian lady beetle that could reduce their numbers (Raak-van den Berg et al., 2014). Several natural enemies attacking adult multicolored Asian lady beetle have been reported, among them the nematode *Parasitylenchus bifurcatus* Poinar and Steenberg, 2012 (Tylenchida, Hexatylina: Iontonchioidea, Parasitylenchidae), entomopathogenic fungus *Hesperomyces virescens* Thaxt. (Ascomycota: Laboulbeniomycetes: Laboulbeniales), ectoparasitic mite *Coccipolipus hippodamiae* (McDaniel and Morrill, 1969) (Acarina: Podapolipidae), insect parasitoid *Dinocampus coccinellae* (Schrank, 1802) (Hymenoptera: Braconidae) and bacteria of the genus *Spiroplasma* (Majerus et al., 1999, Raak-van den Berg et al., 2014, Haelewaters et al., 2017).

Adult multicolored Asian lady beetles have been found parasitized by nematodes in Denmark in 2009 and the parasitic nematode was subsequently described as a new nematode species *Parasitylenchus bifurcatus* (Harding et al., 2011, Poinar and Steenberg, 2012). Diagnostic morphological characters of *Parasitylenchus bifurcatus* are a straight stylet lacking basal thickenings, an excretory pore opening at the level of or somewhat posterior to the nerve ring and a gubernaculum, a narrow bursa in the males and a forked tail tip in the vermiform (infective) females and juvenile males. The characteristic cleft tail tip giving rise also to the name of this new species separates it clearly from a similar species *Parasitylenchus coccinellinae* Iperti and van Waerebeke. Molecular marker often used as a character for nematode identification, the sequence of nearly full length SSU rDNA is available in public domain (Raak-van den Berg et al., 2014).

Different developmental stages of *P. bifurcatus*, first generation parasitic females, subsequent generation parasitic females, vermiform (infective) females, males and juvenile nematodes, occurred together in the body cavity of both female and male *H. axyridis* (Harding et al., 2011, Poinar and Steenberg, 2012, Raak-van den Berg et al., 2014). The prevalence of infected adult ladybirds collected in nature ranged from 2 - 47 % and increased up to 60 % when field-collected ladybirds were incubated in the laboratory for 30 days (Poinar and Steenberg, 2012, Haelewaters et al., 2017). These high rates of parasitism imply that *P. bifurcatus* is a potential biological control agent of *H. axyridis* as parasitism results in depletion of the fat body and partial or complete atrophy of the reproductive organs of the host. Further, *P. bifurcatus* parasitism has been shown as significant mortality factor of *H. axyridis* in the laboratory conditions (Poinar and Steenberg, 2012) and strong association between female failure to reproduce and infection with *P. bifurcatus* has been documented (Raak-van den Berg et al., 2014). Interestingly, *P. bifurcatus* parasitizes only adult host, while larvae and pupae are not included in its life cycle. With several subsequent generations of nematodes within the host, the numbers of nematodes can reach several hundred or even thousand juveniles. It is not documented how *P. bifurcatus* finds and enters uninfected adult beetles, but it is proposed that infective vermiform females with thick cuticle leave infected ladybirds while these are aggregated and enter other uninfected ones (Poinar and Steenberg, 2012).

The origin of *P. bifurcatus* infection in *H. axyridis* is unknown. One possibility is that it could have arrived with *H. axyridis* from Asia or North America, while the other possibility is that infection was acquired from endemic European ladybirds (Poinar and Steenberg, 2012, Raak-van den Berg et al., 2014).

So far, *P. bifurcatus* infecting *H. axyridis* has been documented from Denmark (Poinar and Steenberg, 2012), the Netherlands (Raak-van den Berg et al., 2014), the Czech Republic and Poland (Haelewaters et al., 2017), Kentucky and West Virginia in USA (Tove Steenberg, personal communication, May 28, 2015).

The objective of this study was to examine *H. axyridis* in Slovenia and to check whether it is parasitized by nematodes.

2 MATERIALS AND METHODS

2.1 Samples

In years 2015 and 2016 around 200 samples of adult ladybird species *Harmonia axyridis* from six different locations in Slovenia were collected and identified using morphological keys and species descriptions (Reitter, 1911; Freude et al., 1967; Chapin and Brou 1991; Riedel and Bastian, 2005; Polak, 2013). Ladybirds were dissected one to four days after sampling and individual nematodes were collected under the binocular in 0.9 % NaCl solution. The nematodes for morphometrical analysis were heat killed (at 65 ˚C), fixed in 4 % formalin and transferred on slides for further examination and measurements. Nematodes for molecular analysis were placed into 1 µl of dH_20 in 1.5 ml tubes and stored at -20 ^oC until isolation of DNA.

2.2 Morphomerics

Morphometric analysis encompassing measurements of common nematode body features was performed on 10 fixed nematode specimens of each nematode life stage using Nikon TiE microscope.

2.3 Molecular identification

2.3.1 DNA extraction

DNA was extracted from twenty nematodes. The Promega Genomic DNA Wizard purification kit (Madison, WI, USA) was used after a slight modification of the manufacturer's instructions. Extracted DNA was diluted in 20 µl of dH_20.

2.3.2 PCR amplification

For the amplification of 18S rDNA fragment, two set of primers, 1096F and 1912R, and 1813F and 2646R described by Holterman et al. (2006) were used. Both primers sets give overlapping sequences and together produce an 18 rDNA gene sequence of approximately 1.600 bp. PCR reactions contained 1 µl of isolated DNA, 1x GoTaq buffer (Promega), 1.5 mM MgCl2, 2.5 mM of each of the dNTPs, 1 µM of each of the primers, 1 U GoTaq Flexi DNA Polymerase (Promega) and distilled water up to 25 µl. The amplification was carried out in a thermal cycler Veriti (Applied Biosystems) using the amplification program as described by Holterman et al. (2006). Electrophoresis was performed on a 1 % TBE agarose gel to detect and inspect the amplified DNA product.

2.3.3 Cloning

PCR products were cloned using a pGEM®-T Easy vector kit (Promega) and transformed into competent cells of *E. coli* JM109 (Promega) according to the manufacturer's instructions. White colonies were selected in the blue/white colour screening for further analysis.

2.3.4 Isolation of plasmid DNA and sequencing

Selected clones were grown in 5 ml of LB medium with ampicillin (150 µg/ml) incubated overnight at 37 °C on a rotation shaker at 300 rpm. Overnight cultures were centrifuged at 2.700 rcf for 10 min, and the pellet used for isolation of plasmid DNA by GeneJet Plasmid Miniprep (Thermo Scientific) according to the manufacturer's instructions. The isolated plasmids were sequenced by Macrogen Inc. (Korea) using universal primers SP6 and T7.

2.3.5 Sequence analysis

DNA sequences were assembled using the computer software BioEdit v. 7.0.5.2 (Hall 1999). NCBI blastn suite was used to determine sequence similarity to other sequences in the public domain.

3 RESULTS AND DISCUSSION

In year 2015 none of the dissected ladybirds' specimens exhibited nematode parasites. In 2016 parasitic nematodes were recovered from *H. axyridis* collected at two locations, Brdo pri Lukovici and Trboje. The incidence of parasitized ladybirds from the samples was around 30%, which is in accordance with previous studies finding up to 35 % incidence in Denmark (Poinar and Steenberg, 2012). Even higher incidence up to 47 % was reported from the Czech Republic, (Haelewaters et al., 2017), while lower incidence was reported for the Netherlands (Raak-van den Berg et al., 2014).

Three different nematode life/developmental stages within the individual host were found, including subsequent generation parasitic females, vermiform (infective) females and males (Figure 1). First generation parasitic females were not encountered in analysed samples. Numbers of nematodes found in ladybirds varied, but up to several hundreds of nematodes could be found in some specimens. Contemporaneity of different developmental stages within a host and large nematode populations within a single beetle have been observed before (Poinar and Steenberg, 2012).

Common nematode body features were measured in morphometric analysis. The nematodes extracted from ladybirds were identified as *P. bifurcatus* (Table 1, Figure 1). The characteristic observed features of *P. bifurcatus* nematodes were straight stylet lacking basal thickenings, a narrow bursa and a forked tail tip in the vermiform females and juvenile males. Subsequent generation parasitic females from Trboje had smaller body length of 886.4 µm (782.0-1098.0) and body width of 72.3 µm (59.0-81.0) compared to 1300 µm (920 - 1600) and 195 µm (158-271) of *P. bifurcatus* nematodes from Denmark, respectively. All the other morphometrical characters were in the range of *P. bifurcatus* species description (Poinar and Steenberg, 2012). It can therefore be concluded that *P. bifurcatus* species morphometric characters of subsequent generation parasitic females may have somewhat higher variability than previously reported.

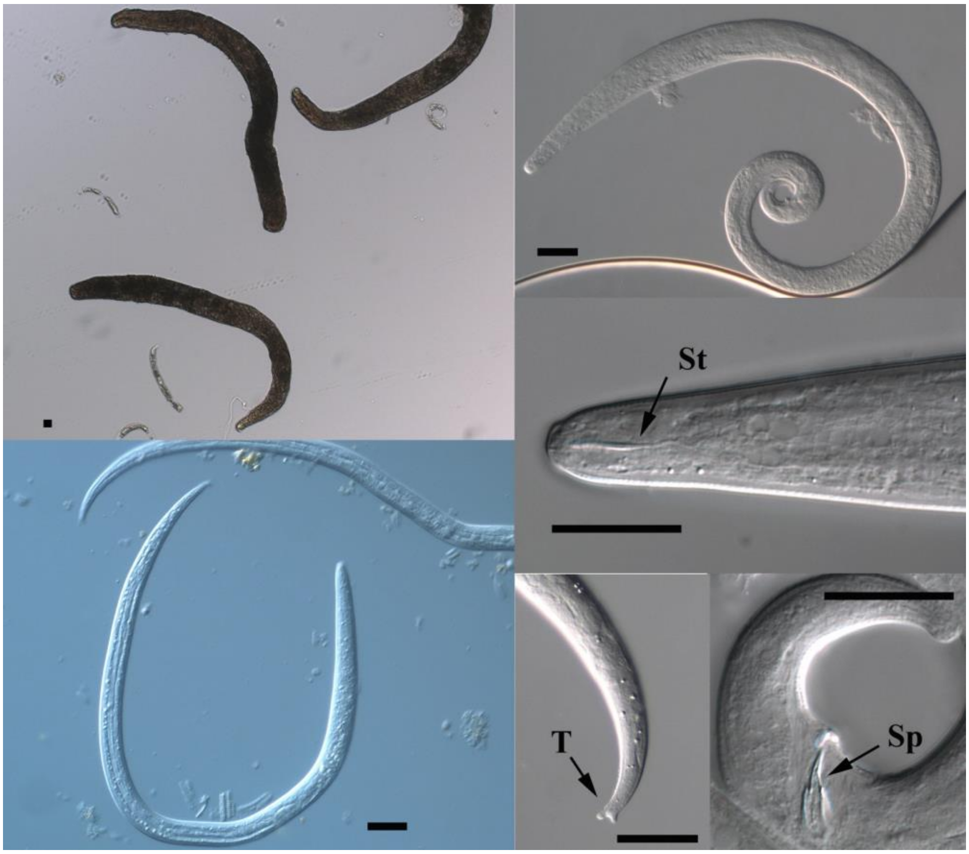

Figure 1: Different developmental stages of *Parasitylenchus bifurcatus* nematodes, parasites of *Harmonia axyridis* ladybirds. Arrows indicating straight stylet lacking basal thickenings (St), spicule (Sp) and a forked tail tip (T). Scale bars = 20µm

Table 1: Morphometric characters of subsequent generation parasitic females, vermiform (infective) females and males of *P. bifurcatus* nematodes isolated from *H. axyridis* from Trboje. All measurements are in µm, presented as mean ± standard deviation, with the range in parentheses

Character	Subsequent generation parasitic females (n = 10)	Vermiform (infective) females (n = 10)	Males (n=10)
Body length	886.4 ± 124.2 (782.0-1098.0)	646.9 ± 60.5 (573.0-752.0)	605.6 ± 58.8 (530.0-689.0)
Body width	72.3 ± 8.1 (59.0-81.0)	16.8 ± 1.6 (14.4-18.6)	21.2 ± 3.3 (17.9-25.8)
Stylet length	13.3 ± 0.5 (12.7-13.9)	12.6 ± 1.4 (10.6-14.2)	9.4 ± 0.3 (9.1-9.7)
Tail length	32.3 ± 1.3 (30.9-34.0)	37.8 ± 2.9 (35.2-41.0)	38.3 ± 4.6 (33.0-44.2)
Tail width	37.1 ± 3.3 (34.2-41.0)	10.7 ± 1.1 (9.5-11.8)	15.7 ± 1.6 (13.7-17.6)
Distance from head to excretory pore	219.3 ± 13.0 (206.5-234.0)	108.5 ± 3.5 (101.5-114.0)	103.6 ± 9.5 (90.9-117.0)
Vulva position %	93.9 ± 0.8 (93.0-94.8)	87.5 ± 0.8 (86.2-88.0)	-
Spicule length	-	-	11.8 ± 0.5 (11.3-12.2)
Spicule width at base	-	-	3.7 ± 0.3 (3.4-4.0)
Gubernaculum length	-	-	4.1 ± 0.4 (3.8-4.8)
Bursa length	-	-	8.6 ± 0.9 (7.3-9.2)

Two sequences were determined from nematodes extracted from the sample of *Harmonia axyridis* from Brdo, Slovenia. Two determined sequences of 18S rDNA region are 1.623 bp long and differ among them in 1 bp position. Both determined sequences belong to *P. bifurcatus* based on a high similarity to the *P. bifurcatus* sequences in the public domain determined with NCBI BlastN tool (99.94 % identity to *P. bifurcatus* sequence with acc. no. KC875397.1). Sequences obtained in this study were deposited at the European Nucleotide Archive, EMBL Nucleotide Sequence Database with the accession numbers LT629306 and LT629307. While no sequences are available for the species *P. coccinellinae* Iperti and Waerebeke to assess the phylogenetic relationship of these two related species, the bifurcated tail has not been reported for *P. coccinellinae* (Iperti and van Waerebeke, 1968, Poinar and Steenberg, 2012). Therefore, based on morphological and molecular characters this is a first report of nematodes *P. bifurcatus* parasitizing multicolored Asian lady beetles *H. axyridis* in Slovenia.

4 CONCLUSIONS

Multicolored Asian lady beetles collected in Slovenia in 2016 were found to be parasitized by nematode *P. bifurcatus.* Identification was confirmed with both, morphological and molecular characters.

Nematode species *P. bifurcatus* compromises fecundity of its host and has therefore a potential to be used as a biological control agent to control high abundance of invasive ladybirds of *H. axyridis* species. However, future studies on biology, possible transmission and effect of this parasitic nematode for native species of ladybirds are needed before any practical application. It is critical that the introduced biological control agent does not become pest themselves as has happened in the past with the intentional introduction of *H. axyridis* into North America and Europe. To our knowlede *P. bifurcatus* is at the moment not used anywhere to manage *H. axyridis*, but studies needed for justification and approval of such use are in progress (T. Steenberg, personal communication, December 1, 2015).

In EU there is no unified federal legislation regulating use of biological control agents (BCA) (Loomans, 2015). Some EU member states have national regulations, which are based on international standards, but are implemented in different ways (Hunt et al., 2011 cited in Loomans, 2015). Introduction of exotic BCA is covered in the following international acts: the International Plant Protection Convention, the Plant Protection Product Acts and the Convention on Biodiversity (Loomans, 2015). In Slovenia the use of biological control agent is regulated with the Plant Health Act (Zakon o zdravstvenem varstvu rastlin, Uradni list RS št. 62/07- uradno prečiščeno besedilo, 36/10 in 40/14 - ZIN-B). Detailed procedures for introduction, cultivation, trade and use of invertebrate BCA are subject to the Rules on biological control of plant pests (Pravilnik o biotičnem varstvu rastlin, Uradni list RS, št. 45/06, 28.4.2006). BCA are classified in two lists: the List of indigenous and the List of non-indigenous species of invertebrate organisms for biological control. The lists are maintained by the Administration based on the EPPO list (PM6/3) and on the basis of the results of researches, professional or scientific articles. Provisions of these regulations do not apply for the introduction and use of microorganisms for biological control. They are regulated by legislation in the field of plant protection products and are subject to different risk assessment in the registration procedure. Microorganisms are regulated by EU Regulation (EC) No. 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market or equivalent regulations. *P. bifurcatus* is not included on the lists of approved BCA in Slovenia. Further studies on safety and efficacy would be needed to include *P. bifurcatus* on this list.

Use of parasitic nematodes in plant protection is an accepted practice. Entomopathogenic nematodes are widely used as biocontrol agents to control several harmuful insect species in integrated pest management programs and slug-parasitic nematodes are used to control certain slugs and snails. Both groups are available as comercial biocontrol products and have been used for decades. Further, there are several studies exploring potential of predatory nematodes to control plant parasitic nematodes and potential of fungal-feeding nematodes for the control of soilborne plant pathogens (Grewal et al., 2005).

Although *P. bifurcatus* is a parasite of an insect species it is not an entomopathogenic nematode. Entomopathogenic nematodes kill their hosts relatively quickly (typically within 24–48 h of infection) with the help of their associated bacteria. On the other hand, *P. bifurcatus* is a typical parasite which can co-exist with its host for an extended time without killing it, but it does cause depletion of fat body as well as partial or complete atrophy of the insect's reproductive organs (Poinar and Steenberg, 2012, Haelewaters et al., 2017). As discussed above, *P. bifurcatus* may have potential as a biocontrol agent of *H. axyridis*.

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Influence of altered temperatures on allelopatic properties of *Amaranthus cruentus* L.

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ABSTRACT

The relationships between allelochemicals and environmental factors are a key factor for the growth of plants under rotation. We investigated the allelopathic potential of *Amaranthus cruentus* L, grown under different temperature conditions in *in vitro* bioassays. An inhibitory effect on germination and growth of lettuce (*Lactuca sativa* L.), tomato (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.) and cucumber (*Cucumis sativus* L.) was observed when seeds were subjected to the leaf litter of *Amaranthus cruentus*. Analysis from our study indicated that germination percentage was significantly affected by growth temperatures (T) of the amaranth ($P < 0.0001$), litter concentration (C) ($P < 0.0001$), vegetable type (V) ($P < 0.0001$), the T × V interaction ($P = 0.0041$) and V × C interaction ($P < 0.0001$). Pepper was the most sensitive with a decline in germination percentage at increasing concentrations (0, 1 and 5 mg ml^{-1}) of litter. Hypocotyl and seminal root lengths were adversely influenced by the plant litter for all the temperature treatments, although effects were most severe when exposed to the leaf litter of the hot temperature treatment. The inhibition caused by the litter was dependent on growth temperature and concentration, while each vegetable species showed different levels of sensitivity.

Key words: *Amaranthus cruentus*; allelopathy; vegetables; germination; hypocotyl; seminal root; crop rotation

IZVLEČEK

VPLIV SPREMENJENIH TEMPERATUR NA ALELOPATSKE LASTNOSTI MEHIŠKEGA ZRNATEGA ŠČIRA *Amaranthus cruentus* L.

Odnosi med alelokemikalijami in okoljskimi dejavniki so odločilni za rast rastlin v kolobarju. V razmerah in vitro poskusa je bil preučevan alelopatski potencial mehiškega zrnatega ščira (*Amaranthus cruentus* L.) v različnih temperaturnih razmerah. Inhibitorni učinek na kalitev in rast vrtne solate (*Lactuca sativa* L.), paradižnika (*Solanum lycopersicum* L.), paprike (*Capsicum annuum* L.) in kumar (*Cucumis sativus* L.) je bil ugotovljen, kadar so bila semena teh vrtnin izpostavljena listnim ostankom mehiškega zrnatega ščira. Analize so pokazale, da so na odstotek kalitve značilno vplivali rastna temperatura (T) ščira (T) ($P < 0.0001$), količina listnih ostankov (C) ($P < 0.0001$) in vrsta vrtnine (V) ($P < 0.0001$) kot tudi interakciji T × V ($P = 0.0041$) in V × C ($P < 0.0001$). Najbolj občutljiva je bila paprika z upadom odstotka kalitve pri naraščanju koncentracije ščira (0, 1 in 5 mg ml^{-1}). Na dolžino hipokotila in semenske korenine so ostanki ščira vplivali negativno pri vseh temperaturah, vendar so bili učinki izrazitejši pri visokih temperaturah. Inhibicija, ki so jo povzročili ostanki ščira, je bila odvisna od njihovih količin in rastnih temperatur, vendar je vsaka od vrtnin pokazala različno občutljivost.

Ključne besede: *Amaranthus cruentus;* alelopatija; vrtnine; kalitev; hipokotil, semenska korenina; setveni kolobar

1 INTORODUCTION

Amaranthus cruentus L., also known as "Mexican grain amaranth" belongs to the family Amaranthaceae and is characterised by being an annual, pseudo-cereal with broad leaves which are used as a leafy vegetable and a forage crop (Saunders & Becker, 1984; Steckel, 2004; Yaacob et al., 2004.). *Amaranthus* spp. have been traditionally used in medicine for the treatment of various diseases and their antioxidant properties have been recorded by various authors (Olumayokun et al., 2004; Dhellot et al., 2006; Mensah et al., 2008). The habitat where amaranth is found differs dramatically and the temperatures that they can grow ranges from 20-

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This article is part of a Master thesis entitled » Influence of abiotic stress on allelopathic properties of *Amaranthus cruentus* L. «, issued by Ingrid Allemann, Supervisor Dr Maria Elizabeth Cawood, Ph. D., Co-Supervisor Dr James Allemann, Ph.D.

35 °C (Grosz-Heilman et al., 1990; Guo & Al-Khatib, 2003). Given its several advantages as a crop, its unique nutritional properties, and its use as food and feed (Ayo, 2001; Bavec & Bavec, 2006; Grobelnik et al., 2009), grain amaranth is receiving increasing attention as an alternative crop worldwide. *Amaranthus cruentus* is one of three amaranthus species cultivated as vegetable and grain source. The other two are *Amaranthus caudatus* L. and *Amaranthus hypochondriacus* L. (Olaniyi, 2007; Olaniyi et al., 2008). Recently, Mlakar et al. (2012) compared the allelopathic activities of extracts prepared from fresh roots, stems, leaves, and inflorescence with seeds of weedy and grain amaranths. It was found that at varying concentrations, the aqueous extracts of the grain amaranth, *A. cruentus*, exert allelopathic activity. Compared to the pigweed amaranth, the grain species displayed a stronger inhibitory effect on the germination process, and root elongation of garden cress. Allelopathy can affect a whole range of aspects in agroecosystems namely, weed management, plant reproduction, species associations, the mulching effect on crops and the succession and rotation of cultivated species (Chon et al., 2006; Rawat et al., 2017). These results point out the problematic consequence when amaranth and more specific, *A. cruentus*, is cultivated in crop rotation systems. Understanding this biological occurrence could help to improve applications in both natural and agricultural systems and is helpful for planning and managing cropping systems (Gronle et al., 2015). Furthermore, climate change influences a plant's chemical response and the ecological function of plant allelochemicals (Harvey & Malcicka, 2015). Sudden changes in temperature may influence the production of chemical compounds and the allelopathic properties of a plant (Maqbool et al., 2013). The allelopathic potential of *Amaranthus retroflexus* increased when grown at high latitudes compared to plants grown at low latitudes, indicating the role of temperature on the expression of allelochemicals (Wang et al., 2017). Therefore, the aim of this study was to investigate the relationship between temperature variation and allelopathic effects of different concentrations of plant litter of *A. cruentus* on the germination and growth of some vegetable species in order to determine the crops' feasibility for intercropping and in rotation systems.

2 MATERIALS AND METHODS

2.1 Plant material

Amaranthus cruentus 'Anna' seeds were planted in pots containing a soil-compost (80:20 v/v) mixture and grown at 28/21 °C; day/night temperatures in climate controlled chambers at The Department of Agriculture, University of the Free State. Fifty pots per chamber with an average of three plants per pot were kept in three different chambers and daylight was set at 12 hours in order to prevent flowering. The plants were watered every second day and fertilised with Nutrifeed solution (Starke Ayres). After three months plants were subjected to cool (14/7 °C) and hot (33/40 °C) temperatures. Stress continued for 14 days where after aerial parts of the plants were harvested and the plant material lyophilised, ground into a fine powder and kept at 4 °C until further analyses. Optimum (28/21 °C) temperature grown plants serve as temperature controls.

2.2 Vegetable seeds

Seeds were obtained from Starke Ayres: Tomato 'Money Maker', Sweet Pepper 'California Wonder', Cucumber v 'Ashley' and Lettuce 'Great Lakes'.

2.3 Allelopathy bioassay

The 'sandwich method' of Fujii et al. (2003) was used for determining the *in vitro* phytotoxicity of the leaf litter from the different temperature treatments of *A. cruentus* on the various vegetable seeds. For this method a 5 ml layer of 0.5 % (w/v) sterile water agar was poured into each well of a sterile multi-dish plate and allowed to set. The *A. cruentus* leaf litter was placed on this bottom layer and a second layer of 5 ml sterile water agar was added on top. This made a sandwich of powdered leaf litter between the two layers of agar (10 ml in total). This method was used in order to physically separate the *A. cruentus* samples from the tested seeds, however allows for diffusion of any active component from the sample through the barrier agar layer. The phytotoxicity of 10 and 50 mg of the powdered leaf litter was measured (1 and 5 mg ml^{-1} litter per well) against the different vegetable seeds. Controls contain no leaf material.

Vegetable seeds were surface sterilised by washing in 96 % ethanol for 1 minute followed by 1.30 min in 3.5 % NaCl and finally back into ethanol for 30 seconds. After sterilization the seeds were placed on sterile filter paper and left to dry within a laminar flow cabinet (Labotec, airflow from above). The seeds were then positioned vertically on the top layer of agar in each well, plates were closed and incubated at 25 °C in black bags (full darkness) for 3-7 days depending on the vegetable used. On the 3^{rd} to 7^{th} day the germination percentage and length of the seminal root and hypocotyl was measured. Each of the experiments was done in triplicate and presented as the mean of the replicates.

2.4 Statistical analysis

The results were expressed as means with least significant difference (*LSD*). Analysis of variance (ANOVA) was performed using SAS 9.3 (Institute Inc., Cary, NC, USA, 2008) statistical programme for data and Tukey-Kramer's *LSD* procedure for comparison of means. Significance of differences compared to the control groups was determined using the t-test (Steel & Torrie, 1980).

3 RESULTS AND DISCUSSION

A. cruentus is used as a food crop, and both its grain and leaves being consumed. This plant is now being considered as a new agricultural crop; it is therefore important to understand how this plant will interact within a changing environment and how this will influence other plants. In addition to the increase in average temperatures, global warming is also characterized by an increase in the frequency of the occurrence of extremely high and low temperatures (Wagner, 1996; IPCC 2007). This study showed that allelopathic activity of *A. cruentus* could be affected by environmental temperatures, which can affect other crops negatively in rotation systems. Highly significant ($P < 0.01$) differences in germination percentage of the various vegetables exposed to the litter of amaranth plants grown under stress or optimal temperature conditions can be seen in Figure 1. Vegetables exposed to plant litter of amaranth grown under hot temperature conditions showed the most significant inhibition in germination (Figure 1). Peppers were the most sensitive with 60 % germination inhibition. Lettuce was the least affected with only 22 % inhibition and affected more negatively by amaranth litter from plants cultivated at optimal temperatures than by that of plants grown at stress temperatures (Figure 1). It appeared as though germination of cucumber, pepper and tomato seeds were generally more sensitive to amaranth litter than lettuce. Steinsiek et al. (1982) reported a more severe allelopathic effect of an aqueous extract of common wheat (*Triticum aestivum* L) straw on the inhibition of germination and growth of selected weed species at 35 °C than at 25 °C or 30 °C. Einhellig & Echrich (1984) claimed that grain sorghum (*Sorghum bicolor* (L.) Moench.) and soybean (*Glycine max* (L.) Merr.) were more susceptible to ferulic acid when they were grown at higher temperatures. It was also observed that at 26/22 °C (day/night) temperature, the allelopathic inhibition of chlorogenic acid or tomatine was greater on the growth of an insect herbivore, *Manduca sexta* (Linnaeus, 1763) than at 26/14 °C (Stamp & Osier, 1997).

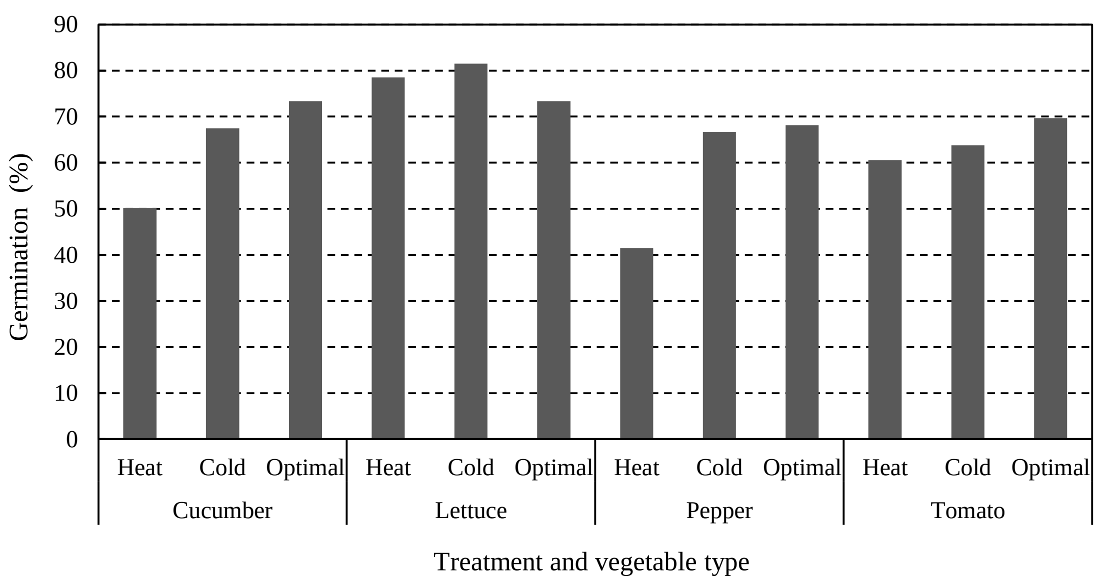

Figure 1: Response of the germination percentages of various vegetables to amaranth litter from plants grown at different temperatures. The data are expressed as average values. $P < 0.01$ ($LSD_{(T:0.05)} = 2.71$). $n = 96$.

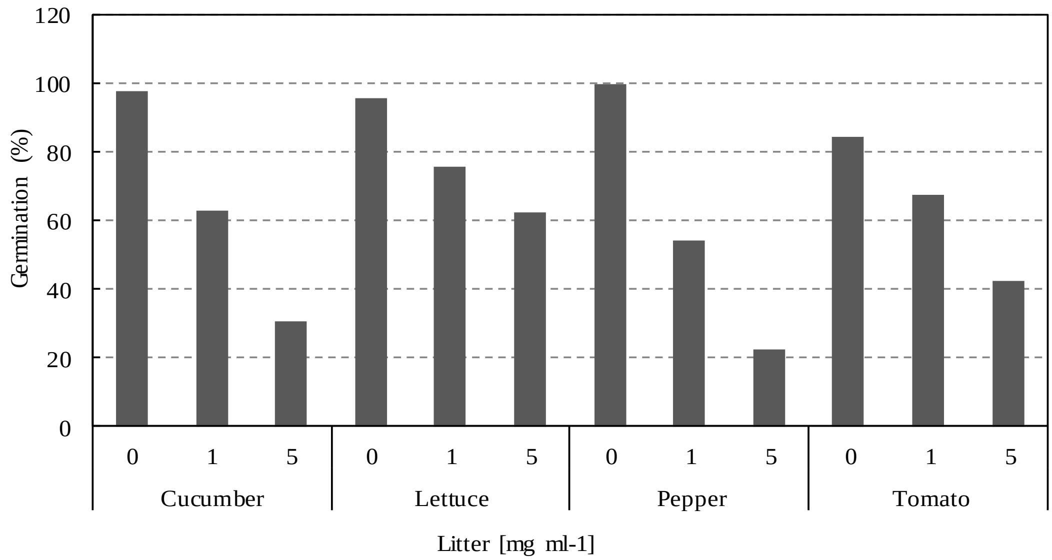

Figure 2: Influence of increasing litter concentration on the germination percentage of four vegetables ($LSD_{(T:0.05)}$ = 2.71). n = 96

Our results emphasise the importance of *A. cruentus* litter concentration on germination and growth of vegetables. The significant interaction between amaranth litter concentration and the effect on the four vegetable species is illustrated in Figure 2 and Table 1. Here it is clear that germination percentage and growth of all four vegetables decreased significantly with increased concentration of amaranth litter that was added to the growth medium, however, each vegetable species showed different levels of sensitivity. Peppers were the most sensitive to amaranth litter with the germination percentage falling from 100 % through 54 % to 22 % at the 0, 1 and 5 mg ml^{-1} litter concentrations respectively. Tomatoes were the least affected, with germination inhibition of only 17 % and 43 %, followed by 19 % and 33 % for lettuce and 36 and 66 % for cucumber at 1 and 5 mg ml^{-1}. From Table 1, an inhibition of 34 %, 76 % and 78 % in hypocotyl growth and 35 %, 68 % and 85 % in seminal root growth occurred in cucumber, pepper and tomato respectively at 1 mg ml^{-1}. An increase in concentration to 5 mg ml^{-1} resulted in growth inhibition of 65 % for cucumber and more than 90 % for pepper and tomato (Table 1).

Table 1: Effect of different concentrations of amaranth litter on the average hypocotyl and seminal root lengths of various vegetables

Leaf litter [mg ml^{-1}]	Hypocotyl length (mm)		
	Cucumber	Pepper	Tomato
0	33.15 ± 23.82	18.49 ± 13.99	39.56 ± 13.82
1	21.74 ± 24.62	4.39 ± 5.45	8.62 ± 9.93
5	11.87 ± 15.19	1.67 ± 2.64	2.05 ± 4.77
AVG	22.25	8.18	16.74
$LSD_{(T \leq 0.05)}$	11.06	4.44	4.67
Leaf litter [mg ml^{-1}]	Seminal root length (mm)		
	Cucumber	Pepper	Tomato
0	23.59 ± 10.32	13.38 ± 5.08	26.69 ± 6.67
1	15.36 ± 15.94	4.33 ± 5.76	4.08 ± 3.09
5	8.82 ± 10.58	1.23 ± 2.06	2.54 ± 2.89
AVG	15.92	6.31	33.31
$LSD_{(T \leq 0.05)}$	6.45	2.33	2.28

n = 13.

According to Qasem (2010), allelopathy plays a significant role in seed dormancy, seed germination and seedling emergence. The influence of allelochemicals on the balance of plant populations and species stability, microorganisms, natural enemies and insect populations, and the spread of pathogens is another important role of allelopathy (Qasem, 2010).

From the results it is clear that germination of all vegetables tested, was adversely affected by both concentrations of amaranth litter irrespective of the temperature treatment to which the plants were exposed (Figure 3). Analysis from our study therefore indicated that germination percentage was significantly affected by growth temperatures (T) ($P < 0.0001$) of the amaranth, litter concentration (C) ($P < 0.0001$), vegetable type (V) ($P < 0.0001$), the T × V interaction ($P = 0.0041$) and V × C interaction ($P < 0.0001$). The T × V × C ($P = 0.0540$) interaction showed no statistical differences.

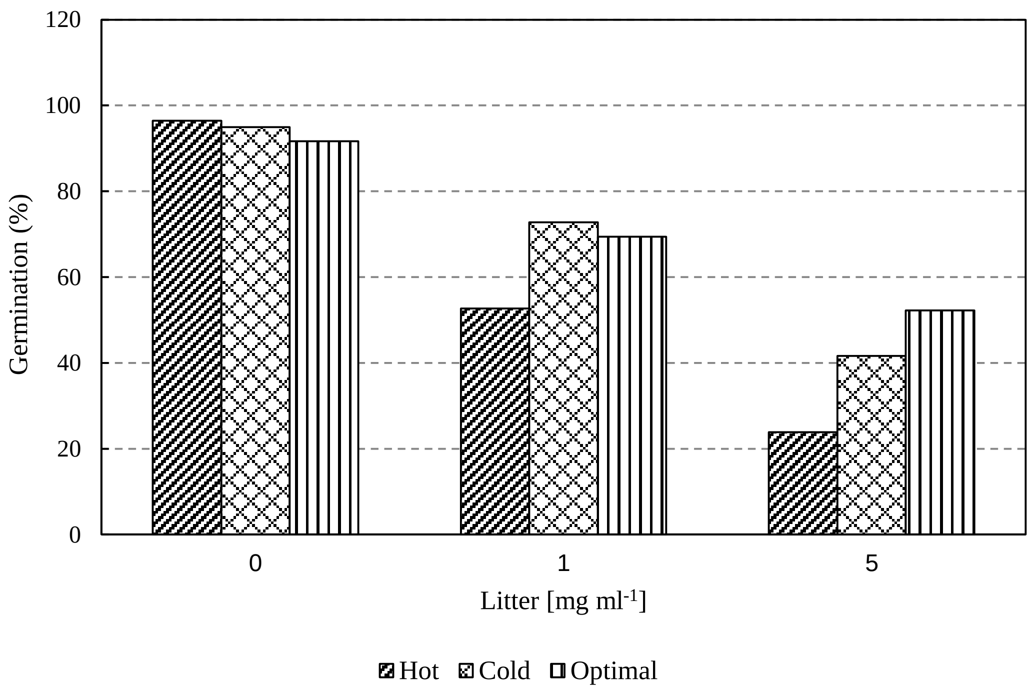

Figure 3: Two way interaction (ANOVA) between litter concentration and temperature on germination percentage of vegetables. $P < 0.01$ ($LSD_{(T:0.05)} = 2.73$). $n = 96$

Lettuce growth exhibited a two way interaction between concentration of plant litter and temperature treatment (Figure 4). Litter concentration and temperature treatments were highly significant in reducing hypocotyl ($LSD_{(T \leq 0.05)} = 3.43$) and seminal root ($LSD_{(T \leq 0.05)} = 3.41$) lengths respectively. From Figure 4 it is clear that the hot and optimal temperatures at both concentrations of litter, had a more severe effect on growth than the cool temperature treatment. Concentrations of 5 mg ml^{-1} for all the treatments inhibited hypocotyl and seminal root development almost completely.

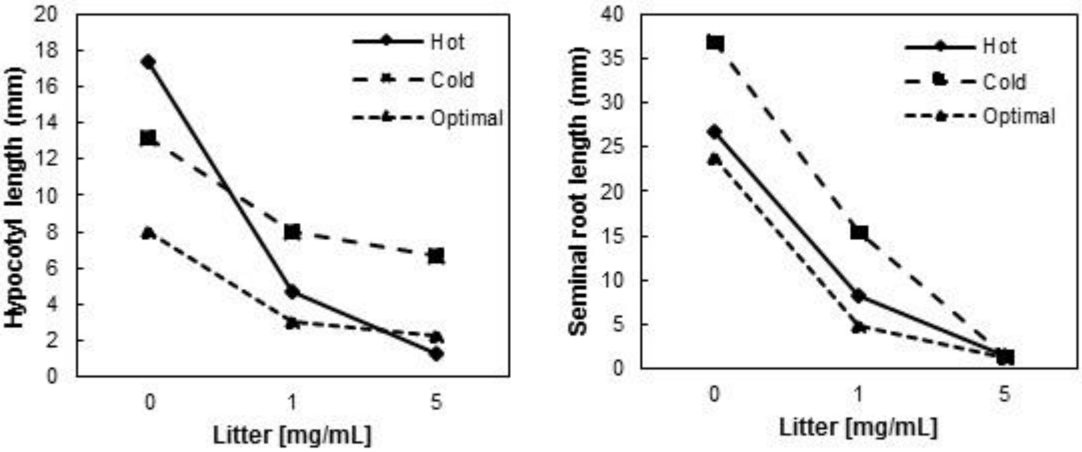

Figure 4: Results of two-way ANOVA of the effect of litter concentration and temperature and their interaction on hypocotyl ($LSD_{(T \leq 0.05)}$ = 3.43) and seminal root lengths ($LSD_{(T \leq 0.05)}$ = 3.41) of lettuce seedlings. *n* = 13.

It is demonstrated in this study that the environment for the cultivation of *A. cruentus* is important and that compounds responsible for allelopathy, are present even at optimal growth conditions. Furthermore, with increased concentrations of litter a decrease in germination and seedling development occurred. Therefore, it is possible that more leftover plant material in a field can have a more severe allelopathic effect on the following harvest. It was also clear that vegetables displayed diversity in reaction towards the temperature treatments and type of extract. In crop rotation systems it is important, because if germination is affected by compounds *of A. cruentus* produced under stress conditions, farmers can incur financial losses.

4 CONCLUSION

The *in vitro* bioassay results of the plant litter from *A. cruentus* indicated that the allelopathic compounds inhibiting germination and growth of vegetables are produced by amaranth irrespective of cultivation temperature, although the litter of plants grown under stressful conditions have a greater allelopathic effect than those at optimal environments. A difference in the response of vegetables and sensitivity to increasing concentration may have a negative impact in crop rotation systems. Results showed that it is vital to understand under which conditions amaranth was cultivated. Field trials will contribute to the confirmation of the phytotoxicity of the leaf litter and will be conducted in future studies. This will add to the understanding of allelopathic properties of *A. cruentus* in crop rotating systems.

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Tiskano / Printed: Ovrednotenje genskih virov belih sort žlahtne vinske trte (*Vitis vinifera* L.) = Evalution of genetic resources of white grapevine varieties (*Vitis vinifera* L.) / Radojko Pelengić … [et al.].- Ilustr. – Dostopno tudi na: http://aas.bf.uni-lj.si/december2012/19Pelengic.pdf, URN:NBN:SI:doc-GTI6AHLW, http://www.dlib.si/details/URN:NBN:SI:doc-GTI6AHLW. – Besedilo v slov. – Bibliografija: str. 437-438. – Abstract; Izvleček

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