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Assessment of genetic diversity of Turkish and Algerian native sheep breeds

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Assessment of genetic diversity of Turkish and Algerian native sheep breeds

Abstract: In Algeria and Turkey, the sheep production systems are based on the under extensive rural conditions and their genetic management has led to increased homozygosity and hence productivity loss. The identification of inter-breed and intra-breed genetic diversity plays a key role in the shaping of conservation and breeding programs. The present study was conducted to investigate the genetic diversity of native sheep breeds reared in Turkey and Algeria. A total of 240 animals from four Algerian (Hamra, Ouled Djellal, Sidaou, and Tazegzawt) and four Turkish (White Karaman, South Karaman, Karacabey Merino, and Kıvırcık) native sheep breeds were genotyped with fourteen microsatellite markers recommended by FAO. A total of 340 alleles were detected from fourteen markers studied. All the eight breeds exhibited moderate to high levels of genetic diversity, with a slight superiority of the Algerian sheep breeds. Overall FIS value was low, but highly significant ($p < 0.001$). It may have been due to the high inbreeding within the population. The mean global coefficient of gene differentiation (GST) showed that approximately 94.0 % of the genetic variation was within-population. The highest number of private alleles with a frequency above 5 % was observed in Ouled Djellal sheep. Structure analysis of populations studied revealed the most appropriate K with four genetic clusters. As the result, the dendrogram showed that the Algerian sheep breeds were completely separated from the Turkish sheep breeds furthermore the Bayesian clustering revealed a high level of admixture, especially in Algerian sheep populations.

Key words: small ruminants; native sheep breeds; genetic diversity; microsatellite; genetic distances

Pregled genetske pestrosti turških in alžirskih avtohotnih populacij ovac

Izvleček: Sistemi reje ovac v Alžiriji in Turčiji temeljijo na ekstenzivni kmečki reji, upravljanje s temi populacijami pa je privedlo do povečanja homozigotnosti in s tem do poslabšanja proizvodnih lastnosti. Ocena medpasemske in znotrajpasemske genske pestrosti igra ključno vlogo pri oblikovanju programov za ohranjanje teh pasem. Pričujoča študija je bila izvedena z namenom raziskovanja genske pestrosti avtohtonih pasem ovac v Turčiji in v Alžiriji. Skupno 240 živali štirih alžirskih (hamra, ouled djellal, sidaou in tazegzawt) in štirih turških (white karaman, south karaman, karacabey merino in kıvırcık) pasem je bilo genotipiziranih s štirinajstimi mikrosatelitnimi markerji, ki jih priporoča FAO. Na štirinajstih analiziranih markerskih lokusih je bilo zabeleženih 340 alelov. Vseh osem pasem je imelo zmerno do visoko stopnjo genske pestrosti z rahlo prevlado alžirskih pasem ovac. Skupna vrednost FIS je bila nizka, vendar statistično značilna ($p < 0,001$). To je verjetno posledica visokega inbridinga v populaciji. Povprečni globalni koeficient diferenciacije genov (GST) je pokazal, da je približno 94,0 % genske pestrosti znotraj populacije. Največ privatnih alelov s pogostostjo nad 5 % je bilo ugotovljenih pri ovcah pasme ouled djellal. Analiza strukture populacij je pokazala, da je K, ki predvideva štiri genetske klastre najprimernejši. Dendrogram, ki je rezultat študije, je pokazal, da so alžirske pasme ovac popolnoma ločene od turških, poleg tega pa je Bayesovo klastriranje pokazalo visoko stopnjo križanja, zlasti v alžirskih populacijah ovac.

Ključne besede: drobnica; avtohtone pasme ovac; genska pestrost; mikrosateliti; genetske distance

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1 INTRODUCTION

Domestic sheep (*Ovis aries*) has been a very important farm animal species for the people economically and culturally since its domestication time around the world (Ryder, 1983). Algeria and Turkey are endowed with diverse farm animal genetic resources including the sheep breeds they are favored by the different climates existed and vegetation, which has since generated a very specific sheep breeding practice. The majority of the sheep population of Turkey and Algeria is composed of multipurpose native breed producing meat, milk, and wool. According to Turkish Statistics Agency, there are 31.2 million sheep heads distributed among twenty sheep breeds have been officially registered while Algeria has a large local sheep population with around 28 million head (Faostat, 2016), the latter contains twelve local ovine breeds with very different phenotypic characteristics (Ameur Ameur et al., 2017).

Turkey has a great genetic diversity that can be characterized by numerous sheep breeds are categorized into four main groups: Fat-tailed, thin-tailed, crossbreeds and extinct breeds (Yilmaz et al., 2013). South Karaman sheep breed, which is one of these breeds and especially raised in the Taurus Mountains located in the Mediterranean region, is a fat tail native sheep breed. It was reported that the hides of this breed, which is very similar to the Karagül breed, could be used in making inner fur because of having a curly pattern of fleece (Ertuğrul et al., 2009; Kiraz et al., 2014). White Karaman sheep breed is the most commonly used breed in central Anatolia in Turkey for lamb production, with a population of around 16.000,000 it makes up more than 50 % of the national sheep herd (TUIK, 2018). Kıvırcık and Karacabey Merino sheep breeds are especially raised in western part of Turkey are known for their meat quality, wool, and meat production (Öner et al., 2014; Yilmaz et al., 2011, Karaca et al., 2009). Karacabey Merino was developed by crossbreeding of Kıvırcık and German Black Head Mutton (Yalcın, 1986).

Algerian domestic sheep breeds were classified according to many different methods such as phenotypic data, morphological and molecular descriptions throughout history (Chelig, 1992; Djaout et al., 2017). The Ouled Djellal breed (white Arabian breed), which constitutes more than half of the Algerian sheep population and is widely raised in Algeria. This breed has a good body conformation, has a high level of adaptability to different climatic conditions (Chelig, 1992; Djaout et al., 2017). The Hamra sheep breed known as Deghma is a very important sheep breed for meat tenderness in Algeria (Chelig, 1992; Djaout et al., 2017). The Sidaou breed, known as Targuia, is raised in the Sahara between Libya-Niger and southern part of Algeria with several more than one million head (Chelig, 1992; Djaout et al., 2017). Tazegzawt sheep breed which is constituted 0.02 % of the total population raised in Kabyle and Ham in the region of Mechria (Chelig, 1992; Moulla, 2015; Djaout et al., 2017). In recent years, non-systematic cross-breeding practices and changes in consumer habits have triggered a quantitative reduction of this breed and quickly faced the risk of extinction threat (Moulla, 2015).

Autosomal microsatellites are a well-known effective and powerful tool to investigate genetic structure and diversity have been widely used in sheep breeds, in all over the world, for Turkish sheep breeds (Koban, 2004; Gutiérrez-Gil et al., 2007; Yılmaz and Karaca, 2012; Yilmaz et al.; 2013; Cemal et al., 2013; Yilmaz et al., 2014) and for the Algerian sheep breeds (Gaouar et al., 2014; Gaouar et al.,2015; Gaouar et al.,2016a; Ghernouti et al., 2017; Ameur Ameur et al., 2018).

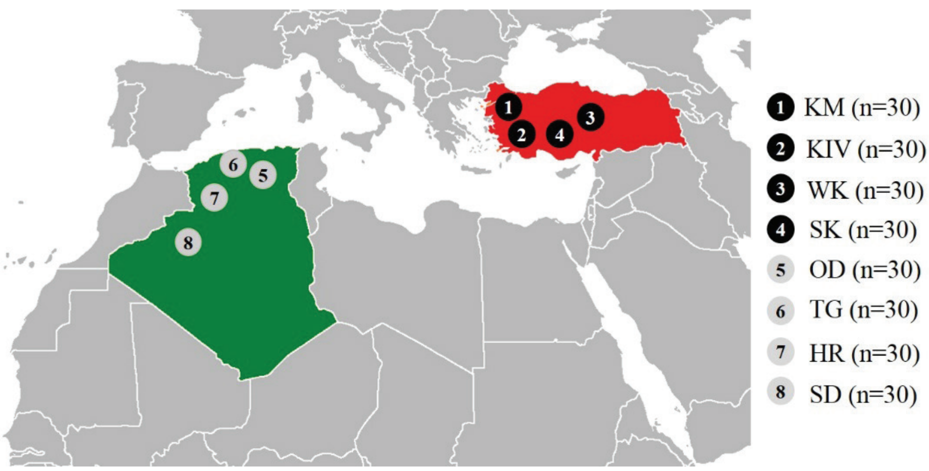

Figure 1: Geographical location of Algeria and Turkish sheep breeds WK: White Karaman, SK: South Karaman, KM: Karacabey Merino, KIV: Kıvırcık, HR: Hamra, OD: Ouled Djellal, SD: Sidaou, TG: Tazegzawt

The first step for a well-structured and sustainable animal breeding and conservation program is to reveal detailed information on intra and inter-breed genetic diversity. This situation indicates how important it is to reveal the genetic structure of breeds. The objective of the present study was to determine genetic diversity and population structure of different native sheep breeds raised in two different countries.

2 MATERIALS AND METHODS

2.1 ANIMAL MATERIAL AND DNA ISOLATION

Blood samples were obtained from 240 sheep, which consist of Hamra (30), Ouled Djellal (30), Sidaou (30), Tazegzawt (30) raised in Algeria and White Karaman (30), South Karaman (30), Karacabey Merino (30) and Kıvırcık sheep (30) breeds raised in Turkey (Figure 1).

2.2 SAMPLING METHOD AND DNA ISOLATION

Blood samples were obtained from 240 head sheep. Blood samples were collected from Vena jugularis into tubes containing K3-EDTA as anticoagulant and stored at −20 °C until DNA extraction. DNA was extracted by using the salting-out technique reported by Miller et al. (1988) and Montgomery and Sise (1990). NanoDrop 2000 (Thermo Scientific, Waltham, MA) spectrophotometer device was used to determinate the quality and quantity of DNA samples.

2.3 PCR AND FRAGMENT ANALYSIS

Fourteen microsatellite markers labeled with a fluorescent dye (D2, D3, and D4) were used according to the recommendation of FAO (2011). Two multiplex groups were created according to the fragment length of microsatellites. Touchdown PCR protocols reported by Hecker and Roux, (1996) were used for the amplification of specific genomic regions (Table 1). The total volume of the amplification mixture amounted to 25 µL. Amplification mixture contained 0.1 µM/each primer, 0.2 mM dNTPs (Applied Biological Materials Inc., Canada), 2.0 mM $MgCl_2$, 1X PCR buffer, 1U of Taq DNA polymerase (Applied Biological Materials Inc.. Canada) and ~50 ng genomic DNA. Capillary electrophoresis was used for the separation of the PCR fragments labeled with fluorescent dye in the Beckman Coulter GeXP genetic analyzer (Beckman Coulter. Inc. USA).GenomeLab™ DNA Size Standard Kit 400 was used for the determination of fragment size.

2.4 STATISTICAL ANALYSIS

The polymorphism statistics such as number of alleles per locus (Na), mean number of alleles (MNa), effective number of alleles (Ne), observed heterozygosity

Table 1: Thermal cycling conditions according to Touchdown PCR

Loci (Dye)	Multiplex group	First denaturation	Denaturation	Annealing	Extension	Cycle	Final extension
OarFCB193 (D3) OarFCB304 (D3) INRA0023 (D3) OarCP34 (D4) D5S2 (D4) BM1818 (D4)	1	95 °C (5 min)	95 °C (40 sec)	63–54 °C (40 sec)	72 °C (60 sec)	40	72 °C (10 min)
BM8125 (D3) McM0527 (D3) CSRD0247 (D3) OarFCB128 (D2) BM1329 (D2) HSC (D2) OarJMP29 (D4) MAF214 (D4)	2	95 °C (5 min)	95 °C (40 sec)	60–50 °C (40 sec)	72 °C (60 sec)	34	72 °C (10 min)

(Ho), expected heterozygosity (He), Wright's F-statistics (F_{IT}, F_{IS}, F_{ST}), Hardy–Weinberg equilibrium and Principal component analysis (PCoA) was performed using the software GENALEX (Peakall & Smouse, 2006) were calculated using GenAlEx (Peakall and Smouse, 2012). Polymorphic information content (PIC) and null allele frequencies were calculated using CERVUS 3.0.3 (Marshall, 1998). Populations 1.2.32 (Langella, 1999) and FigTree 1.4.2. (Rambout, 2006) software was used to generate neighbor-joining (NJ) tree phylogenetic tree between breeds according to Nei's Da distance matrix (Nei et al., 1983). Robustness of the dendrogram topology was tested by bootstrap resampling (n = 1000). FSTAT version 2.9.3 software (Goudet, 2001) was used to obtain values belong to genetic diversity statistics such as Nei's gene diversity (H_T), diversity between breeds (D_{ST}), and coefficient of gene differentiation (G_{ST}). Analysis of molecular variance (AMOVA) was performed using the ARLEQUIN v3.5.2.2 (Excoffier and Lischer, 2010). The STRUCTURE software was used to analyze population structure using independent allele frequencies and an admixture model (burn of 20.000, followed by 100.000 MCMC iterations with 20 replicate runs for each K) (Pritchard et al., 2000). The appropriate number of clusters was identified using ΔK values (K = 2 to 8) that expressing the proportion of alteration in the logarithmic probability Pr(X|K) (Evanno et al., 2005). The most suitable K value was determined according to ΔK value calculated by the STRUCTURE harvester program (Earl and Vonholdt, 2012). The CLUMPAK program reported by Kopelman et al. (2015) was used to find the best alignment from the obtained STRUCTURE results.

3 RESULTS

In this study, a total of 340 alleles were identified from fourteen microsatellites used in the present study. Molecular genetic statistical parameters obtained from the fourteen microsatellites used was given in Table 2.

The number of alleles ranged from 17 (OarCP34, D5S2) to 32 (CSRD0247), while the average number of effective alleles was 10.99. Observed heterozygosity (Ho) values varied from 0.67 (OarFCB304) to 0.85 (OarCP34). PIC values were found to be between 0.87 and 0.93. F_{IS} values that use the local gene pool as a reference point were obtained as positive in thirteen of fourteen microsatellites. The F_{IT} value expressing general heterozygosity loss was higher in BM1329 (0.254) locus than the other. F_{ST} described as an indicator of genetic variation among individuals within the population was observed varied from OarJMP29 (0.041) to BM1329 (0.107) with a mean of 0.068. Mean value of D_{ST} indicating genetic diversity between breeds, G_{ST}, which is an important indicator of

Table 2: Genetic polymorphism parameters of the fourteen investigated loci in sheep breeds studied

Locus	N	Na	Ne	PIC	Ho	He	F_{IS}*	F_{IT}*	F_{ST}*	D_{ST}	G_{ST}	H_T	HWE	F(Null)
OarFCB304	237	25	8.22	0.87	0.67	0.88	0.172^{***}	0.252^{***}	0.096^{***}	0.074	0.084	0.88	***	0.134
OarFCB193	239	23	9.17	0.89	0.80	0.89	0.066^{**}	0.105^{***}	0.042^{***}	0.033	0.036	0.89	***	0.053
BM1818	234	28	14.17	0.93	0.83	0.93	0.047^{*}	0.118^{***}	0.075^{***}	0.061	0.065	0.93	***	0.059
INRA0132	240	21	11.92	0.91	0.82	0.92	0.052^{*}	0.113^{***}	0.064^{***}	0.052	0.056	0.92	***	0.057
OarCP34	239	17	7.68	0.86	0.85	0.87	-0.029^{ns}	0.027^{ns}	0.055^{***}	0.042	0.049	0.87	***	0.008
D5S2	207	17	8.19	0.87	0.73	0.88	0.109^{**}	0.181^{***}	0.080^{***}	0.061	0.069	0.88	***	0.092
CSRD0247	230	32	13.21	0.92	0.76	0.92	0.120^{***}	0.186^{***}	0.075^{***}	0.062	0.067	0.93	***	0.103
MCM0527	232	20	9.59	0.89	0.68	0.90	0.207^{***}	0.252^{***}	0.056^{***}	0.044	0.049	0.90	***	0.140
BM8125	240	20	12.70	0.92	0.78	0.92	0.094^{***}	0.169^{***}	0.083^{***}	0.068	0.074	0.92	***	0.083
HSC	231	24	14.48	0.93	0.72	0.93	0.191^{***}	0.230^{***}	0.048^{***}	0.039	0.042	0.93	***	0.122
BM1329	237	31	14.13	0.93	0.70	0.93	0.164^{***}	0.254^{***}	0.107^{***}	0.088	0.094	0.93	***	0.137
OarFCB128	240	22	10.22	0.90	0.76	0.90	0.116^{***}	0.162^{***}	0.052^{***}	0.041	0.046	0.90	***	0.086
OarJMP29	240	31	11.20	0.91	0.81	0.91	0.081^{***}	0.119^{***}	0.041^{***}	0.033	0.036	0.91	***	0.058
MAF214	240	29	8.92	0.88	0.77	0.89	0.071^{*}	0.142^{***}	0.076^{***}	0.060	0.068	0.89	***	0.077
Mean		24.29	10.99	0.90	0.76	0.90	0.104	0.165	0.068	0.054	0.060	0.91		

N: Number of genotyped individuals, Na: number of alleles, Ne: effective number of alleles, PIC: polymorphic information content, F_{IT}, F_{IS}, F_{ST}: Wright's F-statistics, Ho: observed heterozygosity, He: expected heterozygosity, HWE: Hardy-Weinberg Equilibrium, F(Null): null allele frequency, H_T: Nei's gene diversity, D_{ST}: the diversity between breeds, G_{ST}: coefficient of gene differentiation, *: Wright's statistics according to Weir and Cockerham (1984), *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

Table 3: Genetic polymorphism parameters according to studied Turkish and Algerian sheep breeds across 14 loci

		Mean Heterozigosity				NPA		
Breeds	MNA	Ho (SE)	He (SE)	F_{IS}	HWE	Freq. ≥ 5%	Freq. < 5%	Total
WK	9.93	0.78 (0.040)	0.80 (0.016)	0.047^{ns}	5	5	3	8
KIV	9.57	0.58 (0.056)	0.81 (0.012)	0.326^{***}	9	3	1	4
KM	10.21	0.67 (0.058)	0.76 (0.030)	0.114^{***}	11	1	5	6
SK	9.57	0.72 (0.063)	0.74 (0.059)	0.055^{**}	2	2	3	5
HR	14.71	0.84 (0.023)	0.89 (0.006)	0.055^{***}	8	1	5	6
OD	17.00	0.83 (0.022)	0.90 (0.005)	0.075^{***}	5	-	17	17
SD	13.86	0.82 (0.028)	0.87 (0.010)	0.025^{ns}	4	1	7	8
TG	15.07	0.83 (0.028)	0.86 (0.014)	0.038^{*}	7	1	11	12

WK: White Karaman, SK: South Karaman, KM: Karacabey Merino, KIV: Kıvırcık, HR: Hamra, OD: Ouled djellal, SD: Sidaou, TG: Tazegzawt, MNA: number of alleles, Ho: observed heterozygosity. He: expected heterozygosity, HWE: number of loci not in the Hardy–Weinberg equilibrium ($p < 0.05$), NPA: private alleles, within-breed, F_{IS}: heterozygote deficiency, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

the relative magnitude of genetic differentiation, and H_T, described as total genetic diversity, values were found as 0.054, 0.060 and 0.91, respectively. Microsatellite loci genotyped in the present study were tested using the χ^2 test in terms of compliance with HWE. All fourteen loci deviated from the HWE ($p < 0.001$). Null allele frequency belonging to microsatellite loci used were found to be below 20 %. The results of genetic diversity statistics for each breed are summarized in Table 3.

The mean number of alleles varied from 9.57 (KIV and SK) to 17.00 (OD). The highest expected heterozygosity was observed in OD (0.90) sheep breed reared in Algeria. All the studied breeds showed positive F_{IS} values. Although a total of 66 private alleles have been identified in all breeds studied, only fourteen of them have a frequency greater than 5 %. Analysis of Molecular Variance (AMOVA), which is a method to detect population differentiation utilizing molecular markers, was performed to detect genetic variation between individuals and populations (Table 4).

Table 4: AMOVA of the eight sheep breeds genotyped with fourteen microsatellite markers

Variation Sources	DF	SS	VC	PV (%)	FI
Among population	7	204.88	0.3893 Va	6.71	F_{IS} = 0.092
Among individuals within populations	232	1370.53	0.4964 Vb	8.56	F_{ST} = 0.067
Within individuals	240	1179.50	4.9145 Vc	84.73	F_{IT} = 0.153
Total	479	2754.91	5.800		

DF: degree of freedom, SS: sum of square, VC: variance components, PV: percentage of variance, FI: fixation index

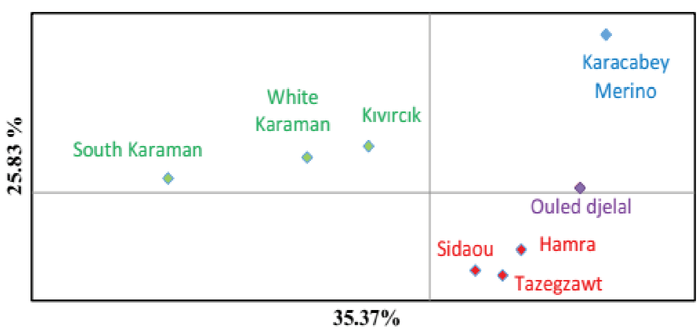

Figure 2: Principal component analysis. Plot of the first (PC1: X axis) and second (PC2: Y axis) principal components for 8 sheep populations.

It was revealed 84.73 % of the total variance was found within individuals while 8.56 % among individuals within populations and 6.71 % among the population.

In the PCA analysis (Figure 2) of the Nei's genetic distance, the first two axes represent 35.38 % and 25.83 % of the total genetic variability, respectively. The phylogenetic network of eight sheep breeds (Figure 3) confirmed and complemented the PCA analysis results. Four cluster belonging to eight breeds studied was revealed in dendrogram based on Nei's Da distance matrix. The first cluster consisted of WK,

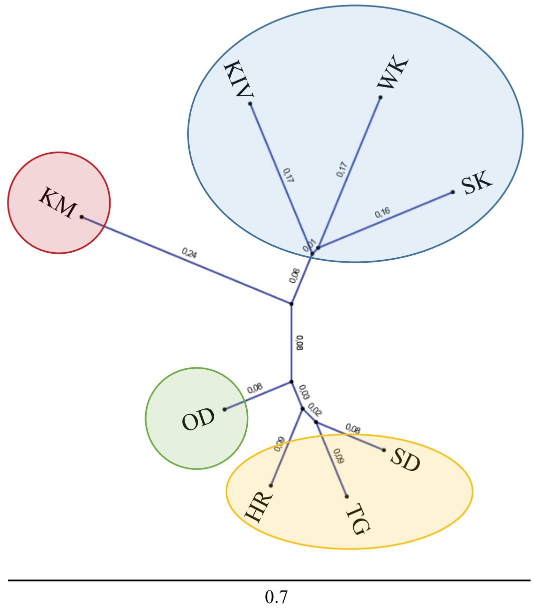

Figure 3: Dendrogram based on Nei's Da distance matrix in studied sheep breeds (bootstrap resampling methodology (1000 replicates)) (WK: White Karaman, SK: South Karaman, KM: Karacabey Merino, KIV: Kıvırcık, HR: Hamra, OD: Ouled djellal, SD: Sidaou, TG: Tazegzawt)

SK and KIV, the second cluster was formed by KM sheep breed raised in Turkey, the third cluster was formed by OD sheep breed and fourth cluster was formed by HR, TG and SD sheep breeds sampled from Algeria.

The results of the Population structure analysis containing different numbers of clustering (K = 2–8) and performed to determine the population structure of the studied breeds are given in Figure 4.

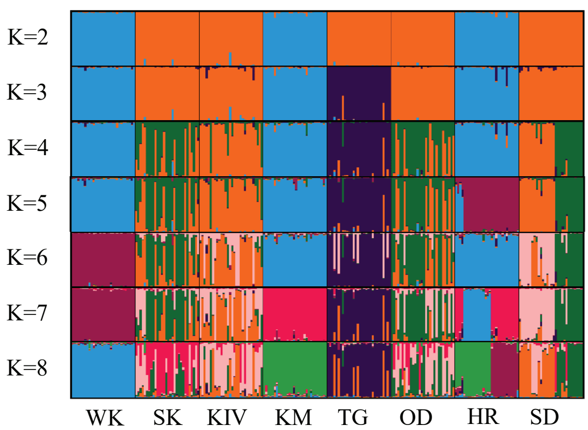

Figure 4: Estimation of the population structure with different K values (WK: White Karaman, SK: South Karaman, KM: Karacabey Merino, KIV: Kıvırcık, HR: Hamra, OD: Ouled Djellal, SD: Sidaou, TG: Tazegzawt)

Table 5: Estimated posterior probabilities [Ln Pr(X|K)] for different numbers of inferred clusters (K) and ΔK statistic

K	Mean LnP(K)	ΔK
2	−16.760,605	—
3	−16.232,850	3.7415
4	−16.025,320	6.1730
5	−16.209,625	0.5631
6	−15.647,060	3.9293
7	−15.638,650	2.5657
8	−16.353,680	—

To present the suitable cluster number (K) in structure analysis results were given in Table 5.

The results obtained from the STRUCTURE analysis were similar to the dendrogram drawn according to Nei's Da distance matrix (Nei et al., 1983) as expected. It is seen that the optimal number of groups was 4 considering the value of ΔK obtained by the method reported by Evanno et al. (2005).

4 DISCUSSION

Today, conservation of farm animal species and the determination of the genetic diversity is currently on the agenda of animal breeders. In this context, conservation activities and characterization of genetic diversity for animal genetic resources have become very important phenomena in all over the world. Genetic variation needed for genetic improvement of domestic animals is a basic requirement for animal breeding (Askari et al., 2011).

Mean number of alleles and polymorphic information content values observed in the present study were higher than values obtained from the other sheep breeds (Yilmaz et al., 2015; Guang-Xin et al., 2016; Kırıkçı et al., 2018). This situation can be regarded as an important indicator of high genetic diversity in sheep populations studied.

F_{IS} values, which is a measure of the deviation of genotypic frequencies from panmixia in populations in terms of heterozygous deficiency or excess, showed that loss of heterozygosity at just one microsatellite locus (OarCP34). The value of overall F_{IS} was low, but highly significant ($p < 0.001$). It may have been due to the high inbreeding within the population. The local inbreeding coefficient (F_{IS}) values, which is used the local gene pool as the point of reference, was low but highly significant ($p < 0.01$) except OarCP34.

Similar findings have been found in previous experiments, conducted in different sheep breeds (Yilmaz et al., 2015). Overall F_{ST} value indicated that a moderate genetic drift occurred in populations. It can be said that this situation is an expected finding given that populations are reared under extensive conditions and are mated freely.

The global G_{ST} value showed that 94.00 % of the total genetic variation can be explained by genetic differences among individuals. It can be accepted that the overall genetic diversity value (DST) obtained from the present study was an indication that the inter-population variability is not high. This finding supported the previously mentioned FST and GST results.

Overall H_T described as Nei's gene diversity value was 0.91 which was higher than the values obtained from Algerian native sheep breeds (Ameur Ameur et al., 2018), Turkish native sheep breeds (Yilmaz et al., 2015) and Albanian native sheep breeds (Hoda and Marsan, 2012) This finding supported Na and PIC values is an indication that goats have a high genetic diversity of populations studied.

The Hardy–Weinberg equilibrium (HWE), which is stated that allele and genotype frequencies in a population will remain constant from generation to generation in the absence of other evolutionary mechanisms, was analyzed using χ^2 test. Test results showed that allele distributions of all studied loci deviated from Hardy-Weinberg equilibrium. This is an expected finding due to the intensive selection studies conducted in the studied populations for long years.

Null allele, which was first introduced by Paetkau and Strobeck (1995), causes to misreading of the microsatellite peaks. It has been reported by Dakin and Avise (2004) that null allele frequencies below 0.20 have no significant effect on molecular genetic studies performed with microsatellites. When the null allele frequencies obtained are examined, it is seen that the null allele frequency values of fourteen microsatellites to be studied are below 0.20. Taking this value into consideration, it has been demonstrated that working locus can be used safely in genetic diversity.

In the present study, the calculated MNa values belonging to Algerian sheep populations studied were found to be higher than the values stated in some studies on domestic and foreign breeds (Gaouar et al., 2016b; Loukovitis et al., 2016; Naqvi et al., 2017) but MNa values observed in Turkish sheep breeds were lower than those of native sheep breeds raised in Tunisia (Sassi-Zaidy et al., 2016). This is thought to be due to the difference in the number of microsatellites and sampling methodology used in this study. FIS value, defined as the inbreeding coefficient, indicated that there is no loss of heterozygosity in populations. Deviations from the Hardy-Weinberg equilibrium should be regarded as a natural consequence of the intensive animal breeding activities that have been practiced in the populations for many years. Although the number of private alleles defined as the source of genetic diversity which has a frequency above 5 % are limited, it can be said that they have sufficient efficiency to identify populations studied.

It is seen that the essential genetic diversity is realized within individuals when the results of the analysis of molecular variance (AMOVA) are examined. Fixation index values give an idea in terms of the inbreeding coefficient and population differences. Analysis of molecular variance (AMOVA) results pointed out that these eight native sheep breeds can be differentiated weakly. The F_{ST} value obtained from the AMOVA analysis was parallel to the G_{ST} value. This finding proves that most of the genetic diversity is caused by the difference between individuals in the present study.

It was noticed that there were four clusters when the dendrogram was examined. Dendrogram, which is showed the position of the Turkish sheep breeds in the present study was different from the findings obtained from the study conducted on the same breeds by Yilmaz et al. (2015). It is known that the Karacabey Merino breed was obtained by crossbreeding the sheep breeds of Kıvırcık reared in the Marmara region and German Black Head Mutton (Sezenler and Özder, 2009). It has a large number of local Kıvırcık form that is adapted to the different regions in Turkey (Öner et al., 2014). Kıvırcık sheep breed which is used as animal material in the study material is raised in the Aegean region. In the context of this information, it is expected result that these two breeds will take place in different clusters in the obtained dendrogram.

STRUCTURE results showed a low level of differentiation and a high level of admixture, especially in Algerian sheep populations. The value of ΔK obtained by the method reported by Evanno et al. (2005) shows that the optimal number of groups is four as in the dendrogram. This revealed that STRUCTURE analysis and dendrogram results supported each other. It can be said that there was a high gene flow between Algerian sheep populations when examined the results obtaoined by CLUMPACK software.

5 CONCLUSION

Domestic sheep reared all over the world, are raised for meat, milk, or fiber production, or conservation purposes. Non-systematic cross-breeding practices applied to increase the production capabilities of domestic animals carried out by breeders are among the main problems of animal husbandry in North African countries as in Turkey (Karaca et al., 2009). This type of practice leads to one of the major threats such as the disappearance of local genetic diversity (Herold et al., 2012).

The present study, which was carried out for the first time, was not described any genetic similarities between Algeria and Turkey sheep breeds. However, the result shows that populations studied have a low level of differentiation and a high level of admixture. Results obtained from the present study revealed that Algerian and Turkish sheep breeds have a high genetic variability. While the knowledge of genetic diversity between breeds is important, the benefit of understanding the genetic variation within a population is considerable. There is no research focused on within and between breed genetic variations in Algerian and Turkish sheep breeds. Microsatellites used in the study have a highly accurate identification potency for the genetic diversity of the studied breeds.

In conclusion, the present study has revealed an important knowledge about genetic diversity and the relationship between some sheep breeds raised in Algeria and Turkey. The information obtained in the study has made a significant contribution to future animal genetic conservation and breeding programs.

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7 CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Comparative study of amaranth species (*Amaranthus* spp.) in the temperate continental climate of Russian Federation

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Comparative study of amaranth species (*Amaranthus* spp.) in the temperate continental climate of Russian Federation

Abstract: Field experiments were carried out in the Chuvash Republic, which is located in the center of the European part of Russia on the banks of the Volga River and has a moderately continental climate with warm summers, cold winters, well-defined transitional seasons and average annual rainfall of 500 mm. There are many cultural and wild species of amaranth in nature, differing in morphological and biological features. The objects of research were four common species of amaranth (*Amaranthus* spp.): *A. cruentus* L., *A. caudatus* L., *A. hybridus* L., *A. spinosus* L.. Particular attention in the experiment was paid to the morphological and biological characteristics of plants according to the growth stages during growing season and the formation of the yield of green mass and grain during 3 years of research. As a result of the experiment, the possibility of cultivating a heat-loving amaranth culture in the climatic conditions of the Chuvash Republic was proved and adaptive species for growing green mass (*A.cruentus* and *A.caudatus*) and for grain (*A.cruentus*) were identified.

Key words: *A. cruentus*; *A. caudatus*; *A. hybridus*; *A. spinosus*; climatic conditions; growing season; growth stages

Primerjalna raziskava vrst ščira (*Amaranthus* spp.) v zmernem kontinentalnem podnebju Ruske Federacije

Izvleček: Poljski poskus je bil izveden v Čuvaški republiki, v osrednem delu evropskega dela Rusije, na bregovih Volge, ki ima zmerno kontinentalno podnebje s toplimi poletji, mrzlimi zimami, z dobro izraženimi prehodnimi obdobji in s povprečno letno količino padavin 500 mm. Na območju uspeva več gojenih in podivjanih vrst ščira z različnimi morfološkimi in biološkimi lastnostmi. Predmet raziskave so bile štiri pogoste vrste ščira: *A. cruentus* L., *A. caudatus* L., *A. hybridus* L., *A. spinosus* L.. Posebna pozornost je bila posvečena morfološkim in biološkim lastnostim rastlin glede na fazo v rastni sezoni, tvorbi biomase in zrnja v 3 letih raziskav. Kot rezultat poskusa je bila dokazana možnost gojitve toploljubnih ščirov v klimatski razmerah Čuvaške republike, za zeleno biomaso vrsti *A.cruentus* and *A.caudatus* in za zrnje vrsto *A.cruentus*.

Ključne besede: *A. cruentus*; *A. caudatus*; *A. hybridus*; *A. spinosus*; rastna sezona; faze rasti; prilagodljiva vrsta

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1 INTRODUCTION

Agriculture faces a great pressure to produce greater quantities of food, feed and biofuel on declining land resources for the projected nine billion people on the planet by 2050 (Godfray et al., 2010). It is predicted that agricultural production has to increase by 70 % by 2050 to cope with an estimated 40 % increase in world population. So, utilization of underutilized food crop performs a massive function for providing food, feed and vitamins to such increasing population (Bruinsma, 2009). In recent years producers and consumers have been interested in new plant species, which are referred as alternative plants or new crops. Whether they are actually new or just recently rediscovered, these species not only create valuable crude material for a number of industrial branches but they also constitute an important source of renewable energy. In addition, they add to the human menu, making it more diverse (Rastogi & Shukla, 2013). Amaranth is one of the few multipurpose crops who provide grain, leafy vegetable, fodder, and greater diet then the predominant staple crops. Nutrition value and use of grain amaranth is a potential future application in bread making (Mlakar et al., 2009).

It is a tremendously short-lived annual, which develop vigorously, drought resistant and adapt effortlessly to new environments. It originates from tropical America, archeological excavations proved it also in India but from tropical America is has spread all over the tropical World, as China, Nepal, Italy, Greece, Africa, and Australia (Ozsoy, 2009). Amaranth is not a "true cereal" such as wheat, corn or barley, but it is considered as "pseudo-cereal" like buckwheat (*Fagopirum esculentum* Moench) and quinoa (*Chenopodium quinoa* Willd.). Amaranth belongs to the order of Caryophyllales and family of Amaranthaceae and to the genus of *Amaranthus*. Amaranth leaves have excellentl chemical composition with mild spinach-like taste so it comes beneath an accurate leafy vegetable (Amicarelli & Camaggio, 2012).

Amaranth species (*A. blitum* L., *A. caudatus* L., *A. cruentus* L., *A. tricolor* L.) are collectively known as amaranths or pigweed. They have a common name such as African spinach, India spinach and Chinese spinach; approximately 60 species are recognized with inflorescence and foliage ranging from purple and red to gold. Members of this genus share many characteristics and uses with members of the closely related genus *Celosia* (Juan, 2007).

Amaranth originates from South America, from where it was widely distributed in most tropical regions and has been used as a grain, leafy vegetable and forage crop (Ebert, 2010). Amaranths could be divided into two groups, based on their consumption, grain and vegetable amaranths. Species grown for vegetables are represented mainly by *A. tricolor*, *A. dubius*, *A. lividus* L., *A. creuntus*, *A. palmeri* S. Wats. and *A. hybridus*. Three principal species considered for grain include, *A. hypochondriacus* L., *A. cruentus* and *A. caudatus* (Topwal, 2019).

Amaranth is a dicotyledonous, herbaceous plant with an erect stem and large inflorescence. Amaranth is C_4 plant and belongs into group of NAD-malic enzyme-type of C_4 metabolism. Some anatomical characteristics of amaranth and its C_4-photosynthesis pathway result in increased efficiency of the usage of CO_2 below an extensive vary of temperature (from 25 °C to 40 °C), below greater mild intensity, and moisture stress environments. All this contribute to the crop's huge geographic adaptability to numerous environmental conditions (Kaufman, 1992). Amaranth leaves can be used as greens in salads, boiled or fried in oil and mixed with meat or fish. This can be used as side dish in soups or as an ingredient in sauce and baby food (Mlakar et al., 2010). The leaves are high in fibers and contain high concentration of vitamin A, B6 and C, riboflavin and foliate. Minerals include calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese. An amaranth grain can be ground for use in bread, noodle, pancakes, cereals, granola cookies or other flour baked products. More than 40 products containing amaranths are currently on the market in the USA (Putnam, 2007). Leafy vegetables of amaranth supply protein, minerals and vitamins in diet. Their lush, green, succulent crisp are eaten raw or cooked as vegetables in soup, they are best when the plant is young and tender. Amaranth grain is high in protein and contains two essential amino acids: lysine and methionine, which are frequently found in other cereal grains. It is higher in fiber and iron than wheat, and higher in calcium (Uusikua, 2010). Amaranth is a widely adapted genus, and can be grown in different climate conditions. But the more limiting factor is the temperature and less limiting is the rainfall amount during the growing season (Dmitrieva, 2018 b). Amaranth can be grown together with corn and sunflower to obtain high-nutrient silage. To do this, the seeds of amaranth, corn and sunflower are sown in separate rows in one field and cut together for silage without mixing in the future (Dmitrieva, 1993).

The aim of the research was to compare amaranth species (*Amaranthus* spp.) by morphological and biological characteristics and to determine adaptive species among them for cultivation for fodder and grain purposes in the soil and climatic conditions of the Chuvash Republic.

2 MATERIALS AND METHODS

2.1 FIELD EXPERIMENT

Field experiment was conducted in period from

2015 to 2017 at the experimental field of the University located on the right Bank of the Volga river in Cheboksary (53°58'N; 47°15'E). The soil type of the experimental field is light gray forest loam, medium podzolic according to the Russian classification that corresponds to soils with average nutritional values (Shishova et al., 2004). The pH of the salt extract (pH_{kcl}) is 5.4, the humus content according to Tyurin is 2.2 %. The arable layer was characterized by moderate concentrations of phosphorus (12.0 mg 100 g^{-1} of soil), potassium (12.0 mg 100 g^{-1} of soil) and magnesium (4.5 mg 100 g^{-1} of soil). The thickness of the arable horizon is 21-23 cm, relief the fields is flat. Judging by the agrochemical characteristics, the soils of the experimental plot do not have the properties to obtain high yields of amaranth without the application of mineral fertilizers. There was one background of mineral nutrition in the experiment. Before sowing, N, P, K fertilizers were applied to all plots at the following rates: 60 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1} according to soil analysis. The area of the experimental plot was 40 m^2 respectively 10 m^2 for each species of amaranth. The experiment had a randomized block design with four replications.

The analyzed species were four of the most common species of amaranth (*Amaranthus* spp.): *A. cruentus*, *A. caudatus*, *A. hybridus, A. spinosus*. from different groups of plants according to plant height: long-stemmed, medium-stemmed and short-stemmed (Table 1).

Every year, amaranth was sown after potatoes. Since the seeds are very small (mass of 1000 seeds is 0.6 g) amaranth requires thorough soil preparation in the spring (double loosening of the soil to a depth of 6 and 8 cm). For the same reason seeds were mixed with sand at a ratio of 1 g seed to 100 g sand to facilitate the sowing process and to obtain a uniform stand (Dmitrieva, 2018a, Fadeeva & Dmitrieva, 2017). Seeds were sown in late May at soil temperatures above 16-18 ^{0}C to a depth of 1 cm. Method of planting was broadcasting and seeds were spaced at 70 cm between rows. Seeding rate was 1.0 kg ha^{-1}. Weeding was done using hoe. First weeding was carried out at two weeks after sowing and second weeding at 4 weeks after sowing. Harvesting for green mass (silage) and grain was done by cutting the whole plant on different dates depending on the *Amaranthus* spp. and weather conditions (Table 2). Observations of plant growth, plant height, number of leaves per plant, leaf area were carried out in accordance with the growth stages: vegetative state, inflorescence formation, flowering, seed maturation. To measure plant height a meter ruler was used to take the height of four tagged plants of amaranths in each experimental unit, and the mean was calculated and recorded. The height was measured from the ground level to the tip of the plant. Leaves of four tagged plants were counted in each experimental, and the mean was determined and recorded. Only fully opened leaves were counted. To calculate the leaf area, the length and width were multiplied using the constant (6.6) for four tagged plants of each amaranth species. The fresh mass of the plants was determined after cutting of whole plant in the net plots, and then extrapolated to per hectare. The harvested amaranth (dry mass of the plant) was dried on 60-70 ^{0}C in laboratory and weighed to determine the dry mass of the net plot and then extrapolated to per hectare. Seeds were harvested once when inflorescence change color to yellow or pink. Plants were cut, threshed and seed cleaned. Seed harvesting was carried out by cutting inflorescences and threshing seeds only in dry and warm weather for two weeks (Table 2). The content of nutrients in the green mass and grain (protein, lipids, carotene, sugar) was determined in the agrochemical laboratory of the Universityby near infrared spectroscopy (Russian Organization for Standardization, 2012).

2.2 WEATHER CONDITIONS

Chuvash Republic has moderately continental climate with warm summers, cold winters, well-defined transitional seasons and average annual rainfall of 500 mm. Weather conditions during 2015-2017 are presented in Table 2. The information is given together with the dates of sowing and harvesting and respectively the duration of the growing season of *A. cruentus* and with such indicators as the rainfall amount and the amount of active temperatures above 10 ^{0}C accumulated during the growing season ($\Sigma T > 10$ ^{0}C).

Table 2 shows that growing season for the formation

Table1: Description of amaranth species (*Amaranthus* spp.) used in the experiment

Amaranthus spp.	Height group	Origin	Stem colour	Inflorescence form	Inflorescence colour	Seed colour
A. cruentus	long-stemmed	USA	Pink	Erect	Pink	Black
A. caudatus	long-stemmed	USA	Pink	Drooping	Pink	Pink
A. hybridus	medium-stemmed	China	Green	Erect	Green	Cream
A. spinosus	short-stemmed	India	Green	Erect	Green	Cream

Table 2: Weather conditions for the growing season of *A. cruentus* in the years 2015-2017

Years	Seeding date	Harvest date	Growing season	Rainfalls (mm)	$\Sigma T > 10\ ^0$C
Green fresh mass (biomass)					
2015	23 May	20 August	91	165	1496
2016	25 May	17 August	85	178	1654
2017	23 May	18 August	87	269	1636
Average			88	204	1595
Grain (seeds)					
2015	23 May	19 September	120	215	1850
2016	25 May	10 September	109	222	2080
2017	23 May	9 September	111	298	2112
Average			113	245	2014

of green mass for silage in 2016 and 2017 was shorter due to warmer weather ($\Sigma T > 10\ ^0$C - 1654 ^{0}C and 1636 ^{0}C) and was not particularly dependent on rainfall. The same situation was observed in the cultivation of grain amaranth when $\Sigma T > 10\ ^0$C accumulated up to 2080-2112 ^{0}C. In general, it can be noted that the weather conditions of Chuvash Republic were quite optimal for formation of green fresh mass (biomass) and seeds of *A. cruentus* during the years of the experiment.

2.3 STATISTICAL ANALYSIS

The results were processed statistically by analysis of variance (One-Way ANOVA) in the Statistica 12.0 program. The significance of differences was determined with Tukey's test at $p < 0.05$.

3 RESULTS AND DISCUSSIONS

3.1 GROWTH ANALYSIS OF AMARANTH SPECIES (*Amaranthus* SPP.)

Amaranth growing season includes the following growth stages from sowing to mature seeds: seedlings, vegetative state, inflorescence formation, flowering, seed maturation. Each growth stage was determined at the time when it was observed in 75 % of the control plants (Dmitrieva, 2018c). The results of growth stages duration of amaranth species in the years of the experiment are presented in Table 3.

Table 3 shows that all amaranth species (*Amaranthus* spp.) have two longest stages of growth: vegetative state reaching 35-45 days and flowering reaching 28-45 days. Comparing species in the vegetative state when plants form the root system and stems with leaves it is determined that *A. cruentus* passes this period faster by 8-16 days which is a good indicator for temperate climatic conditions of the experiment. These results are compatible with those reported by investigators (Thapa & Blair, 2018; Saratovsky et al., 2018). The same pattern was observed during flowering when the same species *A. cruentus* bloomed 6-17 days earlier compared to other species that made it possible to grow *A. cruentus* for grain in these conditions for three years of experiment. As a result, during the growing season *A. cruentus* formed biomass for silage for 88 days and after 24-29 days of seed maturation formed grain suitable for use for fodder and food purposes. *A. caudatus* having a growing season of about 100 days is also suitable for growing fresh biomass for silage in experimental conditions. But analysis of the growth stages showed their longer duration due to lack of heat that contributed only to the partial maturation of seeds. *A. hybridus* was distinguished by the longest duration of the growing season and did not have time to grow to full flowering due to higher requirements for temperature (Costea et al., 2001). In general, the climatic conditions of the Chuvash Republic allow growing all amaranth species for fresh green biomass but considering the early onset of frost it is preferable to grow species with a shorter growing season (*A. cruentus, A. caudatus).*

The main indicator of the physiological state is the growth of plants. Therefore, the experiment involved the analysis of changes in the linear growth (height) and stem diameter of amaranth species. Amaranth species differed significantly in height and were conventionally divided into three groups: long-stemmed (*A. cruentus, A. caudatus)*, medium-stemmed (*A. hybridus)*, short-stemmed (*A. spinosus)*. The change in plant height during the growing season is presented in Table 4.

Table 4 shows that early stages of all amaranth species growth were characterized by very slow growth of the stem and leaves due to the small supply of nutrients

Table 3: Duration of amaranth species (*Amaranthus* spp.) growth stages (days)

Growth stages	*A. cruentus*	*A. caudatus*	*A. hybridus*	*A. spinosus*
2015				
Seedlings	15	15	15	15
Vegetative state	34	42	45	40
Inflorescence formation	14	14	15	15
Flowering	30	35	45	30
Growing season (biomass)	91	106	120	100
Seed maturation	29	-	-	-
Growing season (grain)	120	-	-	-
2016				
Seedlings	12	12	12	12
Vegetative state	35	42	45	40
Inflorescence formation	12	12	14	14
Flowering	26	32	45	30
Growing season (biomass)	85	98	116	96
Seed maturation	24	-	-	-
Growing season (grain)	109	-	-	-
2017				
Seedlings	12	12	12	12
Vegetative state	36	44	45	40
Inflorescence formation	10	10	12	11
Flowering	29	33	45	31
Growing season (biomass)	87	99	114	94
Seed maturation	24	-	-	-
Growing season (grain)	111	-	-	-
2015-2017				
Seedlings	13	13	13	13
Vegetative state	35	43	45	40
Inflorescence formation	12	12	14	13
Flowering	28	33	45	31
Growing season (biomass)	88	101	117	97
Seed maturation	25	-	-	-
Growing season (grain)	113	-	-	-

in small seeds. During this period, the root system developed very actively from seedlings to inflorescences. After 4-5 weeks, intensive plant growth began in the middle of the vegetative stage and reached 40 cm in long-stemmed, 30 cm in medium-stemmed and 16 cm in short-stemmed of amaranth species (*Amaranthus* spp.). Since the inflorescence formation, the growth of the stem and the increase in its diameter were very active and by the end of flowering when harvesting green biomass for silage reached maximum height of 173-175 cm and a diameter of more than 2.0 cm (*A. cruentus, A. caudatus)*, 150 cm and 1.7 cm (*A. hybridus*), 71 cm and 0.7 cm (*A. spinosuss)*. Plant growth during this period was due to the growth of a long inflorescence and in the previous stages due to the elongation of the internodes of the stem. The same results were reported by other investigators. They noted the maximum growth of plants in the period between formation of inflorescences and flowering (Abbas et al., 2017; Carlquist, 2003; Archipova & Breus, 2004). It was determined that the daily increase in plant height

Table 4: Dynamics of changes in plant height and stem diameter of amaranth species (*Amaranthus* spp.) during the growing season in the year 2016 (cm)

Growth stages	*A. cruentus*	*A. caudatus*	*A. hybridus*	*A. spinosus*
Plant height				
Seedlings	5.5 a	5.5 a	5.2 a	5.4 a
Vegetative state	37.9 a	39.0 a	28.8 b	16.4 c
Inflorescence formation	91.0 a	93.6 a	66.6 b	40.8 c
Flowering	173.3 a	175.2 a	150.0 b	71.4 c
Seed maturation	186.9	-	-	-
Stem diameter				
Seedlings	0.3 a	0.3 a	0. 3 a	0.2 a
Vegetative state	0.7 a	0.7 a	0.6 a	0.4 b
Inflorescence formation	1.6 a	1.5 a	1.2 b	0.7 c
Flowering	2.2 a	2.1 a	1.7 b	1.0 c
Seed maturation	2.2	-	-	-

Values within a lines marked with different letter are significantly different at $p < 0.05$.

Table 5: Dynamics of changes in number of leaves and leaf area of amaranth species (*Amaranthus* spp.) during the growing season in the year 2016

Growth stages	*A. cruentus*	*A. caudatus*	*A. hybridus*	*A. spinosus*
Number of leaves				
Vegetative state	10.5 a	10.0 a	9.5 a	7.0 b
Inflorescence formation	30.9 a	28.7 a	27.2 a	21.4 b
Flowering	59.2 a	57.4 a	54.7 a	39.3 b
Seed maturation	64.9	-	-	-
Leaf area (cm^2)				
Vegetative state	446.9 a	450.2 a	429.4 a	289.6. b
Inflorescence formation	2007.5 a	1988.8 a	1907.6 a	1686.1 b
Flowering	4796.7 a	4773.3 a	4689.8 a	3044.5 b
Seed maturation	5710.4	-	-	-

Values within a lines marked with different letter are significantly different at $p < 0.05$.

was 5 cm in the flowering stage and 2 cm throughout the growing season. *A. cruentus and A. caudatus* had the best and significantly different indicators of growth processes and are preferred for growing in climatic conditions of the experiment.

Plant growth depends on metabolic processes and, above all, on the photosynthetic activity of the plant organism. Therefore, it is necessary to select such forms of plants that have a high capacity of photosynthesis and a high rate of growth processes. Number of plant leaves plays an important role because they manufacture and supply food material synthesized during photosynthesis. Favorable conditions for harvest formation of the plants are created when the leaf area exceeds 3 times the area of the plant and the leaves do not shade each other (Taipova & Kuluev, 2015; Dmitrieva, 2014). The change in number of leaves and leaf area of one plant during the growing season is presented in Table 5.

Analysis of the increase in the number of leaves showed the same pattern as with the height of plants according to the growth periods. The maximum increase in the number of leaves and the leaf area per plant was established after vegetative state and inflorescence formation until the end of flowering at the time of harvesting green biomass for silage (Betschart et al., 2001; Saratovsky et al., 2018). The number of leaves and leaf area in these stages increased by 2-4 times and reached 55-60 leaves and 4600-4800 cm^2 in long-stemmed and medium- stemmed species, 40 leaves and 3000 cm^2 in short-stemmed of amaranth species (*Amaranthus* spp.). The area of one plant in the experiment was 700 cm^2, the leaf area of *A. cruentus and A. caudatus* was 7 times larger and reached more than 5000 cm^2, which indicates the optimal ratio of these parameters for photosynthesis and as a result for better growth and development of plants in the climatic conditions of the experiment. Magomedov (2008) made similar experiments in the Republic of Tatarstan and observed the same pattern in the development of the leaf surface during the amaranth growth.

3.2 YIELD OF AMARANTH SPECIES (*Amaranthus* spp.)

Yield is a comprehensive generalizing indicator of the degree of favorable growth conditions for the cultivation of agricultural plants. To determine the yield it is necessary to analyze the increase in fresh and dry mass of plants during growth periods, which are presented in Table 6.

Table 6 shows that since the growth processes were not so active in the first 50 days after sowing, the increase in fresh and dry biomass of all amaranth species (*Amaranthus* spp.) was not intensive during this period. From the stage of inflorescence formation to the end of flowering within 40-50 days, fresh and dry biomass increased almost 5 times (from 12.4 to 57.2 t ha^{-1} fresh mass and from 2.0 to 10.6 t ha^{-1} dry mass in *A. cruentus*) due to active growth in length, thickening of the stem, increasing the number of leaves and elongation of inflorescences (Pospisil et al., 2009; Ulbricht et al. 2009). The same pattern was observed with other species of amaranth. Comparing amaranth species found a significant increase in biomass in the group of long-stemmed species (*A. cruentus and A. caudatus*) due to more active growth in length, thickening of the stem, increasing the number of leaves and elongation of inflorescences. The dry matter content increased from 16 % in the vegetative state to 18.5 % at the end of flowering when harvesting fresh green biomass (Norman & Shongwe, 1993).

Comparing *Amaranthus* spp. found that the dry matter content did not differ significantly depending on the species of amaranth. By the time of harvesting mature *A. cruentus* seeds, the dry matter content increased by another 1 %. The results of the yield of fresh green biomass at the end of the flowering stage and mature seeds are presented in Table 7.

Yield analysis of fresh green biomass showed that all amaranth species except *A. spinosus* had high biological productivity. Soil and climatic conditions during the experiment allowed the plants to form well-developed stems, leaves, inflorescences that contributed to intensive photosynthesis and significantly high yield especially among long-stemmed species (*A. cruentus* - 53.7 t ha^{-1} and *A. caudatus* - 49.0 t ha^{-1}). Analysis of seed productivity showed that only one species of amaranth (*A. cruentus* - 1.32 t ha^{-1}) had time to form seeds in full maturity in all years of the experiment. In other species, seed maturation in climatic conditions of the experiment was not observed due to higher temperature requirements. Experiments with heat-loving amaranth in other climatic conditions prove the possibility of seed maturation of

Table 6: Dynamics of increase of fresh and dry mass of amaranth species (*Amaranthus* spp.) during the growing season in the year 2016 (t ha^{-1})

Growth stages	*A. cruentus*	*A. caudatus*	*A. hybridus*	A. spinosus
Fresh mass (t ha^{-1})				
Vegetative state	12.4 a	12.9 a	9.3 b	6.3 c
Inflorescence formation	31.2 a	29.7 a	19.4 b	15.0 c
Flowering	57.2 a	53.8 a	39.5 b	28.6 c
Dry mass (t ha^{-1})				
Vegetative state	2.0 a	2.1 a	1.5 b	1.0 c
Inflorescence formation	5.5 a	5.2 a	3.4 b	2.8 c
Flowering	10.6 a	10.0 a	6.3 b	4.6 c
Dry matter content (%)				
Vegetative state	16.1 a	16.2 a	16.0 a	16.2 a
Inflorescence formation	17.6 a	17.5 a	17.5 a	17.6 a
Flowering	18.5 a	18.2 a	18.2 a	18.3 a

Values within a lines marked with different letter are significantly different at $p < 0.05$..

Table 7: Yield of amaranth species (t ha^{-1})

Years	*A. cruentus*	*A. caudatus*	*A. hybridus*	*A. spinosus*
Green fresh mass (biomass)				
2015	48.0 a	42.6 a	37.2 b	25.4 c
2016	57.2 a	53.8 a	39.5 b	28.6 c
2017	55.9 a	50.6 a	40.2 b	28.0 c
Average	53.7 a	49.0 a	39.0 b	27.3 c
Grain (seeds)				
2015	1.11	-	-	-
2016	1.46	-	-	-
2017	1.40	-	-	-
Average	1.32	-	-	-

Values within a lines marked with different letter are significantly different at $p < 0.05$..

Table 8: Chemical composition and nutritional value of biomass (*Amaranthus* spp.)

Indicators	*A. cruentus*	*A. caudatus*	*A. hybridus*	*A. spinosus*
Chemical composition (% in dry mass)				
Protein	20.87	21.56	20.75	21.12
Lipids	1.38	1.50	1.33	1.28
Cellulose	17.54	16.12	18.06	15.90
Ash elements	18.73	18.35	17.98	18.07
Sugar	2.65	2.54	2.76	3.05
Nutritional value (g kg^{-1} dry mass)				
Protein	161.70	162.43	160.74	162.78
Calcium	26.39	26.14	25.98	27.09
Phosphorus	4.19	4.49	3.57	4.54
Carotene (mg)	99	93	79	88
Vitamin C (mg)	23	24	31	36

all amaranth species (Caselato-Sousa & Amaya-Farfan, 2012).

3.3 CHEMICAL COMPOSITION AND NUTRITIONAL VALUE OF AMARANTH SPECIES (*Amaranthus* spp.)

The value of forage crops in agriculture is determined by the nutrient content of green biomass for silage and seeds. Main nutritional information of amaranth species is presented in Table 8.

The chemical composition of biomass indicates a quite good feed value of amaranth species (*Amaranthus* spp.). Protein content did not differ significantly among amaranth species and ranged from 20.75 % to 21.56 % in dry mass. The same pattern is established with other indicators of nutritional value comparing species of amaranth (Andini et al., 2013)It can be stated that the soil and climatic conditions of the experiment were quite favorable for the accumulation of nutrients in the fresh green biomass of all amaranth species (*Amaranthus* spp.).

4 CONCLUSIONS

The results of comparative study of amaranth species (*Amaranthus* spp.) indicated that soil and climatic conditions of the Chuvash Republic were quite favorable for growing all species to produce green biomass for silage. But given that amaranth is more demanding to heat than to moisture it is preferable to grow more rapidly developing species with a growing season of not more than 100 days (*A. cruentus, A. caudatus*) in order to avoid being damaged by early frosts. Comparing the dynamics of changes in growth parameters in 2016 year (height and diameter of stem, number and area of leaves, mass of plants), it was found that long-stemmed species of amaranth (*A. cruentus, A. caudatus*) are more suitable for growing under experimental conditions. Analysis of nutritional value of green biomass did not reveal significant differences among the studied species, but taking into account the higher yield of long-stemmed species (*A. cruentus, A. caudatus*) their advantage over medium- (A. *hybridus)* and short-stemmed species (*A. spinosus*) became evident. As a result it can be concluded that the most adapted amaranth species (*Amaranthus* spp.) for soil and climatic conditions of the Chuvash Republic are *A. cruentus* and *A. caudatus* for fodder purposes and *A. cruentus* for grain purposes.

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Physiological and biochemical responses of selected cowpea (*Vigna unguiculata* (L.) Walp.) accessions to iron toxicity

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Physiological and biochemical responses of selected cowpea (*Vigna unguiculata* (L.) Walp.) accessions to iron toxicity

Abstract: This study aimed to investigate the effect of iron toxicity in cowpea using physiological and biochemical responses of selected accessions. Fifteen accessions of cowpea were exposed to two treatments of iron using $FeSO_4$ solution (100 mg l^{-1} and 400 mg l^{-1}) and distilled water at pH 6.2 as control. The results showed that there was a general reduction in germination morphology; germination percentage among the 400 mg l^{-1} Fe-treated accessions. Seed mortality rates were significantly higher among the 400 mg l^{-1}Fe-treated accessions (> 35 %). Water imbibition capacity and relative mass gained were higher for Fe-treated accessions. Furthermore, significant increase in the total sugar and percentage utilization of sugars was accompanied by an insignificant decrease in chlorophyll a, a significant decrease in chlorophyll b contents and the persistence of foliar chlorosis, among the 400 mg l^{-1} Fe-treated accessions. MDA levels were significantly increased while proline remained unchanged, mean SOD activity was insignificantly increased, whereas Cat decreased among the 400 mg l^{-1} Fe-treated accessions. Documentation of these observable changes in physiological and biochemical parameters will be useful in understanding the impact of elevated iron concentrations on the cultivation of cowpea accessions in soils associated with ferruginous ultisols.

Key words: ferruginous ultisol; *Vigna unguiculata*; cowpea accessions; iron toxicity; plant antioxidants; cowpea tolerance; physiological response; biochemical response

Fiziološki in biokemični odziv akcesij kitajske vinje (*Vigna unguiculata* (L.) Walp.) na toksičnost železa

Izvleček: Raziskava je bila izvedena z namenom preučevanja učinkov toksičnosti železa na kitajsko vinjo na osnovi fiziološkega in biokemičnega odziva izbranih akcesij. 15 akcesij kitajske vinje je bilo izpostavljeno dvema obravnavanjema z raztopino $FeSO_4$ (100 mg l^{-1} in 400 mg l^{-1}) in destilirano vodo pri pH 6,2 kot kontrolo. Rezultati so pokazali, da je bilo splošno zmanjšanje v morfoloških parametrih kalitve in v odstotku kalitve pri akcesijah, ki so bile tretirane s 400 mg l^{-1} Fe. Tudi smrtnost semen je bila pri akcesijah, tretiranih s 400 mg l^{-1}Fe značilno večja (> 35 %). Sposobnost nabrekanja z vodo in relativno povečanje mase sta bila večja pri z železom tretiranih akcesijah. Nadalje je bilo opaženo pri s 400 mg l^{-1} Fe tretiranih akcesijah značilno povečanje vsebnosti celokupnih sladkorjev in njihove porabe, kar je bilo spremljano z neznačilnim upadom vsebnosti klorofila a, z značilnim upadom klorofila b in pojavom listnih kloroz. Pri obravnavanjih s 400 mg l^{-1} Fe se je vsebnost MDA značilno povečala, medtem, ko so vsebnosti prolina ostale nespremenjene, poprečna aktivnost SOD se je neznačilno povečala, aktivnost Cat pa zmanjšala. Dokumentiranje teh sprememb v fizioloških in biokemi*čn*ih parametrih bo koristno za razumevanje vpliva povečanih koncentracij železa pri gojenju akcesij kitajske vinje v tleh povezanih z železovimi ultisoli.

Ključne besede: železov ultisol; *Vigna unguiculata*; akcesije kitajske vinje ; toksičnost železa; rastlinski antioksidanti; strpnost kitajske vinje; fiziološki odziv; biokemični odziv

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1 INTRODUCTION

The importance of proteins in humans cannot be over-emphasized especially in its use for the development of body structures as well as regulation of human metabolism. Dietary proteins can be obtained from either the plant or animal sources according to Ahenkora et al. (1998). Many of African countries are currently struggling economically and as such it has become difficult for the citizens to purchase animal products which are major sources of proteins. Notwithstanding the economic situation especially in Nigeria, there is also the issue of health disorders associated with over-dependence on animal proteins (Spence et al., 2010) and these challenges make it imperative for the drive for cheaper and healthier alternative sources which the plant sources provide (Clinfton, 2011).

During austere times such as is prevalence in most African countries, legumes are among the safer and cheaper plant sources of proteins (Nielsen et al., 1993). Averagely, about 70 million people consume legumes globally and attempts aimed at achieving food security through these sources have prompted the need to set up farmlands and improve the technological inputs for better yields while at same time ensuring that adequate and efficient storage systems for preservation of harvested crops are in place (Diouf & Hilu, 2005).

Though the above efforts in the achievement of nutritional sufficiency are a welcome development, environmental factors have been shown to impact significantly on crop productivity. However, human activities such as mining and other industrial activities may be a major source of heavy metal pollution to soil and these impacts negatively on crop productivity (Ikhajiagbe, 2016). The presence of heavy metal in soils usually above the limit of tolerance leads to metal toxicity and a good example is the presence of high concentrations of iron in ferruginous soils in which the available iron (Fe^{2+}) is greater than 300 mg kg^{-1} (Yamauchi and Peng, 1995; Ratering & Schnell, 2000). At such high concentrations, iron becomes toxic to plants' growth and development as seen in lowland rice which has been reported in various countries including Nigeria, Colombia, Malaysia (Suresh 2005; Mitra et al., 2009).

Reports have shown that most cowpea; about 66 % of global production, are grown in the African continent particularly in Nigeria and Niger. In Nigeria, most of these productions are domiciled in the Northern region (Blade et al., 1997). The conditions needed for the cultivation of cowpea such as above 80 % sandy content are also present in some other states such as Edo State, Nigeria, Edo State but have recorded very low production (IITA, 2003). One of the reasons for this shortfall may be due to the presence of high iron in most of these soils (ferruginous ultisols) as shown by the geological evidence of Ikhile (2016) and collaborated by Imasuen & Onyeobi (2013).

There is a dearth of information on the physiological and biochemical response of cowpea to elevated iron levels as most existing literature focus on rice. Therefore, the study aims to investigate iron toxicity in cowpea using physiological and biochemical responses of selected accessions which will serve as a background for the understanding of the basis of tolerance of cowpea accessions planted in a ferruginous ultisol.

2 MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN, SEED COLLECTION AND PLANTING

Fifteen accessions (TVu-3742, TVu-3769, TVu-5348, TVu-5760, TVu-5768, TVu-5782, TVu-5883, TVu-6102, TVu-6193, TVu-6219, TVu-6290, TVu-10600, TVu-10881, TVu-11114, and TVu-11214) of cowpea were used in this study. The study was undertaken in the greenhouse of the Department of Plant Biology and Biotechnology, University of Benin, Nigeria. The accessions were provided by the Genetic Recourses Centre of the International Institute for Tropical Agriculture (GRC, IITA), Ibadan, Nigeria. The accessions were exposed to two treatments (100 mg l^{-1} and 400 mg l^{-1}) of iron sulphate ($FeSO_4$) solution based on toxicity reference for soil iron (300 mg l^{-1}) and distilled water at pH 6.2 as control. The No. 1 Whatman's Filter papers were placed in Petri dishes moistened with the treatment solutions and thereafter ten seeds (mean mass: 0.18 ± 0.09 g) each of the fifteen accessions were planted in three replicates.

2.2 DATA COLLECTION

2.2.1 Germination

Germination parameters were assessed by methods described by Sadeghi et al. (2011). These include germination percentage, seminal root length, shoot length, leaf area of germinant, seed mortality, mean water imbibitions capacity, water imbibitions rate and relative mass gain.

2.2.2 Total sugars and pigment analyses

The productive capacity and pigmentation of the seeds and cotyledons such as total sugars, chlorophyll a and b were assessed. The total sugar was estimated according to the method described by Nelson (1944) and modified by Sankar & Selvaraju (2015). Chlorophyll a and b contents were investigated according to methods described by Arnon et al (1949) and Maxwell & Johnson (2000).

2.2.3 Characterization of iron toxicity symptoms

For characterization of FE-induced chlorosis, morphological observations of the plant in response to the experimental conditions were recorded on an intermittent basis. These observations include the colour, shape, form or the appearance of the leaves and the stem of the plant as well as the positioning of flowers and nodes. Care was also taken to make sure that the succession of chlorosis was recorded.

2.2.4 Antioxidant activities

Enzymatic (catalase, superoxide dismutase) and non-enzymatic (proline and malondialdehyde) antioxidants parameters were evaluated in the seeds and cotyledons of successfully germinated accessions. SOD activity was determined by the methods of Beauchamp & Fridovich (1971) and described by Ranganayakulu et al. (2013). Catalase activity was measured by the method of Luck (1971) and modified by Esma & Gulnur, (2016). MDA was determined using the thiobarbituric acid assay method described by Health & Packer (1968) and modified by Erja et al (2001). The extraction and estimation of proline were done according to the methods of Bates et al (1973) and Marin et al (2006).

2.3 STATISTICAL ANALYSIS.

Data obtained from this study were subjected to a two-way analysis of variance using the SPSS statistical analysis software (SPSS-20®). Students' T-test and the least significant difference; LSD was used for equality of means while the Levene's test was used for the analysis of equality of variances. All statistical analysis was performed at a confidence limit of 95 % ($p = 0.05$) and values are presented as mean ± standard deviation.

3 RESULTS AND DISCUSSION

3.1 RESULTS

The results obtained from this study showed the germination (%) of TVu-3769, 5760 and 6219 were significantly higher for control as well as the mean germ (%) when compared to the 100 mg l^{-1} and the 400 mg l^{-1} Fe-treated accessions (Fig 1). The seminal root length of TVu-5348, 6219, 11114 and mean value of control accessions were significantly higher compared to the 100 mg l^{-1} and the 400 mg l^{-1} Fe-treated accessions (Fig 2). For shoot length, TVu-3769 and 11214 showed significantly higher values for control accessions whereas TVu-5348, 5760, 5782, 6290 and 10600 were significantly higher for 100 mg l^{-1} Fe-treated accessions. Comparison of the mean values of control, 100 mg l^{-1} and the 400 mg l^{-1}, showed a significantly higher mean for 100 mg l^{-1} Fe-treated accessions (Fig 3).

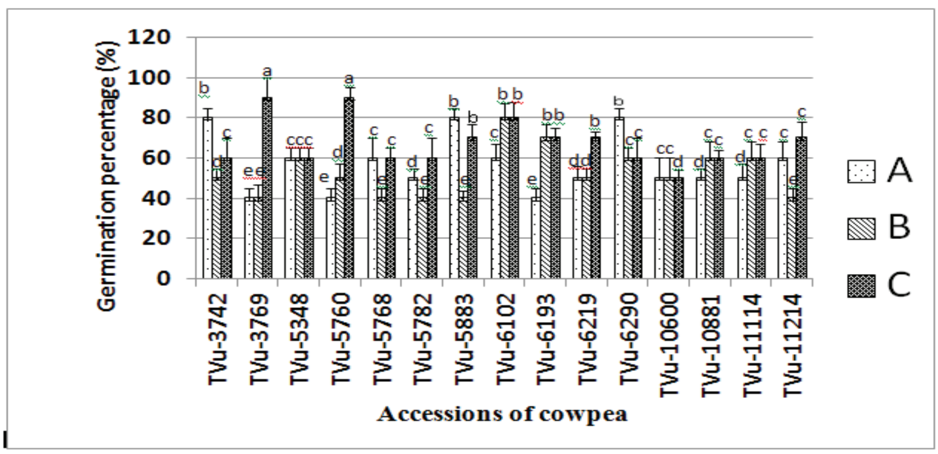

Figure 1: Final germination percentage of accessions after 1 week following germination initiation.
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

The leaf area of TVu-5348, 5883, 6193 and 10600 was significantly higher in control accessions. Also, the mean value of control accessions was higher when compared to 100 mg l^{-1} and 400 mg l^{-1} Fe-treated accessions, the mean differences were statistically insignificant (Fig 4). The mean seed mortality (%) showed a significantly higher value for 400 mg l^{-1} accessions compared to the control and insignificant when compared to 100 mg l^{-1} Fe-treated accessions. The least mean value was observed in the control accessions (Fig 5).

The water imbibition capacity, water imbibition rate and relative mass gain of TVu-10881 were significantly higher in control (Fig 6, 7 and 8) but, comparison of the mean values of the control, 100 mg l^{-1} and 400 mg l^{-1} Fe-treated accessions show no significance (Fig 6). Tvu-3769 showed a significantly higher value for water imbibition rate and relative mass gain for the 100 mg l^{-1} and 400 mg l^{-1} Fe-treated accessions while the comparison of the mean values was statistically insignificant (Fig 7 and 8).

For the plants' productive capacity, the total sugar of germinated seeds was compared to the seeds obtained for studies from the IITA. The results showed that the seeds from IITA had significantly higher total sugar (Fig 9). However, the mean total sugar of 100 mg l^{-1} was higher than 400 mg l^{-1} Fe-treated and control accessions. These mean differences among 100 mg l^{-1}, 400 mg l^{-1} Fe-treated and control accessions were insignificant ($p > 0.05$). Accessing the total sugar contents of the cotyledons showed a significantly higher value for TVu-6219 in control accessions meanwhile comparison the mean values among

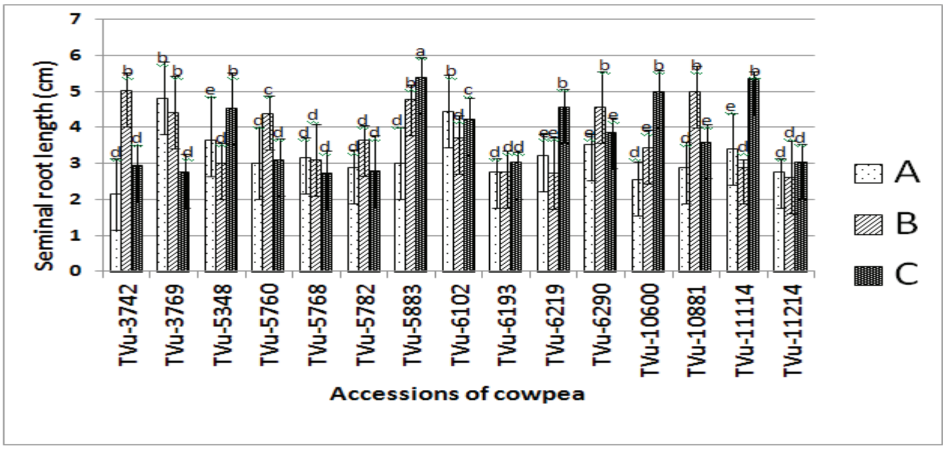

Figure 2: Seminal root length of accessions after 5 days following germination initiation.
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

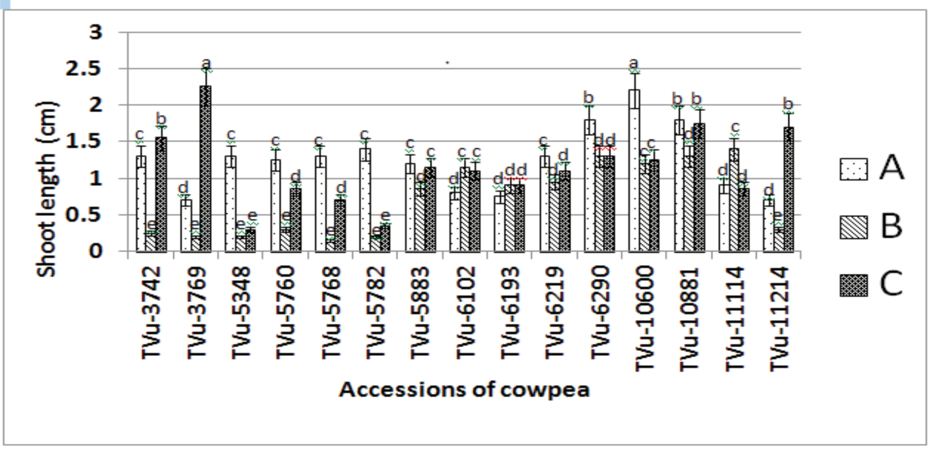

Figure 3: Shoot length of accessions after 5 days following germination initiation.
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

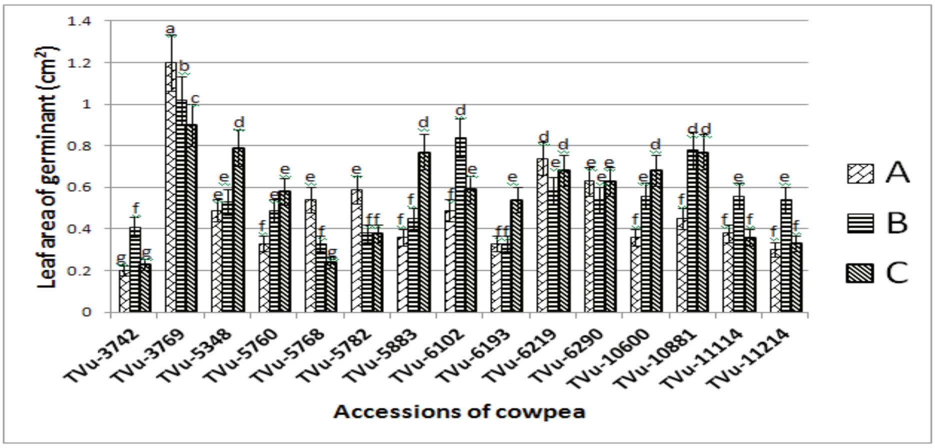

Figure 4: Leaf area of early developing accessions after 1 week following germination initiation.
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance..

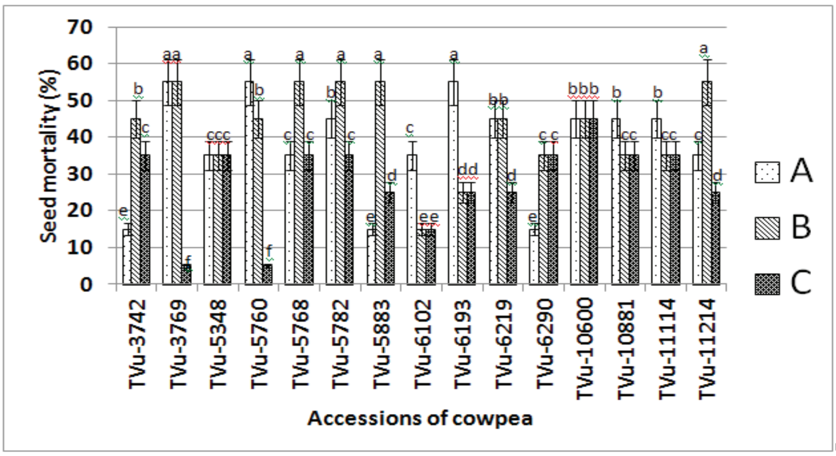

Figure 5: Percentage seed mortality at 21 days after germination initiation.
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

100 mg l^{-1}, 400 mg l^{-1} Fe-treated and control accessions were insignificant ($p > 0.05$) as seen in Figure 10. The percentage utilization of sugar showed a significantly higher mean value for 400 mg l^{-1} compared to 100 mg l^{-1} Fe-treated and control accessions ($p < 0.05$) as seen in Table 1.0.

Table 2.0 showed the Chlorophyll-a (Chl a) and b (Chl b) contents of cotyledon after 1 week following germination initiation. For Chl a, the highest value was seen in Tvu-10881 (0.139 mg g^{-1}) among 100mg l^{-1} Fe-treated accessions and the least in Tvu-10600 (0.032 mg g^{-1}) among control accessions. Statistically, insignificant differential mean values were observed when the control accessions were compared with the Fe-treated accessions. For Chl b, the highest value was observed in Tvu-10881 (0.251 mg g^{-1}) among 100 mg l^{-1} Fe-treated accessions and the least in Tvu-10600 (0.057 mg g^{-1}) among control. The mean chlorophyll b value of 100 mg l^{-1} Fe-treated accessions was higher while the 400 mg l^{-1} Fe-treated was lower compared to the control. These mean differences were statistically significant. The mean total chlorophyll value for the 100 mg l^{-1} Fe-treated accessions was higher while the 400 mg l^{-1} Fe-treated was lower when compared to the control. However, these mean differences are statistically insignificant. The ratio of Chl a to Chl b showed insignificant equal mean values among the 100 mg l^{-1}, 400 mg l^{-1} and the control accessions. Table 3.0 shows the persistence of chlorosis on germinants at 5, 6 and 7 days after germination with the mean persistence of chlorosis being more evident (++ and +++) among the Fe-treated

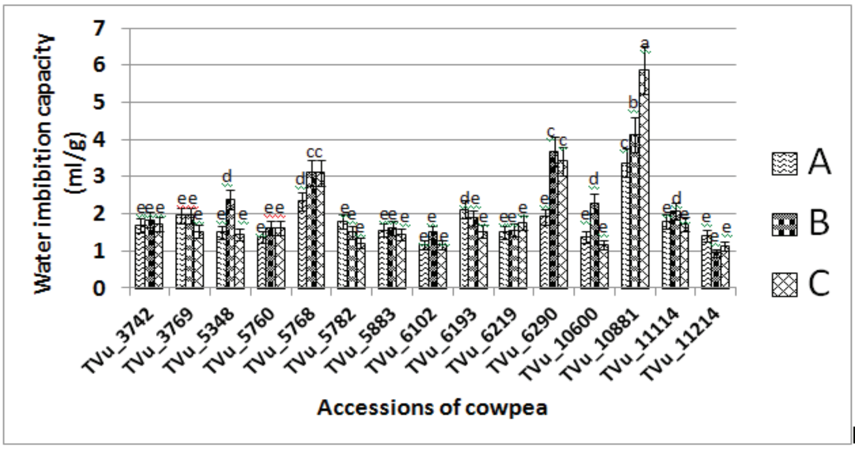

Figure 6: Water imbibition capacity at 4 days after germination initiation.
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

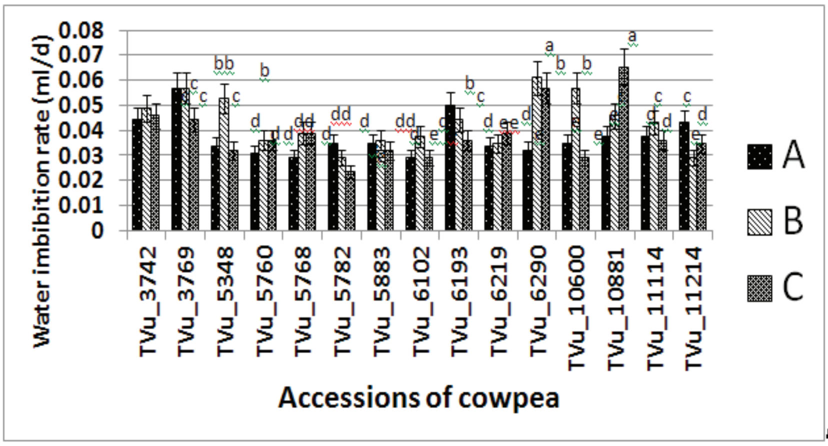

Figure 7: Water imbibition rate 4 days after germination initiation
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance

accessions (100 mg l^{-1} and 400 mg l^{-1}) at early days of germination compared to the controlled accessions (+).

Antioxidant capacities of the accessions showed that the MDA levels of TVu-3769, 6193, 10881 and 11114 were significantly higher among the control group (Fig 11). Comparison of the mean MDA values of 100 mg l^{-1}, 400 mg l^{-1} and control showed that the mean value of control was significantly higher but those of proline were statistically insignificant (Fig 12). Catalase activities of TVu-6193 and 11214 were significantly higher in the control group compared to Fe-treated accessions (Fig 13). Comparison of the mean CAT activities among 100 mg l^{-1}, 400 mg l^{-1} and control accessions showed a significant least value for 400mg l^{-1} Fe-treated accessions while a significantly higher mean value for the 400 mg l^{-1} Fe-treated accessions was observed for SOD activities when compared to the 100 mg l^{-1} and control accessions (Fig 14).

3.2 DISCUSSIONS

The findings of this study showed that iron toxicity significantly reduced germination parameters as evident in the lower germination (%), seminal root and shoot lengths and leaf area of germinants whereas seed mortality is significantly increased. Also, the productive capacities of the accessions were significantly reduced among 400 mg l^{-1} Fe-treated accessions while insignificant mean differences were observed for chlorophyll a. However, Fe-

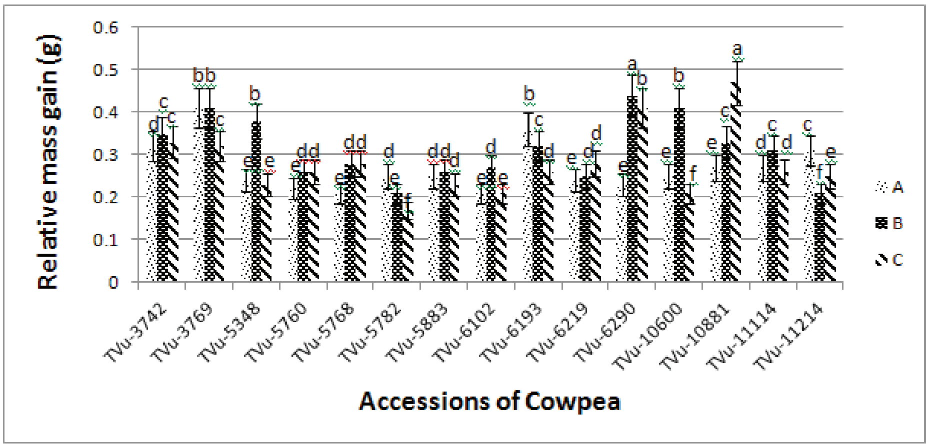

Figure 8: Relative seed mass of imbibed seeds 4 days after germination initiation.
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

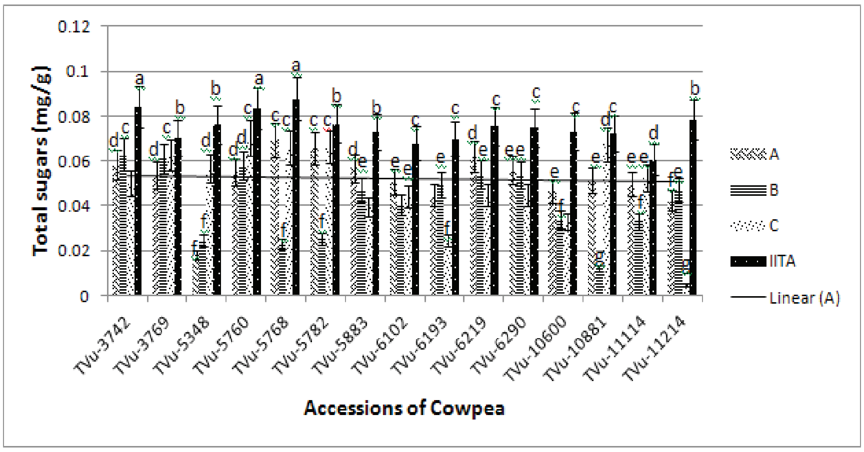

Figure 9: Total sugars of germinated seeds at 4 days following germination initiation.
A: Seeds treated with 100mgl^{-1} $FeSO_4$ Soln., B: Seeds treated with 400mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

toxicity significantly reduced the chlorophyll b content as well as increased persistence of chlorosis among the 400 mg l^{-1} Fe-treated accessions. The plants' antioxidants defence capacity showed significant lower catalase and higher SOD activities among the 400 mg l^{-1} Fe-treated accessions which corresponded with significant higher MDA values while the proline content is unchanged.

Plant germination is usually affected by the difference in genetic make-ups of the different plants as well as environmental factors such as the presence of heavy metals (Bhagyashree et al., 2016). Seed germination is a significant stage in the seedling establishment; it decides successful crop and yield production (Bhattacharjee, 2008). The reduced values for germination parameters obtained from this study is consistent with the results of Ahmad et al. (2012) and Abdel-Haleem (2015). Though arguably, the reduced germination parameters may not be due to iron overload but rather due to general nutrient deficiencies, the presence of iron oxide deposits on the roots of wetland plants could act as a filter for nutrients such as phosphate, thereby causing a deficiency in the aerial parts of the plant. Furthermore, the physic-chemical effects of iron on osmotic balance through the increase of osmotic pressure with the tendency to disrupt seed hydration may be a contributory factor for the reduced seed germination (Nabil & Coudret, 1995; Onyango et al., 2019).

The significance of water in seed germination is critical; thus the capacity for water imbibition by plant seeds is necessary for successful germination initiation

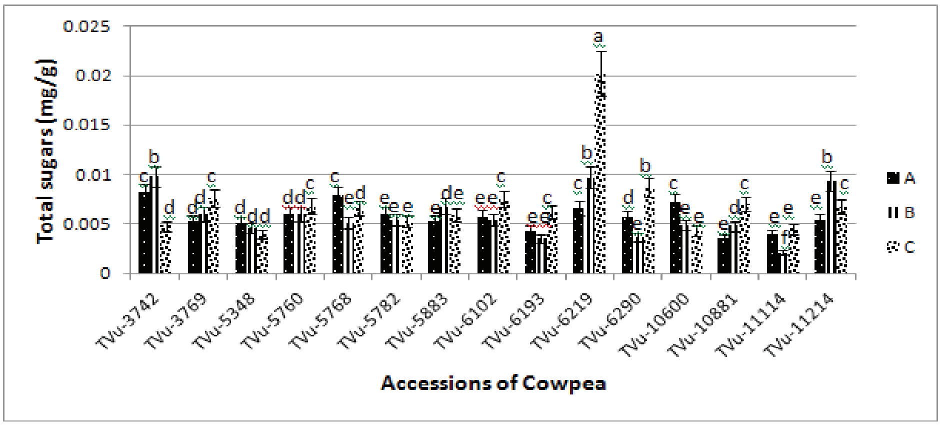

Figure 10: Total sugars of cotyledons at 4 days following germination initiation.
A: Seeds treated with 100mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

Table 1: Percentage utilization of sugars during germination (taken at 4 days after germination initiation)

Accessions	Utilisation (%)			
	Group A	Group B	Group C	*p-value*
TVu-3742	30.76[f]	25.06[g]	40.26[e]	0.192
TVu-3769	23.83[h]	13.33[h]	11.06[h]	0.114
TVu-5348	76.47[b]	67.71[c]	25.88[g]	0.011
TVu-5760	34.01[f]	30.54[f]	15.57[f]	0.089
TVu-5768	20.75[g]	73.66[b]	24.74[g]	0.012
TVu-5782	14.21[h]	66.88[c]	13.69[h]	0.033
TVu-5883	21.81[g]	35.53[e]	45.40[e]	0.051
TVu-6102	25.26[g]	40.23[e]	35.39[e]	0.216
TVu-6193	35.62[f]	29.33[g,f]	64.81[c]	0.037
TVu-6219	17.72[h]	28.31[g,f]	40.61[e]	0.033
TVu-6290	25.37[g]	28.44[g,f]	40.19[e]	0.052
TVu-10600	36.20[f]	53.55[d]	55.05[d]	0.051
TVu-10881	28.63[g]	82.85[a]	6.50[i]	0.001
TVu-11114	18.09[h]	45.39[e]	13.32[h]	0.050
TVu-11214	30.76[g]	25.06[g]	40.26[e]	0.060
p-value	0.023	0.032	0.026	

A: Seeds treated with 100mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

and this has been shown to be affected by the difference in the permeability of the seed testa, seed composition and the availability of water in the environment (Wada and Abubbakar, 2013). Water imbibition capacity responses differed across the accessions. The low osmotic potential has been shown to extend the time needed for imbibition as well as delaying the onset of germination. Results obtained were similar to those of Olasoji et al. (2013) and Araujo et al. (2016) who also observed differential response for the rate of water of imbibition for

Table 2: Chlorophyll contents of cotyledon after 1 week following germination initiation

Accessions	Chl. a (mg g^{-1})			Chl. b (mg g^{-1})			Chl. a+b (mg g^{-1})			Chl.a/b ratio		
	A	B	C	A	B	C	A	B	C	A	B	C
TVu-3742	0.052^{f}	0.111^{d}	0.064^{f}	0.093^{a}	0.200^{a}	0.115^{d}	0.076^{e}	0.161^{b}	0.083^{d}	0.554	0.555	0.555
TVu-3769	0.077^{e}	0.072^{e}	0.060^{f}	0.140^{c}	0.129^{c}	0.108^{d}	0.113^{d}	0.106^{d}	0.084^{d}	0.554	0.555	0.555
TVu-5348	0.080^{d}	0.093^{d}	0.081^{d}	0.144^{c}	0.168^{b}	0.146^{c}	0.118	0.139^{c}	0.121^{c}	0.555	0.555	0.555
TVu-5760	0.043^{f}	0.075^{e}	0.064^{f}	0.077^{e}	0.135^{c}	0.116^{d}	0.063^{f}	0.110^{d}	0.095^{e}	0.555	0.555	0.554
TVu-5768	0.096^{d}	0.055^{f}	0.036^{d}	0.174^{b}	0.099^{d}	0.065^{f}	0.144^{c}	0.079^{e}	0.054^{f}	0.554	0.555	0.555
TVu-5782	0.085^{e}	0.057^{f}	0.068^{e}	0.154^{c}	0.103^{d}	0.122^{c}	0.125^{c}	0.084^{e}	0.100^{d}	0.555	0.554	0.555
TVu-5883	0.062^{a}	0.054^{c}	0.112^{d}	0.111^{d}	0.097^{d}	0.202^{a}	0.090^{d}	0.080^{e}	0.167^{b}	0.555	0.554	0.555
TVu-6102	0.073^{e}	0.048^{f}	0.068^{f}	0.131^{c}	0.086^{d}	0.122^{c}	0.108^{d}	0.071^{e}	0.099^{d}	0.555	0.554	0.555
TVu-6193	0.055^{f}	0.039^{f}	0.079^{e}	0.099^{d}	0.071^{e}	0.142^{c}	0.081^{d}	0.058^{f}	0.118^{d}	0.555	0.554	0.555
TVu-6219	0.075^{e}	0.046^{f}	0.051^{f}	0.135^{c}	0.084^{d}	0.091^{e}	0.110^{d}	0.067^{f}	0.075^{e}	0.554	0.555	0.555
TVu-6290	0.091^{d}	0.092^{d}	0.034^{f}	0.165^{b}	0.165^{b}	0.062^{e}	0.135^{c}	0.134^{c}	0.051^{f}	0.554	0.555	0.554
TVu-10600	0.095^{a}	0.061^{f}	0.032^{f}	0.172^{b}	0.110^{d}	0.057^{f}	0.140^{c}	0.090^{d}	0.046^{f}	0.555	0.554	0.555
TVu-10881	0.139^{c}	0.058^{f}	0.132^{f}	0.251^{a}	0.105^{d}	0.238^{a}	0.202^{a}	0.087^{e}	0.197^{a}	0.555	0.554	0.555
TVu-11114	0.091^{d}	0.055^{a}	0.106^{f}	0.165^{b}	0.098^{d}	0.192^{a}	0.134^{c}	0.081^{e}	0.159^{b}	0.555	0.555	0.555
TVu-11214	0.074^{e}	0.050^{f}	0.061^{f}	0.133^{c}	0.091^{d}	0.110^{d}	0.109^{d}	0.073^{e}	0.091^{d}	0.555	0.555	0.555
p-value	0.094	0.206	0.213	<0.001	0.026	<0.001	<0.001	0.192	0.086	1.000	1.000	1.000

A: Seeds treated with 100mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
[*]Mean values of accessions with different letters show statistical significance.

Table 3: Percentage utilization of sugars during germination (taken at 4 days after germination initiation)

Accessions	Group A			Group B			Group C		
	Days								
	5	6	7	5	6	7	5	6	7
TVu-3742	+++	+	++	++	++	++	++	+	+
TVu-3769	++	++	++	+++	++	++	++	++	+
TVu-5348	++	+	+	+	-	+	++	+	+
TVu-5760	++	+++	++	+	++	++	++	+	+
TVu-5768	++	++	++	++	++	+	++	++	+
TVu-5782	+++	+++	++	+++	+++	++	++	++	+
TVu-5883	+++	+	+	+++	+	+	+++	+	+
TVu-6102	++	++	+	+++	++	++	++	++	++
TVu-6193	+++	++	+	++	+++	+	++	+	+
TVu-6219	+++	++	+	+++	++	++	++	+	-
TVu-6290	+++	+++	+	+++	++	+	+++	++	+
TVu-10600	++	+	++	+++	+	++	++	+	++
TVu-10881	++	+++	++	++	++	++	+	+	+
TVu-11114	+++	+++	++	++	+	++	++	+	+
TVu-11214	++	+++	++	+++	++	++	++	+	++

A: Seeds treated with 100mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

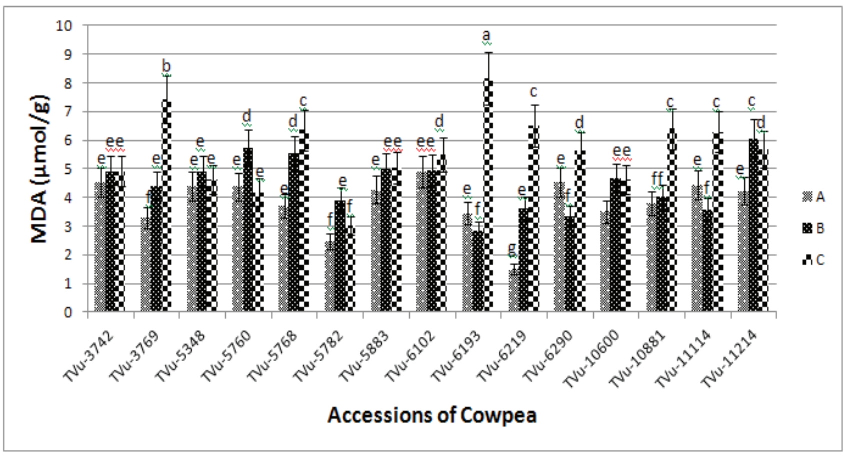

Figure 11: MDA activity of cotyledons of accessions after 1 week following germination initiation.
A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.
*Mean values of accessions with different letters show statistical significance.

plants exposed to heavy metal toxicity and Dufey et al. (2009) who observed decreased relative water content of rice exposed to iron toxicity.

Plant productivity can be assessed using total sugars and percentage utilization of sugar during germination. It can also be important in the determination of the extent of iron toxicity. The increased utilization of sugars in the germinating seeds, as well as the cotyledons with exposure to Fe, may not be unconnected to the increased use of sugar for productivity especially in combating stress posed by the iron toxicity. These findings are inconsistent with those of Priti et al. (2009) and Ezhilvannan et al. (2011) but consistent with the findings of Onyango

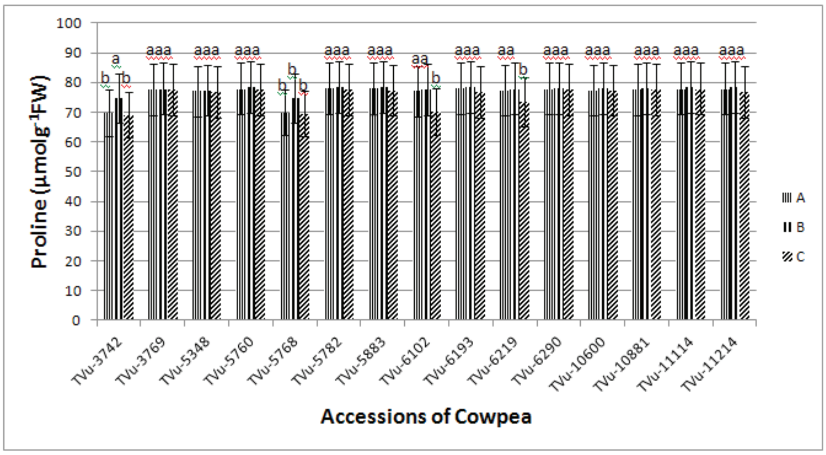

Figure 12: Proline levels in germinants at 7 days after germination initiation. A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.*Mean values of accessions with different letters show statistical significance.

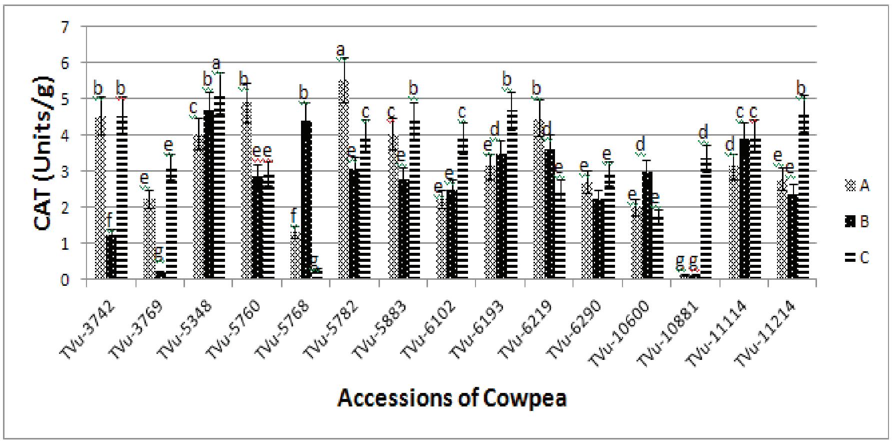

Figure 13: CAT activity of cotyledons of accessions after 1 week following germination initiation.A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.*Mean values of accessions with different letters show statistical significance.

et al. (2019) who observed a reduction in soluble sugar content in rice exposed to iron toxicity.

Development of oxidative stress in plants exposed to heavy metals is largely ascribed to the heavy metal-induced imbalance between the generations of toxic oxygen radicals and their scavenging through the anti-oxidative defence mechanisms. With increasing concentrations of heavy metals, it is expected to observe an increased lipid peroxidation and increased activities of anti-oxidative enzymes such as SOD, GSH and catalase (Priti et al., 2009; Arleta et al., 2012). Activities of SOD and catalase were observed to be concentration-dependent and have an initial increase as concentration increase but a reduced value was later observed upon exposure to Pb-toxicity (Malar et al., 2016). Superoxide dismutase is considered as a first-line defence system against ROS, as it acts on superoxide free radicals, which are produced in different compartments of the cell and are precursors of the other ROS (Priti et al., 2009). It is involved in the dismutation of the oxygen-free radical; O_2^- into H_2O_2 (Sun et al., 2009). Hence, it is expected for its increase during plant stress in response to heavy metal accumulation such as increase Fe concentration. These results are in consistence with the findings of Prasad et al. (2014) but inconsistence with the findings of Sadeghipour et al. (2008) in which reduced catalase activities were recorded.

The H_2O_2 produced from the dismutation of O_2^- is expected to be cleared by peroxidase via the reduction of hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen

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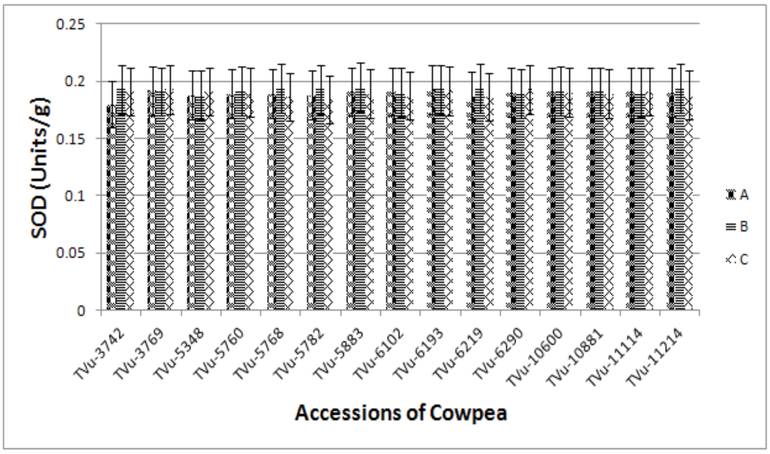

Figure 14: SOD activity of cotyledons of accessions after 1 week following germination initiation.A: Seeds treated with 100 mg l^{-1} $FeSO_4$ Soln., B: Seeds treated with 400 mg l^{-1} $FeSO_4$ Soln. and C: Control.*Mean values of accessions show no statistical significance.

(O_2). At overwhelming levels of H_2O_2, catalase is mobilized for a further breakdown of the H_2O_2 (Gao et al., 2010). Therefore it can be suggestive that the insignificant change in catalase activity may be as a result of the ability of peroxidase to scavenge the hydrogen peroxide produced by SOD. MDA was reduced under Fe exposure which is perhaps an indication of enhanced antioxidant activity. No significant changes in proline content perhaps implied that antioxidant activity may not have been proline-mediated, or affected by changes in proline concentration (Choudhary et al., 2007). The results obtained from this study were inconsistent with the findings Singh et al. (2012) and Krishnaveni et al. (2015).

4 CONCLUSION

The findings of this study showed that iron concentrations greater than 300 mg kg^{-1}, have a negative impact on biochemical processes associated iron metabolism in cowpea. These findings have assisted in understanding the mechanisms, by which there is high yield loss in cowpea due to iron toxicity. The documentation of these observable physiological and biochemical changes will serve as background for further studies into the selection of iron-tolerant cultivars to improve the cultivation and production of cowpea in regions associated with ferruginous soils.

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6 CONFLICT OF INTEREST

There is no conflict of interest as it concerns this research study.

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The response of the sugar beet (*Beta vulgaris* L.ssp. *vulgaris* var. *altissima* Döll) genotypes to heat stress in initial growth stage

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The response of the sugar beet (*Beta vulgaris* L.ssp. *vulgaris* var. *altissima* Döll) genotypes to heat stress in initial growth stage

Abstract: The continuous trend of global warming and increasing interest toward cultivating sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) in tropical regions led us to conduct this study to investigate the effect of high temperature on sugar beet at initial growth stages. Thirty one genotypes were incubated at two temperatures (20 °C and 30 °C) in laboratory for germination test. The same genotypes were assessed for physiological parameters at 30 °C in greenhouse, too. Increasing temperature decreased germination indices with a high variability among the genotypes. Seed vigor index and seminal root length were decreased higher than other indices. The genotypes with higher greenness index had higher total dry mass, leaf area and leaf temperature depression (*LTD*), and those with higher seed vigor index indicated great quantum efficiency of PSII (*Fv/Fm*) values. 'S1-92521' produced high records in both laboratory and greenhouse experiments. Although 'S1-92521' showed good tolerance in both laboratory and greenhouse experiments, totally, sugar beet genotypes had different performance at two experiments. According to the results, seed vigor index could be used as a screening tool in laboratory, and *LTD* and *Fv/Fm* were considered as good criteria for screening heat-tolerant genotypes in greenhouse.

Key words: genotype screening; heat stress; leaf temperature depression; *Fv/Fm*; seed vigor index

Odziv genotipov sladkorne pese (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) na vročinski stres v začetnih fazah rasti

Izvleček: Naraščajoči trend globalnega segrevanja in vse večji interes za gojenje sladkorne pese (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) v tropskih območjih so privedli k izvedbi te raziskave, v kateri je bil preučevan učinek visokih temperature na sladkorno peso v začetnih fazah rasti. 31 genotipov je bilo inkubiranih pri dveh temperaturah (20 °C in 30 °C) v laboratoriju za kalitvene teste. Na istih genotipih so bili v rastlinjaku ocenjeni fiziološki parametri pri temperaturi 30 °C Povišanje temperature je zmanjšalo indekse kalitve z veliko spremenljivostjo med genotipi. Indeks vitalnosti semen in dolžina semenske korenine sta se bolj zmanjšala kot drugi indeksi. Genotipi z večjimi indeksi zelenosti so imeli večjo celokupno suho maso, večjo listno površino in večji upad temperature listov (*LTD*), genotipi z večjimi indeksi vitalnosti semen so pokazali velike vrednosti kvantne učinkovitosti PSII (*Fv/Fm*). 'S1-92521'je imel velike vrednosti vseh merjenih parametrov tako v laboratoriju kot v rastlinjaku. Čeprav je imel genotip 'S1-92521' dobro tolerance na visoke temperature tako v laboratoriju kot v rastlinjaku, so imeli drugi genotipi sladkorne pese zelo različne odzive v obeh poskusih. Glede na rezultate bi lahko indeks vitalnosti semen uporabili kot primerno orodje za preiskušanje genotipov v laboratoriju, parametra *LTD* in *Fv/Fm* pa sta se izkazala kot dober kriterij za preiskušanje genotipov na visoke temperature v rastlinjaku.

Ključne besede: preiskus genotipov; vročinski stres; upad listne temperature; *Fv/Fm*; indeks vitalnosti semen

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1 INTRODUCTION

Production of sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) is often limited by environmental conditions including insufficient water, heat, freezing temperatures and salinity. These conditions lead to decreasing the rates of photosynthesis and canopy expansion (Yang et al., 2017; Zandalinas et al., 2018). Unfortunately, global warming has caused a rise in the world's temperature, and changed the climate in many areas of the world including regions with sugar beet plantations. However, if some genotypes of sugar beet can be proved to be tolerant to high temperature, cultivation of this plant will be less limited due to the climate change. So, there is an interest in summer cultivation of sugar beet and cultivation of sugar beet in tropical regions (El-Kholi, 2008; Ober & Rajabi, 2010). In addition, maximum temperature in semi-arid regions makes it urgent to breed cultivars for high temperature conditions (Karandish et al., 2017). However, there has not been much research and development on heat stress tolerance in this crop.

Seed germination is one of the crop's characteristics that can be used to assess its establishment in field. Physiological processes in germination influence plant metabolism and performance in later stages are mostly affected by abiotic stresses (Gratani et al., 2000; Liu and Huang, 2008; Wang et al., 2008; Silva et al., 2010). High temperature declines germination as well as seedling growth and survival (Beckage & Clark, 2003; Arx von et al., 2013; Fahad et al., 2017). Seed viability and vigor directly affect the performance of seeds and vegetative growth that finally determine the crop yield (TeKrony & Egli, 1991). Seed vigor determines the potential of a rapid and uniform emergence and development of normal seedlings under field conditions (Baalbaki et al., 2009).

It has been proved that heat tolerance at germination time is positively correlated with later growth phases in cotton (Ashraf et al., 1994). However, there are no information about screening criteria of sugar beet genotypes under high temperature, and also the correlation of tolerance at germination time with growth in later growth stages. Biological process such as photosynthesis, is the most sensitive process that is influenced by high temperature (Buchner et al., 2015; Marias et al., 2017). High temperature induces inhibition of photosynthesis, especially PSII activity through declining electron transport activity (Havaux, 1993; Murakami et al., 2000). The quantum efficiency of PSII (*Fv/Fm*) which represents plant nutrient and health status is related to chlorophyll concentration (Mohammadian et al., 2003; Kumar et al., 2017), and has a positive relationship with CO_2 fixation and dry matter production (Sharma et al., 2015). In addition, greenness index and dry mass are important when plants are screened for heat tolerance (Joshi et al., 2007; Nagar et al., 2015; Zhou et al., 2015).

Stomata have a vital role in plant cycle due to CO_2 and water vapor exchange between plant and atmosphere (Miner et al., 2017). Stomata closure and increasing leaf temperature are happening under high temperatures (Rizhsky et al., 2002; Zandalinas et al., 2018) and drought stress (Mohammadian et al., 2003). Canopy temperature with a potential to be a tool for indirect selection of tolerant genotypes to drought and heat (Reynolds et al., 2009) is consistently negatively correlated with yield (Reynolds et al., 1994; Gutierrez et al., 2010; Pinto et al., 2010), and leaf temperature depression (LTD) represents the plant water status (Mohammadian et al., 2001; Balota et al., 2008; Webber et al., 2017).

Early growth stages of plants are important, because a good seedling establishment leads to a better growth and leaf expanding in later stages of growth. As the canopy is responsible for getting the sun light which is necessary for photosynthesis, the sooner a plant canopy is constructed, the more yield the plant will produce (Mohammadian et al., 2005). Early growth stages are more sensitive to abiotic stresses than the other stages as they have an important role in plant adaptation and can be used for germplasm screening (Munns & Tester, 2008; Carpýcý et al., 2009; Pandey & Penna, 2017; Shelke et al., 2017). Screening of genotypes tolerant to specific stresses at early growth stages is rapid, inexpensive and less laborious (Grzesiak et al., 2003; Bafeel, 2014).

There are researches on combined effects of heat and drought stress on sugar beet, but the effect of heat stress, alone, on sugar beet has not been investigated yet. Furthermore, researches lack any study on differences among sugar beet genotypes with regard to heat tolerance. So, this study aimed to investigate the followings by using parameters that are changed under the influence of heat stress: (1) assessing the effect of high temperature on some sugar beet genotypes at germination and seedling establishment stages, (2) determining if genotypes which were tolerant to high temperature in laboratory are heat-tolerant in greenhouse as well and (3) identifying reliable tools for screening tolerant genotypes in heat stress conditions.

2 MATERIAL AND METHODS

This study was conducted in 2015 at Sugar Beet Seed Institute (SBSI), Karaj, Iran. A fundamental germination test was conducted by four sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima* Döll) cultivars (Aria, Paya, FD-415 and Rosaflor) at nine constant temperatures (20, 23, 26, 29, 32, 35, 38, 41 and 44) in order to determine a temperature that can affect the growth of sugar beet. Results of this experiment showed that germination percentage and seed vigor index of these cultivars decreased severely at 30(data are not shown in this article). In addition, average temperature of the hottest month of Iran, July, in regions of spring cultivation of sugar beet is around 30. Based on these two reasons, 30 was selected as a temperature suitable for screening sugar beet genotypes under high temperature. This stage was followed by laboratory and greenhouse experiments using some sugar beet genotypes which were selected in a way that they were genetically different (different origins) as much as possible.

2.1 LABORATORY EXPERIMENT

This experiment was conducted as factorial in a completely randomized design with four replications. The experimental factors included 31 genotypes of sugar beet and two constant temperatures (20, as control, and 30). Characteristics of the genotypes are

Table 1: Properties of used sugar beet genotypes

Genotype	Poloidy	Monogerm/ MultIgerm	Origin	Characteristic	Resistance/Tolerance to
419	Diploid	Monogerm	Iran	O-type	-
7112	Diploid	Monogerm	Iran	O-type	-
31714	Diploid	Monogerm	Iran	O-type	Rhizomania
110-7-8	Diploid	Multigerm	Iran	Half-sib	Drought stress
111-52-25	Diploid	Multigerm	Iran	Half-sib	Drought stress
S1-92685	Diploid	Multigerm	Iran	Inbred line	Rhizomania
S1-92747	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Beet cyst nematode
S1-92748	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Beet cyst nematode
S1-92749	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Beet cyst nematode
S1-92750	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Beet cyst nematode
S1-92751	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Beet cyst nematode
SB27-H-1	Diploid	Multigerm	Iran	Half-sib	Rhizomania- Beet cyst nematode
SB27-H-2	Diploid	Multigerm	Iran	Half-sib	Rhizomania- Beet cyst nematode
SB27-H-3	Diploid	Multigerm	Iran	Half-sib	Rhizomania- Beet cyst nematode
SB33-H-1	Diploid	Multigerm	Iran	Half-sib	Rhizomania- Beet cyst nematode
SB33-H-2	Diploid	Multigerm	Iran	Half-sib	Rhizomania- Beet cyst nematode
SB33-H-3	Diploid	Multigerm	Iran	Half-sib	Rhizomania- Beet cyst nematode
S1-92521	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Rhizoctonia
S1-92615	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Rhizoctonia
S1-92006	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Rhizoctonia
S1-92039	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Rhizoctonia
S1-92128	Diploid	Multigerm	Iran	Inbred line	Rhizomania- Rhizoctonia
7233-P.29	Diploid	Multigerm	Iran	Pollinator population	Salt stress
DR1-HSF14-P.35	Diploid	Multigerm	Iran	Half-sib	Drought stress
Fodder beet	Diploid	Multigerm	Iran	Pollinator population	-
O.T 607	Diploid	Monogerm	Iran	O-type	Cercospora
S1-24	Diploid	Multigerm	Iran	Inbred line	Rhizomania
SB26	Diploid	Multigerm	Iran	Pollinator population	Rhizomania- Beet cyst nematode
SB36	Diploid	Monogerm	Iran	Pollinator population	Rhizomania
SHR01-P.12	Diploid	Multigerm	Iran	Pollinator population	Rhizomania
SHR02-P.4	Diploid	Multigerm	Iran	Pollinator population	Rhizomania

shown in Table 1. Sugar beet seeds, provided from SBSI, were washed for four hours based on 2010 edition of International Seed Testing Association (ISTA) instruction (ISTA, 2010). The seeds were then disinfected with *Carboxin-Thiram 75 % WP* (2 g l^{-1}). To test the germination indices, fifty seeds, 3.5-5 mm in diameter, were individually placed between the folds of pleated filter paper sheets code 3014 (Schleicher and Schuell, Dassel, Germany). After placing the seeds, the pleated filter papers were covered with an additional sheet of filter paper and were sprayed by 60 ml of distilled water. Then, they were placed in plastic germination boxes of 20 × 15 × 7 cm dimensions (l × w × h) which were incubated in a dark seed germinator at abovementioned constant temperatures. After 7 days of incubation, germination indices, length of seminal root, shoot and seedling (total length of shoot + seminal root) were measured for normal seedlings. Seed vigor index was calculated based on Eq (1) (Agrawal, 2003).

Seed vigor index = Germination (%) x Seedling length (cm) (1)

2.2. GREENHOUSE EXPERIMENT

This experiment was conducted as completely randomized design with 5-10 replications. All examined genotypes at laboratory experiment, were used in greenhouse experiment. The goal of this experiment was to compare the reaction of sugar beet genotypes to high temperature (30) during early growing stage. It also aimed to investigate if the genotypes which were tolerant to high temperature in laboratory were heat-tolerant in greenhouse, too. Sugar beet seeds were sown in plastic pots of 20 15 cm (h d) dimension filled with farm soil (organic matter = 1.48 %; EC = 1.07 dS m^{-1}), and put at a steady temperature (30. The temperature of greenhouse controlled automatically during the experiment. Ten seeds were cultivated in each pot, and the number of seedling emergence was counted when the second leaf appeared. At this stage, all seedlings but one were removed so that just one seedling remained in each pot which continued growing to 4-6 leaves stage. All measurements on the leaves were conducted at 4-6 leaves stage. In order to avoid any changes in leaf temperature, plants were irrigated through drop irrigation whenever needed.

Measurements of photosynthesis and photosynthetic characteristics were performed on third and fourth leaves using a portable photosynthesis system (Li-6400, Li-CorInc, Lincoln, NE., USA) in the open system mode (the leaf chamber was configured to track the temperature, humidity, and illumination conditions of the growth chamber) between 11:00 and 14:00. Data were taken when net photosynthetic rate reached the steady state. Net photosynthetic rate (*An*), transpiration rate (*E*), stomatal conductance (g_s) and vapor pressure deficit (*VPD*) were measured on fifth leaves of five replications from each genotypes. Water use efficiency (*WUE*) was calculated using Eq. (2):

WUE=An/E (2)

Leaf temperature depression (*LTD*) was calculated from Eq (3). Air and canopy temperatures were determined using a portable photosynthesis system (Li-6400, Li-Cor Inc, Lincoln, NE., USA).

LTD (°C) = Leaf temperature – Air temperature (3)

Greenness index was measured for the middle part of the third or fourth leaves using SPAD, Minolta SPAD-502 chlorophyll meter (Minolta camera Co. Ltd., Osaka, Japan).

The stomatal samples were collected at 30 on a sunny day during 10:00–11:00 am. Samples were taken from the middle parts of adaxial and abaxial surfaces of the third or fourth leaves, at a similar position for all genotypes, after applying a colorless nail polish on the area. For the stomata to be observed clearly by microscope, a transparent nail polish was smeared on the abaxial and adaxial surfaces at the middle of the leaves. The slides of the leaf epidermal fingerprint with the transplant nail polish method (Bin et al., 2008) were observed by an optical microscope (Olympus DP72, Olympus Inc., Japan). The stomatal pore length (*SPL*), stomatal pore area (*SPA*) and epidermal cell density (*ECD*) were measured with Image-Pro Express software (Olympus Inc., Japan). Stomatal density (*SD*) was calculated using Eq. (4) (Xu & Zhou, 2005; Xu et al., 2009).

SD = stomata/(epidermal cells + stomata) × 100 (4)

The stomatal pore area index (SPAI) is defined as the total stomatal aperture area per unit of leaf area and is calculated from Eq. (5) (Zheng et al., 2013):

SPAI=Stomatal average density × stomatal pore area per stomata × 100 (5)

Chlorophyll fluorescence parameters were assessed using a portable stress meter (Walz GmbH Eichenring, 691090 Effeltrich, Germany). Minimal fluorescence, F_0, and maximal fluorescence, *Fm*, were measured in 30 min dark-adapted leaves. Variable fluorescence ($Fv = Fm-F_0$) and quantum efficiency of PSII (*Fv/Fm*) for dark-adapted leaves were calculated as well (Maxwell & Johnson, 2000).

Each plant was separated into root, leaf and petiole parts after being harvested at 4-6 leave stage (36 days after seeding). Measuring leaf area was done immediately afterwards using a leaf area meter (Delta- T Devices LTD, Burwell, Cambridge, England), followed by measuring fresh and dry mass of the mentioned parts (being dried in an oven at 85°C for 48 h).

2.3. DATA ANALYSIS

The PROC UNIVARIATE within SAS v9.1 software (SAS Institute Inc., Cary, NC, USA) was used for data analysis, and residuals were distributed normally. Significant differences between the means were determined through the least significant difference (LSD) test at the 0.05 probability level. The protected least significant difference (protected LSD) was used to compare the means of main effects for the treatments whose effects were statistically significant at $p < 0.01$ or $p < 0.05$ as the F-test indicated. In addition, principal component analysis (PCA) were performed by XLSTAT 2016.

3 RESULTS

3.1 LABORATORY EXPERIMENT

There was a significant difference between genotypes based on measured traits. As shown in Figure 1A, 15 genotypes were ranked as susceptible and 'S1-92521' was the most tolerant to high temperature, regarding seed germination (Figure 1A). Seed vigor index declined as the temperature rose (Figure 1B). With regards to seed vigor index, 24 genotypes were susceptible to high temperature. This trait did not change significantly in four genotypes and increased in three genotypes (S1-92521, SHR01-P.12, and O.T.607) (Figure 1B). All measured parameters at 20 °C and 30 °C are presented in supplemental data (supplementary material, not presented).

In 83.87 % of sugar beet genotypes, there was a decrease in seminal root length at 30 compared to control (20), while the seminal root length of 6.45 % of genotypes increased and that of 9.68 % of genotypes did not change considerably (Figure 1C). The highest decrease and increase in seminal root length at 30, compared to that at 20, was observed for 'SHR02-P.4' (56.40 %) and 'SHR01-P.12' (13.81 %), respectively (Figure 1C). Genotypes which were not affected by heat stress significantly included S1-921258, S1-92521 and 111-52-25.

Genotypes S1-92747 and SHR02-P.4 had the highest increase and decrease in length of shoot at 30 compared to 20, respectively (Figure 1D). At 30, 23 genotypes produced longer shoot compared with the length of shoot at 20, and three had a decreasing trend in the length of their shoot (Figure 1D). The seedling length of almost half sugar beet genotypes tested in this experiment decreased at 30, with 'SHR02-P.4' (34.42 %) showing the lowest amount of relative seedling length at 30 (Figure 1E).

3.2. GREENHOUSE EXPERIMENT

Sugar beet genotypes showed different performance under high temperature in greenhouse. The highest emergence percentage (88 %) was observed in 'S1-92521' with no significant difference from 'SHR01-P.12', 'S1-92128', fodder beet, 'S1-92685, S1-92039', 'SHR02-P.4' and '7233-P.29'. The lowest emergence percentage were observed in 'DR1-HSF14-P.35' (49 %), 'O.T.607' (53 %), 'S1-92749' (56 %), 'S1-92615' (57 %), '7112' (57 %) and '111-52-25' (59 %) (Table 2).

The highest value of greenness index belonged to 'S1-92747' (34.99), whereas 'DR1-HSF14-P.35' (21.36) had the least value (Table 2). 'SB-33H-3', 'S1-92749', 'S1-92039' and 'S1-92750' indicated high values of greenness index with no significant difference from 'S1-92747'.

The maximum *Fv/Fm* value belonged to 'DR1-HSF14-P.35' (0.627), which had no significant difference from 20 of other genotypes, whereas 'SB26' (0.370), which was not significantly different from 'S1-92615' and 'SB-33-H-1', showed the lowest record of this trait (Table 2).

The minimum values of leaf, root, petiole and whole plant fresh mass were observed in 'S1-92006' (3.32 g), 'S1-92750' (0.60 g), 'S1-92006' (1.70 g) and 'S1-92006' (5.72 g), respectively. 'DR1-HSF14-P.35' had the maximum value of the above-mentioned traits excluding root fresh mass whose maximum value belonged to 'S1-92521' (Table 2). According to the results, 'S1-92521' produced the most values of leaf, root and whole plant dry mass, and the highest petiole dry mass (0.95 *g*) belonged to 'SB33-H-2' (Table 2). The lowest values of leaf, root, shoot and total dry mass were attained for 'S1-92128' (0.25 g), 'S1-92006' (0.10 g), 'S1-92006' (0.13 g) and 'S1-92006' (0.52 g), respectively (Table 2). The highest leaf area was produced by 'S1-92039' (383.2 cm^2) while the lowest value (100.41 cm^2) was observed in 'S1-92747' and 'S1-92006' (Table 2).

A high variability of the *SD* was observed among the sugar beet genotypes. The lowest *SD* belonged to 'O.T.607' (68.37 stoma mm^{-2}) (Table 3). On the contrary, genotypes with high *SD* values (Table 3) were

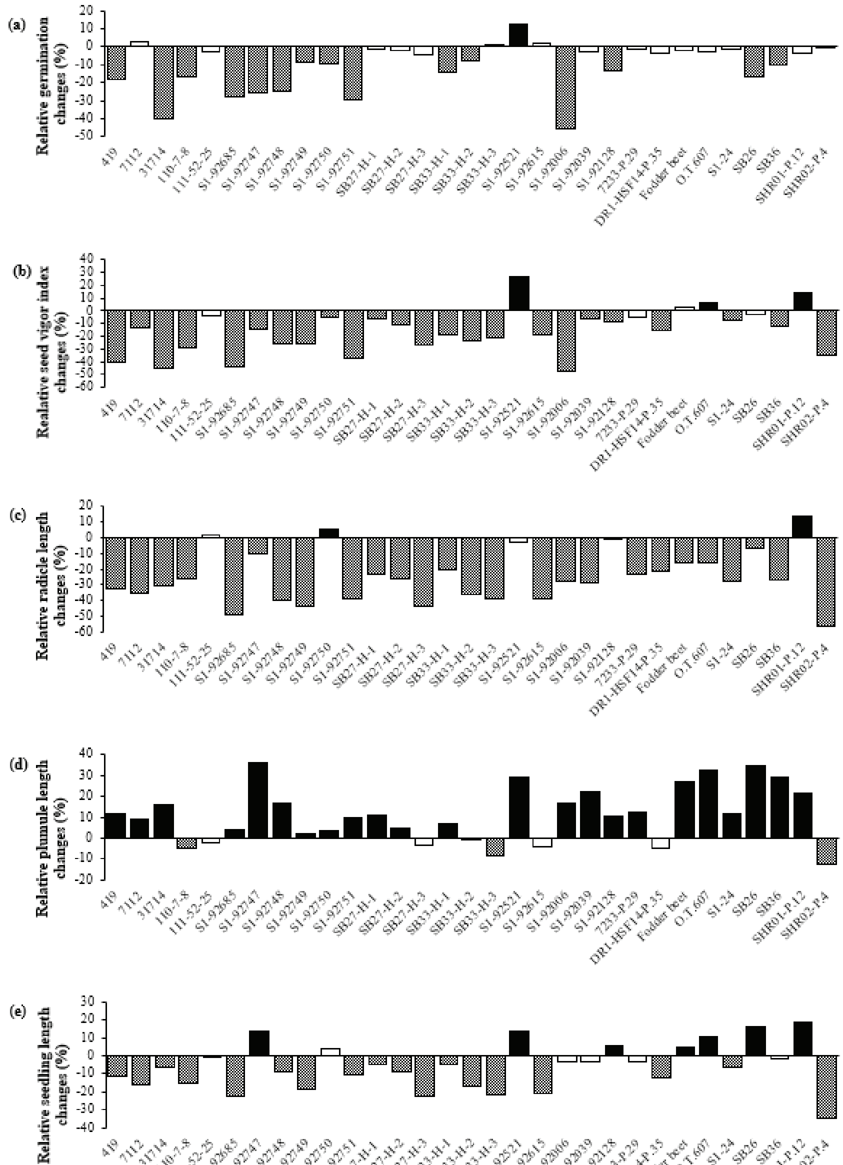

Figure 1: Relative germination (**a**), relative seed vigor index (**b**), relative seminal root length (**c**), relative shoot length (**d**) and relative seedling length (**e**) changes in sugar beet genotypes at 30 in comparison to 20. Solid fill columns, no fill columns (with 5% change) and pattern fill columns respectively indicate increasing, steady and decreasing trend of the traits at 30.

in the order of S1-92749 (223.74 stoma mm^{-2}), SB27-H-3 (211.33 stoma mm^{-2}), SB26, 7112 and 419 (192.67 stoma mm^{-2}).The least value of *ECD* was obtained for 'O.T.607' (366.7 epiderm mm^{-2}). '419', and 'SB26' (1274.1 and 1106.3 epiderm mm^{-2}, respectively) showed the highest *ECD* amount with significant difference from other genotypes (Table 3). Sugar beet genotypes had significantly different *SPA* values ($p < 0.01$). Genotypes 7233.P.29, 111-52-25, S1-92685, SB27-H-1, S1-92751, DR1-HSF14-P.35, fodder beet, S1-92039, O.T.607 and SB33-H-2 showed higher *SPA* values compared to other genotypes. The least *SPA* value was observed in 'SB26' (31μ m^2) (Table 3). The highest and the lowest *SPAI* was observed for 'S1-92749' (0.742 %) and 'S1-92750' (0.260 %), respectively (Table3).

There was a significant difference ($p < 0.01$) in *An* between the first and the last ranks of sugar beet genotypes) (Table 3). Genotypes S1-92685 (15.24 mmol m^{-2} s^{-1}) and S1-92748 (14.57 mmol m^{-2} s^{-1}) showed the highest values of *An* and were significantly

Table 2: Emergence percentage (EP), greenness index (GI), *Fv/Fm*, Leaf fresh mass (*LFM*), root fresh mass (RFM), petiole fresh mass (*PFM*), total fresh mass (*TFM*), leaf area (*LA*), leaf dry mass (*LDM*), Root dry mass (*RDM*), petiole dry mass (*PDM*) and total dry mass (*TDM*) of sugar beet genotypes at 30 in greenhouse.

Genotype	EP (%)	*GI*	*Fv/Fm*	*LFM* (g)	*RFM* (g)	*PFM* (g)	*TFM* (g)	*LA* (cm^2)	*LDM* (g)	*RDM* (g)	*PDM* (g)	*TDM* (g)
419	70 d-k	21.72 jk	0.549 a-f	8.14 d-j	1.56 c-f	3.68 e-j	13.38 d-j	170.2 g-l	0.53 f-k	0.21 g-k	0.23 h-k	0.96 h-m
7112	57 l-o	21.68 jk	0.540 a-f	8.44 b-j	1.34 c-g	5.28 a-f	15.06 c-ij	215.0 e-j	0.59 d-j	0.21 g-k	0.41 c-g	1.21 f-k
31714	76 b-g	27.70 d-g	0.487 def	7.32 f-k	1.38 c-g	4.44 c-i	13.14 e-j	155.0 i-l	0.69 c-i	0.23 g-k	0.37 c-i	1.28 e-j
110-7-8	72 c-j	27.38 d-h	0.522 a-f	9.62 b-f	1.62 c-f	4.54 c-i	16.08 b-g	263.2 b-f	0.79 a-f	0.27 e-i	0.40 c-g	1.46 b-g
111-52-25	59 k-o	23.01 h-k	0.544 a-f	5.84 i-l	0.92 fgh	2.80 hij	9.54 h-k	138.0 jkl	0.43 i-l	0.17 ijk	0.19 jkl	0.79 klm
S1-92685	81 a-e	28.70 c-f	0.486 def	7.74 e-k	1.78 cd	4.22 d-i	13.74 d-j	198.2 e-k	0.65 c-i	0.39 cde	0.35 c-j	1.36 d-i
S1-92747	68 f-m	34.99 a	0.910 a-d	6.98 f-k	1.50 c-f	3.46 f-j	11.94 f-j	187.2 f-k	0.90 abc	0.36 def	0.37 c-h	1.63 a-f
S1-92748	63 h-n	28.02 def	0.525 a-f	7.42 f-k	1.56 c-f	3.52 f-j	12.50 f-j	191.4 f-k	0.71 b-h	0.32 d-g	0.31 d-k	1.34 d-j
S1-92749	56 mno	31.13 a-d	0.557 a-f	8.34 c-g	2.54 ab	6.42 abc	17.30 b-f	230.0 d-i	0.81 a-e	0.53 ab	0.63 b	1.96 ab
S1-92750	75 b-h	33.34 abc	0.515 b-f	6.38 g-l	0.60 h	5.80 a-d	12.78 e-j	159.8 i-l	0.61 d-j	0.13 jk	0.51 bc	1.22 e-k
S1-92751	62 i-n	26.69 d-i	0.493 c-f	7.24 f-k	1.04 e-h	4.66 b-h	12.94 e-j	205.2 e-j	0.63 d-j	0.19 h-k	0.37 c-i	1.16 f-l
SB27-H-1	75 b-h	25.63 f-k	0.553 a-f	5.78 jkl	1.12 d-h	2.90 hij	9.80 h-k	160.0 i-l	0.49 g-l	0.19 h-k	0.23 g-k	0.90 i-m
SB27-H-2	61 j-o	28.94 c-f	0.58 a-d	11.8 abc	1.92 bc	5.24 b-g	18.96 a-d	309.6 a-d	0.95 ab	0.42 bcd	0.47 b-e	1.83 a-d
SB27-H-3	74 c-i	26.02 e-k	0.523 a-f	9.26 b-j	1.64 cde	4.26 d-i	15.16 c-h	252.6 b-g	0.79 a-f	0.29 e-i	0.39 c-h	1.44 c-h
SB33-H-1	66 g-m	29.85 b-f	0.457 efg	8.14 d-j	1.70 cde	3.42 f-j	13.26 e-j	203.0 e-k	0.85 a-d	0.11 k	0.33 c-j	1.29 e-j
SB33-H-2	69 e-l	29.60 b-g	0.516 a-f	11.16 a-e	1.72 cde	4.60 b-i	17.48 a-f	282.6 b-e	0.45 h-l	0.32 d-g	0.95 a	1.72 a-e
SB33-H-3	66 g-m	34.17 ab	0.510 b-f	8.34 c-j	1.04 e-h	4.20 d-i	13.58 e-j	237.6 c-i	0.71 b-h	0.19 h-k	0.37 c-i	1.26 e-k
S1-92521	88 a	28.99 c-f	0.520 a-f	11.86 ab	3.18 a	5.64 a-e	20.68 abc	321.2 abc	0.99 a	0.56 a	0.47 b-e	2.01 a
S1-92615	57 l-o	29.66 b-f	0.447 fg	6.02 h-l	1.26 c-h	3.30 f-j	10.58 g-k	166.8 h-l	0.57 e-j	0.23 g-k	0.29 e-k	1.09 g-l
S1-92006	63 h-n	28.29 def	0.585 a-d	3.32 l	0.70 gh	1.70 j	5.72 j	100.4 l	0.29 kl	0.10 k	0.13 k	0.52 m
S1-92039	76 a-g	30.37 a-e	0.515 b-f	11.64 a-d	3.04 a	6.64 ab	21.32 ab	383.2 a	0.93 ab	0.48 abc	0.49 bcd	1.89 abc
S1-92128	83 abc	29.82 b-f	0.560 a-e	5.86 i-l	1.08 d-h	2.56 ij	9.50 jkl	162.5 h-l	0.25 l	0.23 g-k	0.61 b	1.08 g-l
7233-P.29	82 a-d	23.09 g-k	0.56 0a-e	11.35 a-d	1.58 c-f	5.34 a-f	18.30 a-e	265.0 b-f	0.73 a-g	0.23 g-k	0.33 c-j	1.28 e-j
DR1-HSF14-P.35	49 o	21.36 k	0.627 a	13.46 a	1.58 c-f	7.02 a	22.06 a	337.0 ab	0.77 a-f	0.21 g-k	0.45 b-f	1.42 c-h
Fodder beet	81 a-d	27.38 d-h	0.528 a-f	6.28 g-l	1.16 d-h	4.02 d-i	11.46 g-j	172.6 g-l	0.47 g-l	0.13 jk	0.25 g-k	0.84 j-m
O.T.607	53 no	22.55 ijk	0.610 ab	8.52 b-j	1.46 c-f	4.16 d-i	14.14 d-i	222.4 e-j	0.69 c-i	0.25 f-j	0.31 d-k	1.20 f-k
S1-24	79 a-f	26.32 e-j	0.599 abc	9.60 b-g	1.54 c-f	3.10 hij	14.24 d-i	246.6 c-h	0.77 a-f	0.33 d-g	0.27 f-l	1.36 d-i
SB26	69 e-l	26.64 d-i	0.370 g	8.38 b-j	1.50 c-f	3.20 gj	13.08 e-j	217.8 e-j	0.61 d-j	0.30 e-h	0.21 h-k	1.11 g-l
SB36	59 k-o	22.52 ijk	0.536 a-f	4.34 kl	1.16 d-h	2.76 hij	8.26 j-k	119.2 kl	0.35 jkl	0.17 ijk	0.17 j-k	0.68 lm
SHR01-P.12	87 ab	27.92 def	0.571 a-d	9.38 bh	1.28 c-h	4.14 d-i	14.80 d-i	215.0 e-j	0.69 b-i	0.21 g-k	0.29 e-k	1.18 f-k
SHR02-P.4	76 a-g	27.27 d-h	0.492 c-f	9.32 b-i	1.42 c-f	4.42 c-i	15.16 c-h	231.0 d-i	0.63 c-i	0.23 g-k	0.31 d-k	1.16 f-l

Different letters indicate significant differences using the LSD Test ($p < 0.05$).

Table 3: *SD*: stomatal density (*SD*), stomatal pore length (*SPL*), epidermal cell density (*ECD*), stomatal pore area (*SPA*), stomatal pore area index (SPAI), net photosynthesis rate (*An*), stomatal conductance (g_s), Water use efficiency (*WUE*), leaf temperature depression (*LTD*) and vapor pressure deficit (*VPD*) of different sugar beet genotype at 30.

Genotype	*SD*	*SPL*	*ECD*	*SPA*	*SPAI*	*An*	g_s	*WUE*	*LTD*	*VPD*
	(stomata mm^{-2})	(µm)	(epiderm mm^{-2})	(µm^2)	(%)	(mmol m^{-2} s^{-1})	(mol m^{-2} s^{-1})	(%)	(°C)	(kPa)
419	192.67 abc	14.99 h	1274.1 a	33.46 fgh	0.647 a-d	11.44 c-h	0.057 b-f	0.0085 c-g	-0.161 d-j	3.77 h-k
7112	192.67 abc	16.22 gh	764.4 c-f	34.87 d-h	0.667 abc	11.95 b-g	0.044 c-i	0.0076 c-g	-0.144.0c-j	4.07 a-e
31714	99.44 e-j	16.38 fgh	714.7 c-h	33.59 fgh	0.334 fgh	10.87 e-i	0.042 d-i	0.0064 d-h	-0.035 b-i	3.92 d-i
110-7-8	142.95 c-g	18.11 c-h	696.1 c-h	38.41 b-g	0.548 a-g	11.50 c-h	0.054 b-g	0.0089 b-f	-0.236 e-k	4.11 a-d
111-52-25	149.16 b-g	18.84 b-g	783.1 cde	39.43 a-g	0.588 a-f	9.88 i	0.028 hi	0.0038 h	-0.130 b-j	3.9 e-j
S1-92685	111.87 e-j	21.72 ab	677.4 c-i	43.66 ab	0.492 a-h	15.24 a	0.039 d-i	0.0089 b-f	-0.237 e-k	4.08 a-e
S1-92747	124.30 d-j	19.23 a-g	565.6 e-j	38.80 b-g	0.482 a-h	11.01 d-i	0.036 e-i	0.0058 e-h	-0.126 b-j	4.14 abc
S1-92748	111.87 e-j	17.45 c-h	689.9 c-i	34.71 d-h	0.400 a-h	14.57 a	0.054 b-g	0.011 a-c	-0.068 b-i	4.03 a-e
S1-92749	223.74 a	16.33 fgh	888.7 bcd	32.91 gh	0.742 a	13.16 b	0.041 d-i	0.0079 c-g	-0.367 h-k	4.05 a-e
S1-92750	74.58 ij	17.51 c-h	540.7 e-j	34.79 d-h	0.260 h	12.19 b-f	0.043 c-i	0.0068 d-h	0.038 b-h	3.71 jkl
S1-92751	99.44 e-j	20.49 abc	658.8 c-i	41.20 a-d	0.413 c-h	11.92 b-g	0.055 b-g	0.0088 b-f	-0.172 d-j	3.78 g-k
SB27-H-1	111.87 e-j	21.50 ab	515.8 f-j	43.56 ab	0.487 a-h	12.22 b-e	0.050 b-h	0.008 c-g	0.102 c-f	3.77 h-k
SB27-H-2	186.45 a-d	21.07 a-e	745.5 c-g	38.52 b-g	0.704 ab	11.86 b-g	0.038 d-i	0.0069 d-h	-0.504 jk	4.22 a
SB27-H-3	211.31 ab	17.01 e-h	895.0 bcd	34.15 e-h	0.730 a-b	11.94 b-g	0.039 d-i	0.0066 d-h	-0.045 b-i	3.98 b-g
SB33-H-1	111.87 e-j	16.89 e-h	491.0 hij	34.37 e-h	0.389 d-h	11.94 b-g	0.039 d-i	0.0068 d-h	-0.192 d-j	4.12 a-d
SB33-H-2	149.16 b-g	19.37 a-g	907.4 b-c	40.67 a-e	0.601 a-e	11.51 c-h	0.041 d-i	0.0069 d-h	-0.081 b-j	4.18 ab
SB33-H-3	136.73 c-i	17.15 c-h	783.1 cde	35.37 c-h	0.487 a-h	11.10 d-i	0.036 f-i	0.0053 fgh	0.016 b-h	3.82 f-k
S1-92521	111.87 e-j	16.42 fgh	553.1 e-j	33.92 fgh	0.381 e-h	10.44 hi	0.024 i	0.0036 h	-0.639 k	4.12 a-d
S1-92615	155.38 b-f	18.96 a-g	646.4 d-i	38.38 b-g	0.593 a-f	12.75 bc	0.069 ab	0.0118 ab	0.072 b-g	3.89 e-j
S1-92006	87.01 g-i	15.27 h	733.4 c-h	31.45 h	0.276 h	11.96 b-g	0.086 a	0.0127 a	214 a-d	3.57 l
S1-92039	118.09 e-j	19.54 a-g	602.9 e-j	39.83 a-f	0.472 b-h	10.67 ghi	0.060 bcd	0.0096 a-e	0.300 ab	4.05 a-e
S1-92128	118.09 e-j	17.77 c-h	559.3 e-j	37.22 b-h	0.440 c-h	12.19 b-f	0.039 d-i	0.0068 d-h	-0.119 b-j	4.04 a-e
7233-P.29	93.23 f-j	22.28 a	441.3 ij	45.68 a	0.426 c-h	11.15 d-i	0.059 b-e	0.0091 b-f	-0.030 b-i	3.9 e-j
DR1-HSF14-P.35	93.23 f-j	20.44 a-d	609.1 e-j	41.80 abc	0.399 d-h	10.79 ghi	0.053 b-g	0.0082 c-g	0.212 a-d	4.05 a-e
Fodder beet	118.09 e-j	20.21 a-e	596.6 e-j	41.12 a-d	0.484 a-h	11.24 d-h	0.038 d-i	0.0059 e-h	-0.334 g-k	3.9 e-j
O.T.607	68.37 j	19.64 a-f	366.7 j	39.73 a-f	0.270 h	10.96 d-i	0.036 f-i	0.0056 e-h	-0.320 f-k	4.01 b-f
S1-24	74.58 ij	20.04 a-e	441.3 ij	38.30 b-g	0.286 g-h	11.33 d-h	0.036 f-i	0.0054 fgh	0.070 b-g	3.76 h-l
SB26	192.67 abc	14.83 h	1106.3 ab	31.08 h	0.600 a-e	10.83 f-i	0.034 ghi	0.0049 gh	0.281 abc	3.71 jkl
SB36	80.80 hij	17.08 d-h	497.2 g-j	34.28 e-h	0.277 h	12.23 bcd	0.065 abc	0.0098 a-d	0.144 b-e	3.75 i-l
SHR01-P.12	161.59 a-e	16.60 fgh	733.4 c-h	35.62 c-d	0.576 a-f	13.01 b	0.053 b-g	0.0096 a-d	-0.092 b-ij	3.95 c-h
SHR02-P.4	155.38 d-f	19.53 a-g	652.6 d-i	38.69 b-g	0.600 a-e	11.30 d-h	0.044 c-i	0.0064 d-h	0.635 a	3.66 kl

Different letters indicate significant differences using the LSD Test ($p < 0.05$).

different from other genotypes at 30 regarding this trait (Table 3). The lowest amount of *An* was obtained for '111-52-25' (29.65 mmol m^{-2} s^{-1}). Stomatal conductance (g_s) ($p < 0.001$) of 'S1-92006' and 'S1-92615' (0.09 and 0.07 mol m^{-2} s^{-1}, respectively) indicated the highest values and the minimum level of this trait was related to 'S1-92521' (0.02 mol m^{-2} s^{-1}), with no significant difference from 18 of other genotypes (Table 3). The best *WUE* was observed in 'S1-92006' (0.0130 %) which had no significant difference from 'S1-92615', 'S1-92748', 'SB36', 'SHR01-P.12', and 'S1-92039'. On the other hand, the minimum *WUE* value belonged to 'S1-92521' (0.0036 %) and '111-52-25' (0.0038 %) (Table 3). 'SHR02-P.4' (0.635) produced the highest value of *LTD*. The least value for *LTD* was obtained from 'S1-92521' (-0.639) (Table 3). Also in case of *VPD*, 'S192006' (3.57 kPa) and 'SB27-H-2' (4.22 kPa) had the highest and the lowest values, respectively (Table 3).

In order to have a better assessment of sugar beet genotypes based on important measured traits, the PCA was performed. The PCA revealed that two first components together accounted for 56.82 % (38.23 % and 18.59 %, respectively) (Figure 2). Genotypes placed in the upper quarter on the right (S1-92039, DR1-HSF14-P.35, 7233-P.29, 110-7-8, S1-92749, S1-92685, 7112, SB27-H2 and SB33-H-2) had a high leaf area, total fresh mass, total dry mass, *Fv/Fm* and greenness index. On the other hand, genotypes placed in the upper quarter on the left (S1-92006, S1-92615, S1-92748, SHR01-P.12, SB36, S1-92751 and 419) had high values of *LTD*, *WUE*, g_s and *An*. Also it was revealed that leaf area, total fresh mass and total dry mass were not related to *WUE*, g_s and *An*. However, *Fv/Fm* was positively related to all of these six traits.

4 DISCUSSION

This study was conducted to determine the tolerant genotypes of sugar beet in high temperature, as well as investigating the suitable criteria for screening tolerant genotypes. According to the results, in laboratory experiment, 'S1-92521', '111-52-25', 'SHR01-P.12', 'O.T. 607', fodder beet, '7233-P.29' and 'SB26' demonstrated a better performance, compared to the other genotypes, almost in all traits in high temperature (Figure 1). Records of 'S1-92521', '111-52-25' and 'SHR01-P.12', in 30 were higher than those in 20, or did not change significantly (Figure 1). Specifically, all measured traits of 'S1-92521' showed higher records in 30 except seminal root length which did not change significantly. Similarly, except germination percentage, which did not change significantly, all traits of 'SHR01-P.12' improved in 30 compared with 20. There was not a significant change in any of the measured traits of '111-52-25' either. Genotypes O.T. 607, fodder beet, 7233-P.29 and SB26 performed well in all of the traits, too, except in the case of seminal root length which decreased in 30. In 'SB26', a decline in germination percentage was observed as well which can be compensated through planting more seeds. Genotypes with poor performance in laboratory experiment included 110-7-8, SB33-H2 and SHR02-P.4 (Figure 1). In most of the genotypes, seminal root length was negatively affected by heat stress, but concerning the whole seedling, this loss was, to some ex-

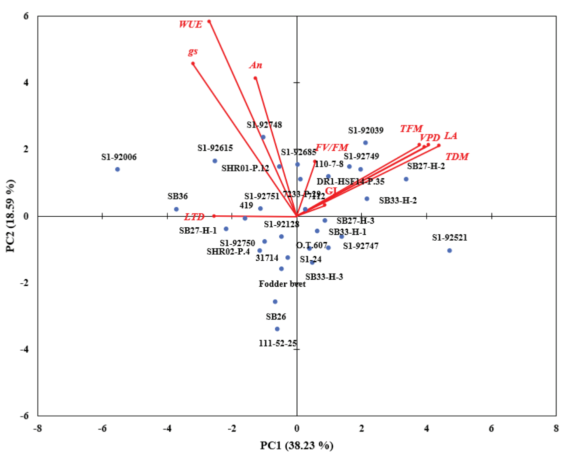

Figure 2: Biplot of the first and second principal component (PC) axes for greenness index (*GI*), leaf area (*LA*), leaf temperature depression (*LTD*), net photosynthesis (*An*), photochemical efficiency of PSII (*Fv/Fm*), stomatal conductance (g_s), total dry mass (*TDM*), total fresh mass (*TFM*) and water use efficiency (*WUE*) traits on sugar beet genotypes at 30.

tent, compensated by increasing shoot length under high temperature (Figures 1C, 1D, 1E).

According to the results, two of the traits could be good criteria for screening tolerant genotypes to high temperature, seed vigor index and seminal root length. Genetically difference among the genotypes is a criteria to select a trait as a screening tool for stress tolerance (El-Hendawy et al., 2007). In the present study, by increasing the temperature, high differences among the genotypes were observed regarding seed vigor index and seminal root length (Figures 1B, 1C). In other words, almost all of the genotypes showed decreased records under heat stress, and just a few of them could show an increase or maintain without a significant change (Figures 1B, 1C). At this point, by having a more precise look, it can be seen that seed vigor index could be a better screening criteria rather seminal root length. Because genotypes which showed a better or unchanged seed vigor, generally had a good record in other measured traits in the laboratory experiment, too (like '7233-P.29' and 'O.T.607'), but this was not observed in the case of genotypes with a better or unchanged seminal root length (like 'S1-92750' and 'S1-92128') (Figure 1). Seed vigor index has been considered as a screening criteria in previous studies, too (ISTA, 2014). Seed germination factors and seedling properties have been considered as evaluation criteria in breeding programs that worked on environmental stresses such as drought stress (Sadeghian & Yavari, 2004).

In greenhouse experiment, high amounts of leaf area, total fresh mass and total dry mass which were accounted for plant yield, were observed in 'S1-92521', 'S1-92039', 'SB33-H-2' and 'SB27-H2' (Table 2). Two other genotypes, S1-92747 and S1-92749, also showed high records for total dry mass and greenness index as well as quantum efficiency of PSII (Table 2, Figure 2). The potential of multivariate analysis technique such as PCA for the identification of tolerant genotypes to environmental stress has been shown in different crops such as rice (Cha-um et al., 2009), sugarcane (Cha-um et al., 2012), tomato (Juan et al., 2005), peanut (Liu et al., 2012) and soybean (Shelke et al., 2017). PCA identifies the probable grouping and establishment of relationships among variables (Martínez-Calvo et al., 2008; Sarabi et al., 2016). Regards lack of significant relationship between stomatal indices and gas exchange parameters maybe due to small number of samples (McElwain et al., 2016), we did not use stomatal characteristics in PCA. According to the PCA analysis, *WUE* and g_s produced the most variance among the genotypes, and the least was related to greenness index and *Fv/Fm* (Figure 2). Furthermore, there was a positive relationship among total dry mass, total fresh mass, leaf area, greenness index and *Fv/Fm* (Figure 2). This implies that with more quantum efficiency of PSII, indicating less stress inside the plants, photosynthesis got more efficient, and consequently more assimilate were produced. *Fv/Fm* had a positive relationship with *WUE*, g_s and *An*, as well, indicating that more tolerance caused the net photosynthesis to rise (Figure 2). There is a positive relationship between g_s and *An*, showing that increasing stomatal conductance leads the *An* to rise (Urban et al., 2017). Stomatal conductance and A_n, did not show much relation with the yield parts of the plants, total dry mass and leaf area which could be because of the fact that these traits were measured instantaneously. In

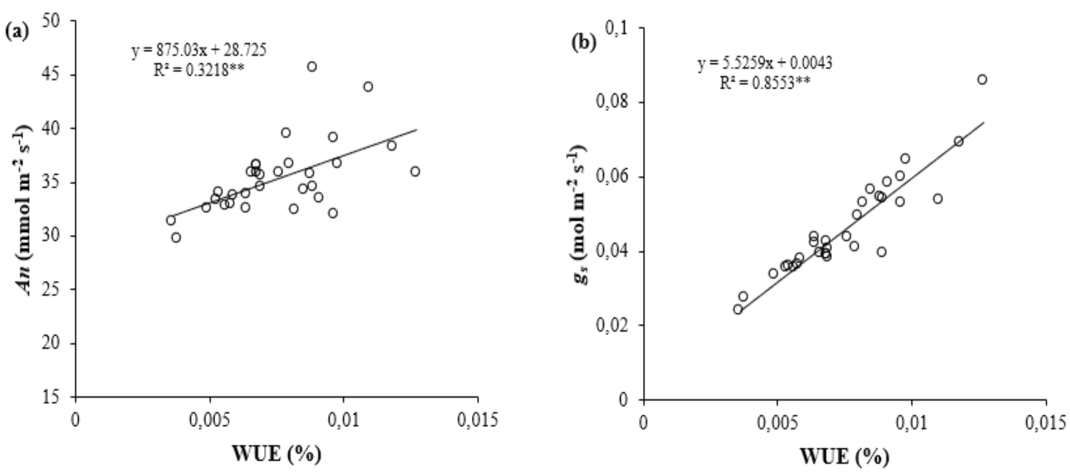

Figure 3: Relationship between *WUE* and *An* (**a**) and *gs* (**b**) in sugar beet genotypes (n = 31). ** indicate significant correlation at $p < 0.05$ and 0.01, respectively

addition, it might be due to the increasing of transpiration under high temperature.

High *VPD* values of 'S1-92749', 'S1-92039', 'S1-92521' and 'SB27-H2' resulted higher total dry mass and leaf area; while, the least values of mentioned traits observed from 'S1-92006' and 'SB36' (Figure 2). Difference between the leaf and air temperatures (*LTD*) showed a negative relationship with yield parameters and also with *VPD* and a positive relationship with *WUE*, g_s and *An* (Figure 3). This indicates that under high temperature, tolerant plants could maintain their stomata open resulting in cooling the leaves as a consequence of transpiration. Stomatal conductance to CO_2 is strictly proportional to stomatal conductance to water (Gilbert et al., 2011). Gas exchange reaction to temperature was related to the interaction effect of some parameters like internal plant water status and *VPD* (Chaves, 1991). In addition, it has been mentioned that high temperature causes rising in g_s (Killi et al., 2017). By increasing *VPD*, we witness an increase in stomatal conductance and leaf transpiration and consequently the mobility of water and nutrient in the plant (Caird et al., 2007). It has been well established that plants regulate rates of transpiration and photosynthesis in parallel, maintaining a balance between g_s and *An* (Lawson et al., 2011). High positive correlation between canopy temperature depression and stomatal conductance has been observed in sugar beet (Mohammadian et al., 2001; Fukuoka, 2005). Under stress-free conditions the water transpired by the plants evaporates and cools the leaves (González-Dugo et al., 2006; Schauberger et al., 2017). In the present study, plants could maintain their transpiration because of the *VPD* and because the plants were not in shortage of water, as sufficient water was supplied for them. However, *LTD* of sugar beet genotypes was varied (Table 3). It was clear that sugar beet genotypes had different ability for cooling their leaves under high temperature.

In order to choose a screening criteria for heat tolerance in the sugar beet genotypes, the level of reaction to heat stress should be taken into consideration, as it was done in the laboratory experiment. High reaction intensities among the genotypes were observed in leaf area, total dry mass, g_s, *An* and *LTD* among which the first two traits need destructive harvest most of the time, so it is better not to choose them as criteria. Stomatal conductance (g_s) and net photosynthetic rate (*An*) are not suitable either, because according to the PCA, they were not related to the yield parameters like total dry mass (Figure 2). Leaf temperature depression (*LTD*), however, could be considered as a screening criteria for heat tolerance, because it does not show the two weakness point mentioned above. Temperature difference between leaves and air has been considered as a screening criteria in previous studies as well (Reynolds et al., 2009). Canopy temperature depression was selected as a suitable screening tool for selecting drought-tolerance cultivars of wheat, because there was a genotypic variation for it, and also it was directly correlated with grain yield (Thapa et al., 2018). Quantum efficiency of PSII (*Fv/Fm*) could be considered as a good screening criterion for heat tolerance, because it was positively related to g_s, A_n, *WUE* and yield parameters (Figure 2). Quantum efficiency of PSII (*Fv/Fm*) has been introduced as a tool for early detection of heat (Zhou et al., 2015) and drought stress (Mohammadian et al., 2003). It was observed that *Fv/Fm*, shoot fresh mass, shoot dry mass and root dry mass of tomato genotypes decreased under heat stress conditions (Zhou et al., 2015). Also, *Fv/Fm* had decreased in sugar beet by drought stress (Mohammadian et al., 2003). In addition, possibility of using biomass, canopy temperature depression, greenness index and *Fv/Fm* for precise screening in heat-tolerant wheat genotypes has been proved (Joshi et al., 2007; Nagar et al., 2015).

A similarity was observed between the performance of some genotypes under high temperature in laboratory and that in greenhouse. Genotype S1-92521 showed a good performance in high temperature in both experiments in all traits (Figure 2 and Table 2 and 3). On the other hand, 'SB-27-H-1', 'S1-92006' and '111-52-25' performed poorly in both laboratory and greenhouse experiments, especially in yield traits, leaf area and total dry mass. However, other genotypes did not show any noticeable similarity in their reaction to heat stress between laboratory and greenhouse experiments.

5 CONCLUSION

The present study investigated the sugar beet genotypes behavior under high temperature condition as well as identifying good screening tools at germination and early growth stages (4-6 leave stage). A high variation was observed among the genotypes in the present study, which is a valuable feature for breeding programs. Based on important traits, 'S1-92039', 'S1-92521', 'SB33-H-2', 'S1-92747', 'S1-92749' and 'SB27-H-2' were tolerant genotypes to high temperature in greenhouse. The only genotype which showed a good tolerance in both laboratory and greenhouse experiments, was S1-92521. Three genotypes, 110-7-8, SB33-H3, and SHR02-P.4, did not perform well in neither of the two experiments. So, in general, except for 'S1-92521', we could not say the genotypes which performed well in laboratory could also tolerate high temperature in greenhouse. In laboratory experiment, seed vigor index was chosen as a good screening tool for selecting heat-tolerant genotypes. In greenhouse, *LTD* and *Fv/Fm* were considered as beneficial non-destructive screening tools to find tolerant sugar beet genotypes to high temperature at early growth stages.

6 ACKNOWLEDGEMENTS

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Modified CTAB protocol for RNA extraction from Lemon balm (*Melissa officinalis* L.)

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Modified CTAB protocol for RNA extraction from Lemon balm (*Melissa officinalis* L.)

Abstract: Ribonucleic acid (RNA) quality and integrity are crucial for many studies in plant molecular biology. High-quality RNA extraction from plants with high levels of compounds such as polysaccharides, polyphenols, and other secondary metabolites are problematic. RNA extraction from Lemon balm tissues can be difficult due to the presence of polyphenolic and polysaccharide compounds or can be done by expensive protocols. This study shows improvement of a CTAB-based protocol which allows rapid and easy isolation of high-quality RNA from Lemon balm plant. The RNA obtained is suitable for cDNA synthesis and RT-PCR experiments.

Key words: *Melissa officinalis*; CTAB; RNA; RT-PCR

CTAB protokol za ekstraskcijo RNK iz melise (*Melissa officinalis* L.)

Izvleček: Kakovost in ohranjenost ribonukleinske kisline (RNK) sta bistveni za mnoge raziskave v molekularni biologiji rastlin. Visoko kakovostni izvlečki RNK iz rastlin z veliko vsebnostjo spojin kot so polisaharidi, polifenoli in drugi sekundarni metaboliti so problematični. Ekstrakcija RNK iz tkiv melise je lahko težavna zaradi prisotnosti polifenolov in polisaharidov ali pa je lahko narejena le z dragimi protokoli. Raziskava predstavlja izboljšanje protokola na osnovi CTAB, ki omogoča hitro in enostavno izolacijo kvalitetne RNK iz melise. Pridobljena RNK je primerna za cDNK sintezo in RT-PCR poskuse.

Ključne besede: *Melissa officinalis*; CTAB; RNK; RT-PCR

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1 INTRODUCTION

The extraction of high quality ribonucleic acid (RNA) is an important step for many studies in plant molecular biology, such as northern blotting, microarray hybridization, both targeted real-time PCR (RT-qPCR) analysis and next generation sequencing (NGS) (Gambino et al., 2008; Guerriero et al., 2016). Extraction of RNA in sufficient quantity and quality from the tissues of aromatic, woody, and aquatic plants is particularly challenging, because of high levels of compounds such as polysaccharides, polyphenols, and other secondary metabolites (Gambino et al., 2008; Jordon-Thaden et al., 2015). Phenolic compounds form high molecular weight complexes tend to co-precipitate with RNA by binding to nucleic acids and polysaccharides in the presence of alcohols. Hence, they contaminate the final extract and interfere with subsequent applications (Gambino et al., 2008).

Conventional protocols for RNA isolation usually involve the use of detergents, such as cetyl trimethyl ammonium bromide (CTAB) or sodium dodecyl sulphate (SDS), denaturing organic solvents (phenol and chloroform), reducing agents (β-mercaptoethanol and dithiothreitol), or denaturing agents (guanidinium isothiocyanate salts) (Gambino et al., 2008). Methods involving CTAB, initially were developed for pine tree tissues (Chang et al., 1993) and subsequently used to extract RNA from a wide range of polysaccharide- and polyphenol-rich plant tissues (Iandolino et al., 2004; Meisel et al., 2005; Gambino et al., 2008).

Lemon balm is one of the oldest and widely used medicinal plant in the mint family (Lamiaceae), native to Southern Europe, Mediterranean region and northern Iran (Döring et al., 2014; Jalal et al., 2015). It is a perennial herb and a rich source of natural antioxidants (Saraydin et al., 2012). Reports indicated that lemon balm had many beneficial effects such as anti-oxidant, anti-bacterial, anti-viral, anti-inflammatory, sedative, mnemonic improvement, reducing excitability, anxiety, stress, gastrointestinal disorders and sleep disturbance. However, the mechanisms underlying these medicine effects remain largely unknown (Jalal et al., 2015).

Nutritional efficacy testing, based on selected biomarker approaches, do not provide global information while application of microarrays and high-throughput qPCR allows quantification of a large number of biomarkers, and consequently enables evaluation of the global effects of nutrients on cells (Jun et al., 2012). Hence, a clean and intact RNA is important for functional genomic studies (Rubio-Piña & Zapata-Pérez, 2011).

The aim of this study was to develop an optimized CTAB-based protocol, to reduce the time and cost of extraction without reducing quality and yield of RNA from polyphenolics and polysaccharide-rich tissues of old lemon balm.

2 MATERIALS AND METHODS

2.1 PLANT SPECIES USED

Leaf tissues was collected from 3 months old lemon balm plant (Agricultural and *Natural Resources* and *Education* Center, West Azerbaijan, Iran) and frozen in liquid nitrogen. Then, leaf samples were transferred to -80 ˚C until analyses. Total RNA was extracted with TRIzol (Sigma Alderich), RNX- plus (Sina Clone) and (CTAB)-based protocol.

2.2 NUCLEIC ACID EXTRACTION

2.2.1 (CTAB)-based protocol

2.2.2 Reagents

Extraction buffer: 2 % (w/v) CTAB (hexadecyltrimethylammonium bromide), 0.1 M Tris-Hcl (pH: 8), 1.4 M NaCl, 20 mM EDTA (pH: 8), 2 % PVP (polyvinylpyrrolidone), BME (β-mercaptoethanol) (to a final concentration of 10 % (v/v), added right before use).

Sodium acetate 3 M (pH: 5)

Chloroform/ IAA (isoamyl alcohol) (24/1)

Phenol/ chloroform/ isoamyl alcohol (25/24/1)

70 % ethanol

RNA/RNase-free water

DNase inactivation reagent

Liquid nitrogen in a Dewar flask

Equipment list

Water bath at 65 °C

Vortexer

24-place centrifuge cooled to 4 °C

Incubator at 37 °C with orbital shaker

Procedure

1- About 80-100 mg of leaf tissue was ground to a fine powder using liquid nitrogen and transferred into a 2 ml centrifuge tube. Then, 900 µl of extraction buffer and 100 µl of β-mercaptoethanol were added. The mixture was shaken for 30 s and then incubated at 65 °C for 10 min, inverting the tube 3-4 times every now and then during incubation.

2- Then, 800 µl of chloroform was added. The mixture was shaken for 30 s and centrifuged at 12000 rpm for 10 min at 4 °C. The supernatant was transferred to a new tube.

3- 800 µl of phenol/chloroform/ isoamyl alcohol (25/24/1) was added and shaken for 1 min. Then, the mixture was centrifuged at 12000 rpm for 15 min at 4 °C (this step was repeated twice).

4- The supernatant was transferred to a new tube and an equal volume of chloroform/isoamyl alcohol (24/1) was added. Samples were shaken for 30 s and centrifuged at 12000 rpm for 10 min at 4 °C.

5- The final supernatant was transferred to a new tube. 1/10 volume of sodium acetate (3 M, pH: 5) and equal volume of cool isopropanol were added to the tube, then kept at -20 °C for at least 1 h. Next, the samples were centrifuged at 13000 rpm for 15 min at 4 °C.

6- The pellets were successively washed with 70 % ethanol for one or two times. After a short drying time at room temperature, the pellets were dissolved in 20-50 µl of DEPC water.

7- Finally, 1 µl of DNase enzyme was added and incubated at 37 °C for 30 min.

2.3 ESTIMATION OF RNA QUALITY

The purified RNA was measured by spectrophotometric analysis (*Nanodrop* 2000c; Thermo Fisher Scientific, Waltham, USA). Contamination due to proteins and phenol/carbohydrates was determined by recording the OD ratios; $A_{260}/_{280}$ and $A_{260}/_{230}$, respectively. In order to verify RNA integrity, extracts were fractioned by electrophoresis in a 1.5 % agarose gel, stained with ethidium bromide and visualized in a gel documentation system (InGenius3, Syngene, UK).

2.4 RT-PCR

Single-stranded cDNA was synthesized from 500 ng total RNA using reverse transcriptase and oligo (dT), following the manufacturer's protocol (Thermo Scientific). The synthesized cDNA was used in a PCR reaction in order to estimate the expression level of the tubulin gene using following primers: Tubulin-FWD: 5´-GCTTTCAACACCTTCTTCAGTG-3´and Tubulin REV: 5´-CTTTCTCAGCTGAGATCACTG G-3´.

3 RESULTS AND DISCUSSION

3.1 ESTIMATION OF RNA QUALITY

The isolation of high-quality RNA from enrich polysaccharides and polyphenols plant tissues is quite challenging (Liao et al., 2014). Lemon balm leaves contain secondary metabolites such as, polyphenolic acid (rosmaric acid, trimeric compounds and some flavonoids), terpenoids, carboxylic acid, essential oils, various sugars, golden and pectic materials (Jafarpour & Fard, 2016) which affect the quality of the isolated RNA. The biosynthesis of secondary metabolites in plants is influenced by season harvesting, growing region, agronomic conditions and type of processing (Wahby, 2016). Therefore, RNA extraction from lemon balm tissues can be difficult due to the presence of these compounds. Polyphenolic compounds (particularly tannins) are readily oxidized to form quinones, which bind irreversibly to nucleic acids and proteins, and may decrease RNA yield, as well as inhibit PCR amplification (Shu et al., 2014). In contrast, polysaccharides can co-precipitate and degrade RNA, constitute the major obstacle of RNA isolation in low ionic strength buffers, and cause the browning effect and make RNA difficult to be dissolved (Shu et al., 2014). In addition, these interfering chemicals severely interfere with RNA-dependent RT and DNA polymerases and cause the RT-PCR to fail. Thus, these contaminating substances must be eliminated during RNA isolation (Hou et al., 2011; Ouyang et al., 2014; Sabzevari & Hosseini, 2014). In this case, successful isolation of intact RNA from tissues rich in polysaccharides and polyphenolic compounds, is a basic requirement for many molecular studies

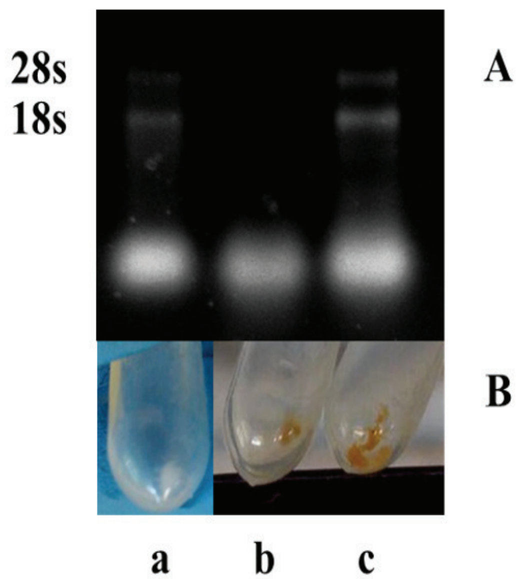

Figure 1: (A): Ethidium bromide agarose gel (1.5 % (w/v)) was used to separate total RNA and (B): pellet of RNA extracted from leaf tissues of three months old lemon balm plant by (a) CTAB; (b) TRIzol and (c) RNX-Plus.

Table 1: Quality of total RNA isolated from three months old leaves of lemon balm based on three different protocols

Method	$A_{260}/_{280}$ value	$A_{260}/_{230}$ value
CTAB	1.96	1.80
TRIzol	1.52	0.54
RNX-Plus	1.67	0.38

(Hunter & Reid, 1999; Martínez-Fuentes et al., 2015). The currently commercial kits such as RNeasy, BioZOL and BIOLINE are simple, rapid, non-toxic with good yields of high-quality RNA from plant tissues, but too expensive. Moreover, successful RNA extraction by RNX- Plus and TRIzol from lemon balm plant has only been reported in seedling stage and explants grown in solid MS medium, respectively (Kim et al., 2011; Nasiri-Bezenjani et al., 2014). Upon this status, we tested three different methods (modified CTAB, TRIzol and RNX- Plus) to evaluate the best and effective RNA extraction method from 3 months old lemon balm plant.

In current study, evaluation of quality and quantity of total RNA isolated from three different protocols was done by agarose denatured gel (Figure 1a) and nanodrop (Table 1), respectively. The CTAB method already showed a much better RNA quality than TRIzol and RNX-Plus methods by only visualizing the pellet. The RNA pellet appeared in white color in CTAB method while TRIzol and RNX-Plus methods produced brown colored pellet (Figure 1b). The brown color indicates the presence of carbohydrates, proteins and phenolic compounds mixed with the extracted RNA (Rubio-Piña & Zapata-Pérez, 2011). In the CTAB method, brown color and contamination (phenolic compounds) were reduced by addition of PVP and high concentrations of β-mercaptoetanol in the extraction buffer. The PVP strongly binds to the polyphenol compounds through hydrogen bonds, and removes phenolic compounds and secondary metabolites from nucleic acid, also preventing browning effect of polyphenols (Rubio-Piña & Zapata-Pérez, 2011; Shu et al., 2014). The strong reductant β-mercaptoetanol is conventionally used at 10 % (v/v) to inhibit RNase activity and prevent any possible oxidation reactions (Ouyang et al., 2014). On the other hand, chloroform: isoamyl alcohol is used to eliminate remaining phenolic compounds and PVP from solution (Shu et al., 2014; Martínez-Fuentes et al., 2015). Total RNA isolated by modified CTAB and RNX- Plus method showed two bright bands (28S rRNA and 18S rRNA). However, the gel electrophoresis result of TRIzol method revealed no production of clear and bright RNA band, indicating low quantity of RNA.

The $A_{260}/_{230}$ ratio detected below 0.6 in both TRIzol and RNX-Plus methods (Table 1), representing high contamination with phenolic compounds or polysaccharides and very low molecular grade of RNA (Martínez-Fuentes et al., 2015). It is important to note that RNA solution with $A_{260}/_{280}$ and $A_{260}/_{230}$ ratios of 1.8~2.0 corresponds to a high quality (Hou et al., 2011; Martínez-Fuentes et al., 2015). However, CTAB based method produced highly purified RNA with $A_{260}/_{280}$ and $A_{260}/_{230}$ ratios of 1.96 and 1.80, respectively (Table 1).

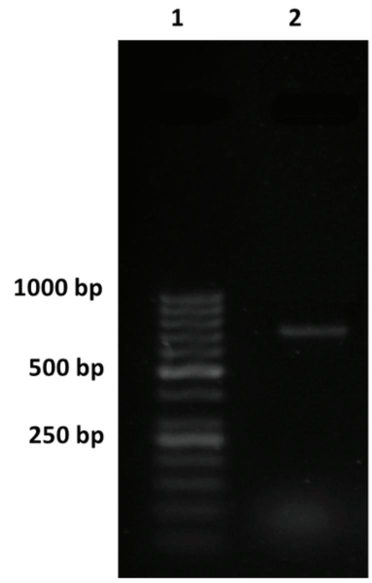

Figure 2: RT- PCR product with tubulin specific primers using total RNA isolated from leaves of three months old lemon balm plant based an CTAB method. Lane 1: 50 bp DNA ladder (Thermo Fisher Scientific), Lane 2: 750 bp amplified Tubulin gene.

3.2 RT-PCR

RNA sample extracted by the improved CTAB method, was further characterized with RT-PCR using tubulin primers. The results showed amplification of an expected fragment of approximately 750 bp (Figure 2), suggesting high quality of extracted RNA at the molecular level. Due to RNA contamination, reverse transcriptase enzyme will fail to produce cDNA, which consequently, RT-PCR could result in failure to amplify the target sequence (Sabzevari & Hosseini, 2014). Thus, the successful synthesis of the first-strand cDNA and PCR amplification (Figure 2) clearly shows that the RNA is free of contaminants.

4 CONCLUSION

Our results demonstrated that, modified CTAB protocol extracted highly qualified RNA from lemon balm old leaves compared to other tested methods. The method is cost effective and may be simply used as an alternative method for RNA isolation from recalcitrant plant tissues.

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Higher yielding varieties of common buckwheat (*Fagopyrum esculentum* Moench) with determinate growth habit (single mutation *det*) manifest higher photosynthesis rate at stage of grain filling

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Higher yielding varieties of common buckwheat (*Fagopyrum esculentum* Moench) with determinate growth habit (single mutation *det*) manifest higher photosynthesis rate at stage of grain filling

Abstract: Comparison of common buckwheat varieties with determinate vs. indeterminate growth habit reveals no differences in leaf photosynthesis rate at stage before flowering. However, at stage of seed filling the difference was significant. Maximal difference was 20 days after early flowering, i.e in period of most intensive seed formation. These results show that determinate varieties have higher sink strength providing by developing seeds. It is correlated with higher yield ability of such varieties. Probably, growth limitation resulting from *det*-mutation leads to some shifts in system of sink priorities of buckwheat plant and allows initiate the development of additional seeds. One more possible cause of alteration of the physiological parameters in determinate varieties is some optimization of plant structure: in terms of physiology the determinate buckwheat is a plant which is more similar to cereals than indeterminate buckwheat. However, underlying physiological changes accompanying the transition from indeterminate toward determinate growth in buckwheat remain almost unknown. Assumption about strong effect of *det*-mutation *per se* on photosynthesis rate was not supported in our work. Alternative assumption about accumulation of additional genes enhancing the sink ability suggests opportunities for additional progress in the selection work using tools evaluating photosynthesis intensity at stage of grain filling.

Key words: *Fagopyrum esculentum*; buckwheat; photosynthesis rate; sink strength; growth habit

Bolj donosne sorte navadne ajde (*Fagopyrum esculentum* Moench) z determinantno rastjo (enojna *det* mutacija) imajo večjo fotosintezo v fazi polnjenja zrnja

Izvleček: Primerjava sort navadne ajde z determinantno in nedeterminatno rastjo ne kaže razlik v fotosintezi listov v fazi pred cvetenjem, vendar je razlika v fazi polnjenja zrnja značilna. Največja razlika je bila20 dni po začetku cvetenja, to je v fazi najbolj intezivnega oblikovanja semen. Ti izsledki kažejo, da imajo determinantne sorte večjo moč ponora, ki jo dajejo razvijajoča se semena. To je povezano tudi s sposobnostjo večjega pridelka teh sort. Verjetno je omejitev rasti posledica *det*-mutacije, kar vodi v nekatere premike v sistemu prioritet ponora v rastlinah ajde in, kar vzpodbudi razvoj dodatnih semen. Nadaljni možni vzrok v spremembi fizioloških parametrov determinatnih sort je v optimizaciji zgradbe rastline, determinatna ajda je v fiziološkem pogledu bolj podobna žitom kot pa nedeterminatni ajdi. Kljub vsemu, pa ostajajo fiziološke spremembe, ki spremljajo prehod od nedeterminantne k determinatni rasti skoraj popolnoma neznane. Domneva o močnemu čimku *det*-mutacije *per se* na fotosintezo v našem delu ni bila potrjena. Alternativna domneva o kopičenju dodatnih genov, ki pospešujejo sposobnost ponora daje priložnosti za nadaljni napredek pri selekcijskem delu z uporabo ovrednotenja jakosti fotosinteze kot orodja v fazi polnjenja zrnja.

Ključne besede: *Fagopyrum esculentum*; navadna ajda; velikost fotosinteze; moč ponora; rastna oblika

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1 INTRODUCTION

Fagopyrum esculentum Moench (common buckwheat) is a species cultivated as groats or grain crop in many countries, mainly in Russia and China (Wang & Campbell, 2004; Fesenko et al., 2016). During a last half of century, crop evolution of common buckwheat have resulted the increasing of the species grain productivity. Physiological basis of the result is mainly unknown. Probably, it was associated with correction of source-sink relationships.

In Russia, breeding of this crop on scientific basis was started in 1900s on Shatilov's Experimental Station (Orel region, Russia). First Russian commercial variety, Bogatyr, was bred by selection of heavier (i.e. larger and better filled) fraction of grain from cultivated local buckwheat. Beginning from 1960s buckwheat breeding in Russia is based on application of several morphological mutations (Fesenko, 1983; Fesenko et al., 2006). Agricultural practice has chosen mainly determinate varieties based on a mutation *d* (*det*) (Fesenko, 1968; Ohnishi, 1990) that limits the generative development of shoots by 3-5 inflorescences without possibility for development of any additional ones (Fesenko, 1983; Fesenko et al., 2009)(Fig. 1). First variety of this type was registered in 1985. Since the beginning of 21st century the share of the determinate varieties in the buckwheat sowing area in Russia was increased from 8.2 % to 56.2 %, which led to an increase in average buckwheat yield by 1.5 times (FAO, 2014). Breeding and research work with determinate type buckwheat was conducted also in Slovenia (Bohanec & Kreft, 1981; Luthar et al., 1986; Kreft, 1989), Serbia (Nešković et al., 1990) and Japan (Kasajima et al., 2016).

The steady increase in productivity of determinate varieties compared to traditional ones implies, among others, changes in their physiology, which probably include correcting some processes associated with regulation of photosynthesis. Although the results of experiments evaluating correlation between photosynthesis rate and plant productivity are not always unambiguous (Peng et al., 1991; Long et al., 2006; Driever et al., 2014), the intensification of the assimilate synthesis looks as one of major factors in plant productivity growth.

Since changes in the intensity of photosynthesis are one of the supposed reasons for a higher grain productivity of buckwheat with determinate growth, we compared the common buckwheat varieties of indeterminate, i.e. traditional, and determinate types on seasonal dynamics of the photosynthesis rate. Also, we evaluated the influence of *det*-mutation itself on photosynthesis rate in buckwheat. The aim of this article was to describe results of this work and to discuss it.

2 MATERIAL AND METHODS

2.1 PLANT MATERIAL

Two local cultivars from Orel region represented by accessions k-406 and k-1709 from collection of Vavilov's Institute of Plant Industry, St.-Petersburg; three varieties of traditional type with indeterminate growth habit (genotype *DET*/*DET*) Bogatyr (registered in 1938), Kalininskaya (1954) and Shatilovskaya 5 (1967); four varieties with determinate growth (genotype *det*/*det*) Demetra (1995), Dozhdik (1998), Dikul (1999) and Devyatka (2004). All the varieties are similar in characteristics of vegetation period and manifest similar time of flowering beginning. F_2 hybrids 'Dikul

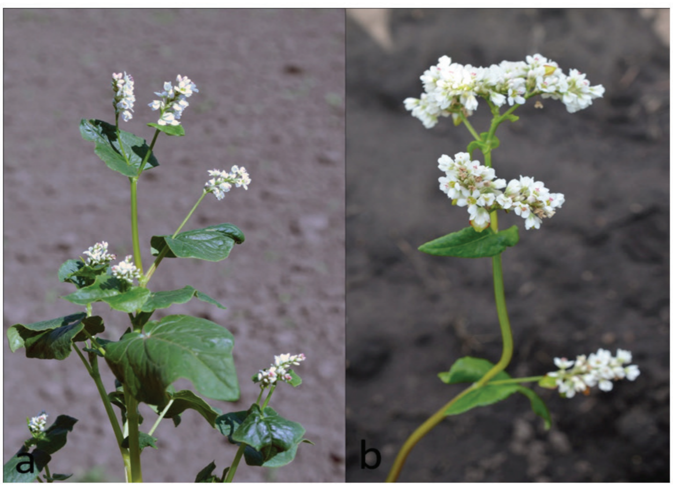

Figure 1: Shoots of a) determinate and b) indeterminate buckwheat shoots

Table 1: Some essential dates of the experiment

Year	Sowing date	Date of seedlings appearance	Varieties groups	*Dates of early flowering
2013	May 23	May 29	Indeterminate	June 19-20
			Determinate	June 19-22
2014	May 21	May 27	Indeterminate	June 15-19
			Determinate	June 18-20
2015	May 29	June 4	Indeterminate	June 28
			Determinate	June 28-29

* 10-15 % of plants have any opened flowers

Table 2: Weather conditions in days when photosynthesis rate was measured

		Air temperature, °C		Air humidity, %	
Year	Date	average	max	average	min
2013	June 13	19.5	24.4	61	41
	June 26	21.8	30.9	81	49
	July 6	23.9	31.1	64	34
	July 16	17.1	21.6	90	73
2014	July 3	21.8	26.5	39	22
	July 14	13.6	18.0	81	61
	July 24	13.6	19.2	74	49
2015	June 20	15.6	18.0	93	90
	July 6	21.2	27.1	60	42
	July 16	17.4	24.2	76	43
	July 26	26.3	33.8	57	37
2018	July 13	22.1	28.8	69	39

× Bogatyr' were used to evaluate the influence of *det*-mutation itself on photosynthesis intensity.

2.2 EXPERIMENTAL DESIGN

The experiment was conducted in 2013-2015 in crop rotation of buckwheat breeding laboratory of the All-Russia Research Institute of Grain Legumes and Groats Crops, Orel, Russia. A plot area was 10m^2. The plots locations were random, with fourfold replication. Sowing rate was 300 seeds per square meter. Dates of sowing and early flowering are presented in Table 1.

The photosynthesis intensity was evaluated on intact plants in real-time regime with a portable gas analyzer Li–COR – 6400 using the original methodology of the company Li–COR. The evaluations were conducted three times in 2014 and four times in both 2013 and 2015 at different developmental stages (see Results). Fifteen plants of every determinate variety and twelve plants of every indeterminate one were analyzed every time of the experiment (60 plants of every type in sum). The measurements were made in order "indeterminate - determinate - indeterminate - etc" with alteration every five plants.

Yield data were obtained by weighting of the grain yield from each plot. All these parameters were used to compare the two groups of varieties, i.e. indeterminate (traditional) vs. determinate. Significance of the differences between the groups was evaluated using t-statistics.

2.3 WEATHER CONDITIONS

Weather conditions deviate during experiments, but in permissible range (Table 2). In addition, gas exchange was evaluated in morning time, 9AM – 11AM, when conditions were maximally suitable. They did not notably influence the results of gas exchange evaluation. For example, photosynthesis rate was not decreased in 2014 July 14, the coldest day of the work, in comparison with results at this developmental stage in two other years.

3 RESULTS

3.1 GRAIN PRODUCTIVITY OF VARIETIES IN THE EXPERIMENT

In this experiment the determinate varieties manifest higher grain productivity than the indeterminate varieties, on average; the difference was significant in all years of the study (Table 3).

3.2 PHOTOSYNTHESIS RATE AT DIFFERENT DEVELOPMENTAL STAGES

At stage before flowering the photosynthesis rate was measured only in 2013 and 2015. In a season scale, in 2013 this stage values of photosynthesis rate were maximal, but in 2015 – minimal. Comparison between 2013 and 2015 reveals at least twice difference (Table 4), but comparison between indeterminate and determinate varieties in every year reveals no any differences.

During period of flowering, i.e. 10, 20 and 30 days followed to early flowering, the measuring of photosynthesis rate was conducted in 2013, 2014 and 2015. Determinate varieties manifest significantly higher mean values of CO_2 exchange in all cases with an exception of 10 days after early flowering in 2014 (Table 4). In all years of the experiment the maximal differences between determinate and indeterminate varieties were at stage of 20 days after early flowering.

Maximal values of photosynthesis rate among determinate and indeterminate varieties sometimes were almost identical, and sometimes were even higher for indeterminants. Therefore, really, the possible maximal CO_2 exchange at level of individual plant of determinate varieties is not always higher, but CO_2 exchange at population level is always sufficiently more consistent at stage of seed filling. It correlates with the sufficiently higher and more consistent grain productivity of determinate varieties in Russia.

Table 3: Grain yield (t ha^{-1}) of varieties with indeterminate and determinate growth habits

Year	Varieties group	X±m	t	P
2013	Indeterminate	1.02±0.12		
	Determinate	1.38±0.11	2.21	0.05
2014	Indeterminate	1.49±0.06		
	Determinate	1.97±0.07	5.21	0.001
2015	Indeterminate	1.25±0.09		
	Determinate	1.52±0.08	2.24	0.05

3.3 THE *DET*-MUTATION *PER SE* DOES NOT AFFECT THE PHOTOSYNTHESIS RATE

The differences between varieties with indeterminate and determinate growth habits in the photosynthesis rate at stage of grain filling may be due to either the effect of the *det*-allele *per se* or the accumulation of additional genes affecting the intensity of gas exchange. We analyzed F_2 hybrids between indeterminate (Bogatyr) and determinate (Dikul) varieties. As expected, all F_1 hybrids were indeterminate; F_2 segregation was Mendelian, 182 indeterminate : 67 determinate ($\chi^2 = 0.48$; $p = 0.49$). For the test 55 plants of each type were selected and labeled. Measurements were made alternately: one determinate plant, one indeterminate plant etc. The experiment shown no differences in photosynthesis rate between the indeterminate and determinate groups of F_2 hybrids: photosynthesis rate was 10.89 ± 0.51 with range 1.32 - 18.22 for indeterminate sample and 10.07 ± 0.57 with range 1.00 - 20.12 for determinate sample from F_2 population ($t = 1.07$; $p > 0.1$). Therefore, the advantages of determinant varieties in photosynthesis rate are not directly conditioned by *det*-allele. Obviously, some other genes were accumulated which increase the photosynthesis rate at stage of grain filling. This indicates the possibility of selection for the intensity of photosynthesis.

4 DISCUSSION

Photosynthesis rate is regulated by sink strength (assimilate demand) and source strength (assimilate supply) (King et al., 1967; Marcelis et al., 2004; Wubs et al., 2009; Borrill et al., 2015; Zhang et al., 2015; White et al., 2016). Obviously, there are limitations for the photosynthesis intensity, which are various between crops. So, maximal values of CO_2 assimilation was 42.5 μmol m^{-2} s^{-1} for sorghum (Salas-Fernandes et al., 2015) and 30-33 μmol m^{-2} s^{-1} for a high-yielding *indica* cultivar of rice (Adachi et al., 2014). Sometimes, photosynthesis rate is restricted by CO_2 concentration in air: several studies on rice revealed polymorphism for reaction on increasing of CO_2 concentration in air that was interpreted as differences in sink ability of filling grain between different varieties (Chen et al., 2014; Zhu et al., 2014).

However, photosynthetic apparatus usually does not work at full capacity. So, reported maximum indi-

Table 4: Leaf photosynthesis rate (μmol m^{-2} s^{-1}) of buckwheat plants at different stage of their life cycle in field conditions

			Indeterminate varieties	Determinate varieties	t	P
Year	Developmental stage	Date	X ± m (range)	X ±m (range)		
2013	Before flowering	June 13	14.04 ± 0.75 (8.54 – 21.85)	14.78 ± 1.05 (8.45 – 21.97)	0.57	-
	10 days after early flowering	June 26	9.59 ± 0.62 (4.14 – 17.30)	11.84 ± 0.64 (5.77 – 16.25)	2.53	0.02
	20 days after early flowering	July 6	9.03 ± 0.80 (4.25 – 15.69)	14.68 ± 0.51 (10.75 – 18.60)	5.96	0.001
	30 days after early flowering	July 16	9.54 ± 0.47 (5.32 – 12.98)	12.08 ± 0.43 (8.43 – 15.47)	3.96	0.001
2014	10 days after early flowering	July 3	10.24 ± 0.60 (6.23 – 17.30)	11.04 ± 0.51 (7.98 – 14.67)	1.02	-
	20 days after efflores-cence	July 14	11.76 ± 0.62 (5.77 – 17.62)	13.56 ± 0.57 (8.81 – 17.54)	2.14	0.05
	30 days after early flowering	July 24	8.55 ± 0.27 (6.05 – 11.99)	9.63 ± 0.46 (6.44 – 14.00)	2.02	0.05
2015	Before flowering	June 20	6.50 ± 0.09 (5.87 – 6.96)	6.24 ± 0.13 (5.58 – 6.78)	1.64	-
	10 days after early flowering	July 6	11.44 ± 0.19 (10.48 – 12.96)	12.57 ± 0.27 (11.21 – 14.28)	3.42	0.001
	20 days after early flowering	July 16	12.19 ± 0.18 (11.14 – 13.10)	14.07 ± 0.17 (13.33 – 14.70)	7.59	0.001
	30 days after early flowering	July 26	8.27 ± 0.19 (6.97 – 9.30)	9.38 ± 0.15 (8.32 – 10.17)	4.59	0.001

vidual leaf net CO_2 assimilation rates for *V. vinifera* L. and other *Vitis* species approach 20 μmol m^{-2} s^{-1}(Roper & Williams, 1989; Gamon & Pearcy, 1990). But more commonly reported maximum rates fall in the range of 8 to 13 μmol CO_2 m^{-2} s^{-1} (Downton et al., 1987; Correia et al., 1990). On *Eucalyptus globulus* Labill. excision of several leaves causes increased photosynthesis in the remained leaves (Eyles et al., 2013). In addition, the increased assimilate demand also enhances photosynthesis (Aranjuelo et al., 2013). Finally, total sink strength can be increased as result of interactions with other organisms: for example, soybean plants inoculated with two different strains of *Bradyrhizobium japonicum* (Kirchner,1896) Jordan, 1982 had 14–31 % higher rates of photosynthesis than N-fertilized plants (Kaschuk et al., 2012).

Different groups of buckwheat varieties were not different in both sink and source strength at stage of vegetative development. However, at stage of seed filling the significant differences in photosynthesis rate were revealed between varieties with determinate vs. indeterminate growth habits. At present time the varieties with determinate growth habit cover more than a half of sowing area under buckwheat in Russia. Earlier, the higher productivity of such varieties was interpreted only in terms of shift in balance between competitive sinks, i.e. vegetative growth and seed development. Our work elucidates that grain filling in buckwheat is limited by itself sink capacity rather than source capacity of leaves and competitive interactions with other growing organs.

All buckwheat varieties produce redundant number of flowers. Obviously, not all of the flowers produce seeds. It was discussed that there is often a hierarchy among sinks (Wardlaw, 1990), i.e. some organs have priority and suffer less from a reduction in assimilate supply than other organs. Such hierarchy usually is resulted from evolution of certain strategy of a species adaptation (Wardlaw, 1990). Since main adaptive property of common buckwheat is ability to continuous intensive growth, primary sinks in buckwheat plant are shoot meristems; seed production is only secondary sink (Fesenko, 1983). It explains the very little increasing of buckwheat seed productivity due to selection of most vigorous and productive plants. Progeny of such plants also had vigorous growth (maybe more vigorous than parental population), but competition between plants in the canopy was also very strong, and seed production was poor: only few plants produce sufficient number of seeds, and productivity of whole canopy remains low (Fesenko et al., 2006).

Determinate varieties manifest higher and more consistent yield obviously due to set several additional seeds per plant in comparison to indeterminate varieties. Setting the additional seeds on determinate plants could be explained by essentially reduced competition from vegetative growth at time of seed formation in comparison to indeterminate ones. However, this hypothesis does not explain why seed filling together with indeterminate growth in varieties of traditional type drive less sink strength than seed filling together with reduced vegetative growth in varieties of determinate type. Besides, it does not answer a question, why indeterminate varieties do not set additionally seeds with possible following growth of photosynthesis rate?

Probably, growth limitation resulting from *det*-mutation leads to some shifts in the priorities and allows initiate the development of additional seeds. One more possible base of the alteration of physiological and grain yield parameters in determinate varieties is some optimization of plant structure: determinate buckwheat is a plant, which is more similar with cereals than indeterminate buckwheat (*det*-mutation is a first step of buckwheat to became "cereal" in terms of physiology). However, underlying physiological changes accompanying the transition from indeterminate toward determinate growth in buckwheat remain almost unknown.

Attempting to determine any genes influencing photosynthesis rate led to discovering QTLs affecting, for example, chlorophyll content, stomatal resistance, transpiration rate (Teng et al., 2004; Wang et al., 2015), mesophyll conductance, and root surface area determining hydraulic conductance (Adachi et al., 2014). A mutation of rice *erect panicle 3* (*ep3*) decreases photosynthesis due to reducing stomatal conductance (Yu et al., 2015).

Assumption about strong effect on photosynthesis rate of *det*-mutation *per se* was not supported in our work. Possible alternative explanation for higher photosynthesis rate together with higher seed productivity of the determinate varieties is accumulation of some additional genes enhancing the sink ability of filling seeds. The mechanisms of functioning of these genes are currently unknown. However, such assumption suggests opportunities for additional progress in the selection work using tools evaluating photosynthesis intensity at stage of grain filling.

5 CONCLUSION

The present study revealed the buckwheat varieties with determinate growth habit (a mutation *det*) manifested higher photosynthesis rate at stage of grain filling compared to varieties with indeterminate growth habit. The mutation *det* itself is not determining the difference. Perhaps, some other genes increasing photosynthesis rate at stage of grain filling, i.e. sink strength of developing seeds pool, were accumulated in determinate varieties. Probably, there are some possibilities to continue the selection for photosynthesis rate in buckwheat.

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Winter wheat growing in Ukraine: ecological assessment of technologies by the influence on soil fertility

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Winter wheat growing in Ukraine: ecological assessment of technologies by the influence on soil fertility

Abstract: Modern technologies of winter wheat growing need to be improved taking into account the results of ecological evaluation of their impact on soil fertility indices. We aimed to assess the technologies of winter wheat growing in different soil and climatic conditions of Ukraine by their influence on soil fertility. It is known that in order to estimate ecological safety of crop growing technologies, it is advisable to use a method based on identifying negative impacts on soil fertility. We propose the group of deviation values from the optimum as follows: (i) strong, which leads to an unsatisfactory ecological condition (> 50 %), (ii) average that provides a satisfactory state (> 25 %, but < 50 %), (iii) moderate, which provides a normal state (≤ 10 %, but < 25 %), (iv) absent, an optimal condition is provided (< 10 %).

It is revealed that technologies of winter wheat growing in the conditions of Polissya, Forest-steppe and Steppe Zones of Ukraine can have a negative influence on potassium regime in soils, the influence by intensity can vary from moderate to strong. In Polissya and Forest-steppe, winter wheat growing can lead to deterioration of soil pH status. In Steppe, along with the potassium regime, the technologies can negatively influence soil nitrogen status and the effect may be characterized as strong.

Key words: ecological assessment; growing technology; limiting factors; soil fertility

Pridelava ozimne pšenice v Ukrajini: ekološka ocena tehnologij glede na vplive na rodovitnost tal

Izvleček: Moderne tehnologije pridelave ozimne pšenice je potrebno izboljšati glede na ekološko ovrednotenje njihovega vpliva na indekse rodovitnosti tal. Namen raziskave je bil oceniti tehnologije pridelave ozimne pšenice na različnih tleh in klimatskih razmerah Ukrajine po njihovem vplivu na rodovitnost tal. Ugotovljeno je bilo, da je za oceno ekološke varnosti pridelave poljščin priporočljivo uporabiti metode, ki temeljijo na prepoznavanju negativnih učinkov na rodovitnost tal. Predlagamo naslednje skupine glede na odstopanja od optimalnega stanja: (i) močno odstopanje, ki vodi v nezadovoljive ekološke razmere (> 50 %), (ii) povprečno odstopanje, ki daje zadovoljivo stanje (> 25 %, a manj kot 50 %), (iii) zmerno odstopanje, ki daje normalno stanje (≤ 10 %, a manj kot 25 %), (iv) odstopanja ni, optimalno stanje (< 10 %).

Ugotovljeno je bilo, da lahko imajo tehnologije pridelave ozimne pšenice v razmerah con polesja (Polissya), lesostepe (Forest-steppe) in stepe (Steppe) v Ukrajini negativni vpliv na režim kalija v tleh, jakost vpliva se spreminja od zmerne do močne. V polesju in lesostepi lahko pridelava ozimne pšenice vodi do poslapšanja pH tal. V stepi lahko tehnologije ob vplivu na režim kalija še močno negativno vplivajo na status dušika v tleh.

Ključne besede: ekološko ovrednotenje; tehnologija pridelave; omejujoči dejavniki; rodovitnost tal

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1 INTRODUCTION

At the International Congress on Environmental Issues in Agriculture (Lefebvre et al., 2005) where the most important issues of modern agrotechnologies were discussed, it was stated that their ecological safety is one of the main requirements nowadays. It has been shown by researches (Davidson, 2000; Arshad and Martin, 2002; Ţenu et al., 2009; Killebrew and Wolff, 2010; Makarenko and Bondar, 2012) that it is impossible to identify environmental risks and to develop technologies that would meet the modern environmental standards without studying the mechanisms of negative influence of agrotechnical methods of crops growing on ecosystem components (Karlen et al., 2003; Killebrew and Wolff, 2010; Makarenko and Bondar, 2013). Winter wheat - is one of the leading crops for Ukraine. This crop is grown on an area of 6.4 million hectares and occupies about 37-40 % in the structure of crops (according to the Ministry of Agrarian Policy of Ukraine, 2018).

In spite of its role as a major food in Eastern Europe (Petrenko et al., 2017) wheat is growing by technologies that causes environmentally negative impact. Thus, soil tillage, application of pesticides, mineral fertilizers, growth regulators, which can lead to violation of natural processes in the soil system and to soil degradation (Puskás and Farsang, 2009; Killebrew and Wolff, 2010; Mueller et al., 2012).

Baliuk and Medvediev pointed out that soil degradation is a result of maintaining old technologies in Ukrainian agriculture (Baliuk et al., 2012; Ukraine: Soil fertility to strengthen climate resilience. Preliminary assessment of the potential benefits of conservation agriculture. 2014). Researches stated that pesticides application leads to soil and terrestrial ecosystems missfunction (Wasim et al., 2009). Application of agrochemicals violating the optimal doses, with expired dates, inappropriate proportions of nutrient elements will - decrease the effective and potential soil fertility, pollute natural water resources by toxicants and reduce the quality of agricultural products (Shang et al., 2019). Taking into account that the vast majority of agrochemicals are recycled products of industrial wastes. For instance, low enriched agro-ores have a high probability of impact on living organisms and ecosystems due to the presence of impurities of heavy metals, radionuclides, organic and inorganic substances (Hazrat et al., 2019). Therefore, the study of the influence of wheat growing technologies on the soil conditions of agroecosystems is of high importance from scientific and practical points of view.

The main purpose of the study was to identify (1) the negative processes in the soils of agroecosystems of different climatic zones of Ukraine for winter wheat cultivation; (2) the implementation of environmental regulation of the effects of winter wheat cultivation technology on the soils in agroecosystems; (3) application of the obtained results for the development of recommendations for winter wheat cultivation in the conditions of Ukraine.

2 MATERIALS AND METHODS

2.1 SITE DESCRIPTION

The research was carried out within the framework of scientific and technical program of National Acade-

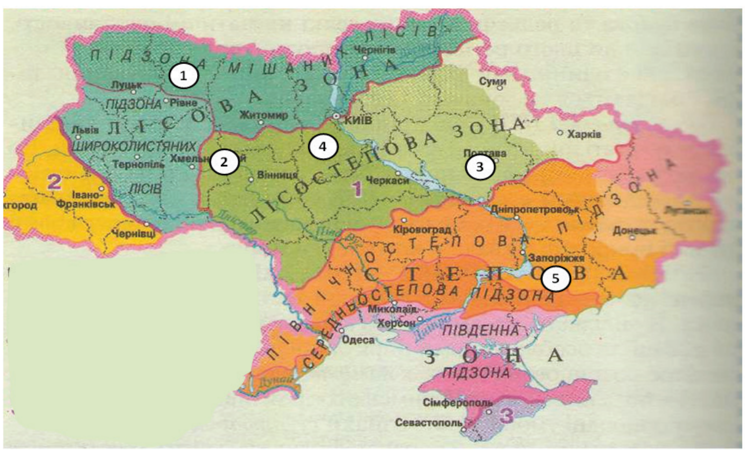

Figure 1: Mapping scheme of long-term field experiments location in various soil-climatic zones of Ukraine: 1 - Rivne SARS, 2 - Khmelnytskyi SARS, 3 - Poltava Institute AIP named after M. Vavilov, 4 - SS NULES of Ukraine "Agronomic Research Station", 5 - Zaporizhzhya RS IOS NAAS.

Table 1: Characteristic of technologies of winter wheat growing in different soil-climatic zones of Ukraine

Parameter	Characteristic of the parameter
Poltava Institute AIP named after M. Vavilov	
Soil type	chernozem typical low-humus heavy loamy
Soil tillage	surface tillage, plowing, pre-sowing cultivation
Variety / biological yield Planting rate / method Preceding crop	Kosoch / 5.93 t ha^{-1} 200 kg ha^{-1}/ conventional sowing pea
Fertilization system	organic fertilizers (manure 10 t ha^{-1}) and mineral fertilizers $N_{52}P_{52}K_{52}$
Protection system	recommended for this soil-climatic zone
Khmelnytskyi SARS	
Soil type	chernozem podzolized slightly eroded medium loamy with deep groundwater occurrence
Soil tillage	plowing, disking
Variety / biological yield Planting rate / method Preceding crop	Astet / 6.85 t ha^{-1} 135 kg ha^{-1} /conventional sowing pea
Fertilization system	organic fertilizers (manure 16 t ha^{-1} and 8 t ha^{-1}) and mineral fertilizers ($N_{116}P_{60}K_{120}$ and $N_{55}P_{30}K_{60}$)
Protection system	recommended for this soil-climatic zone
SS NULES of Ukraine "Agronomic Research Station"	
Soil type	chernozem typical low-humus medium loamy
Soil tillage	surface tillage, plowing, pre-sowing cultivation
Variety / biological yield Planting rate / method Preceding crop	Natsionalna / 6 t ha^{-1} 150 kg ha^{-1} /conventional sowing pea
Fertilization system	mineral fertilizers $N_{60}P_{60}K_{60}$
Protection system	recommended for this soil-climatic zone
Rivne SARS	
Soil type	dark grey podzolized
Soil tillage	surface tillage, plowing, pre-sowing cultivation
Variety / biological yield Planting rate / method Preceding crop	Poliska 90 / 6.5 t ha^{-1} 145 kg ha^{-1} / conventional sowing pea
Fertilization system	mineral fertilizers $N_{90}P_{60}K_{60}$, organic (manure 10 t ha^{-1}), organic-mineral (straw + green manure + $N_{90}P_{60}K_{60)}$
Protection system	recommended for this soil-climatic zone
Zaporizhzhya RS IOS NAAS	
Soil type	chernozem ordinary low-humus
Soil tillage	basic cultivation, plowing, pre-sowing cultivation
Variety / biological yield Planting rate / method Preceding crop	Dalnytska / 7 t ha^{-1} 200 kg ha^{-1} / conventional sowing black fallow
Fertilization system	mineral fertilizers in the following doses: $N_{75}P_{50}K_{25}$, $N_{50}P_{50}K_{50}$, $N_{15}P_{10}K_{5}$, organic fertilizers: semi-decomposed manure 5.7 t ha^{-1}
Protection system	recommended for this soil-climatic zone

my of Agrarian Sciences of Ukraine (NAAS) "Scientific and practical substantiation of sustainable development of agroecosystems in Ukraine", which envisaged integrated research of crops growing technologies in various soil and climatic zones of Ukraine. The following institutions were involved in this study: Institute of Agroecology and Environmental management of National Academy of Agrarian Sciences (NAAS, 48°18′N, 25°56′E); Rivne State Agricultural Research Station (Rivne SARS, 50°37′N, 26°15′E); Khmelnytskyi State Agricultural Research Station (Khmelnytskyi SARS, 49°25′N, 27°0′E); Poltava Institute of agro-industrial production (Poltava Institute AIP, 49°35′N, 34°34′E) named after M. Vavilov; Zaporizhzhya Agricultural Research Station of the Institute of Oilseeds (Zaporizhzhya RS IOS NAAS, 47°50′N, 35°10′E); Separated subdivision of National University of Life and Environmental Sciences of Ukraine (SS NULES of Ukraine, 50°10′N, 30°19′E) "Agronomic Research Station". The research was conducted during 2007 - 2018 years in the conditions of long-term field experiments (established more than 20 years ago) located in Polissya, Forest-Steppe and Steppe regions of Ukraine (Figure 1).

2.2 METHODOLOGY OF TECHNOLOGIES ASSESSMENT

The basic technology (BT) of winter wheat growing which envisaged soil tillage depending on its type, selection of regional variety, seed rates and method of sowing, system of plant protection. In addition, technologies with application of mineral and organic fertilizers in various combinations were studied, taking into account soil-climatic conditions of the of the experiments location (Table 1).

The basic agrochemical parameters of the soil were determined according to standardized methods: pH_{KCl} - by potentiometric method (displacement of exchange ions H^+ and Al^{3+} 1N KCl (pH = 5.56) ratio of soil to solution was 1 : 2.5), nitrogen content that is easily hydrolysed - by Cornflind (the method is based on alkaline hydrolysis of organic nitrogen-containing compounds of soil under the action of 1N NaOH for two days at 260 °C. The released ammonia is absorbed by boric acid and is determined by titration with sulfuric acid), content of exchangeable potassium and labile phosphorus - according to the modified Machigin method (the method is based on the removal of mobile compounds of phosphorus and potassium from the soil with a 1 % $((NH_4)_2\ CO_3)$, (pH 9.0) at the soil:solution ratio 1 : 20 and a temperature of 25 ± 2 ° C. The soil suspension was shaken for - 5 minutes and than, insulating the soil in solution - 20 hours. Before determining the phosphorus, the colored soil extraction was discolored with activated carbon. Phosphorus was determined calorimetrically after ammonium molibdate addition. Potassium was determined by flame photometry. Soil organic matter (SOM) was determined - by Tyurin method (the method is based on the oxidation of organic carbon to CO_2 by a solution of potassium bichromate and sulfuric acid, the excess of which is titrated with Mora salt).

2.3 STATISTICAL ANALYSIS

Data are presented as mean values of four replicates and standard deviations. The significance of the experimental data was estimated by the analysis of variance (two-factor ANOVA) following calculation of the least significant difference LSD_{05}.

3 RESULTS AND DISCUSSION

Following the concept of limiting factors, a method for evaluating the technology was proposed the intensity of its negative influence on soil fertility indices:

strong influence, which leads to an unsatisfactory ecological state of components of agroecosystems or individual processes that occur in them (the deviation from the optimum in the direction of deterioration is more than 50 %);

medium influence that provides a satisfactory state of components of agroecosystems or individual processes that occur in them (the deviation from the optimum in the direction of deterioration is greater than 25 % but does not exceed 50 %);

moderate influence, which ensures a normal state of components of agroecosystems or individual processes that occur in them (the deviation from optimum in the direction of deterioration more than 10 % but not exceeding 25 %);

no influence, which ensures an optimal state of components of agroecosystems or individual processes that occur in them (the deviation from the optimum in the direction of deterioration does not exceed 10 %) is provided.

The optimal soil parameters by fertility indices were determined according to DSTU 4362: 2004 "Soil quality. Soil fertility indices". The optimal parameters of fertility indexes are presented in Table 2.

According to the results of our research, the technologies of winter wheat growing in different soil and climatic conditions of Ukraine influenced the formation of edaphic soil parameters of the agroecosystem,

Table 2: The range of soil parameters and respective ecological state

Ecological state	Nitrogen that is easily hydrolyzed	Labile phosphorus	Exchangeable potassium	pH $_{KCl}$	SOM, %
	mg kg^{-1}				
chernozem typical low-humus heavy loamy					
Standard	35–45	45–60	300–400	6.3–7.0	4.5–5.7
Optimal	32–34	41–44	270–299	5.7–6.2	4.1–4.4
Normal	27–33	34–43	228–269	4.8–5.6	3.4–4.0
Satisfactory	18–26	23–33	150–227	3.2–4.7	2.3–3.3
Unsatisfactory	< 18	< 23	< 150	< 3.2	< 2.3
chernozem podzolized medium loamy slightly eroded					
Standard	35–45	150–200	120–170	5.7–6.4	2.8–4.2
Optimal	34–32	135–149	108–119	5.1–5.6	2.5–2.7
Normal	33–27	113–134	90–107	4.3–5.5	2.1–2.4
Satisfactory	18–26	75–112	59–89	2.9–4.4	1.3–2.0
Unsatisfactory	< 18	< 75	< 60	< 2.9	< 1.4
chernozem typical low-humus medium loamy					
Standard	35–45	45–60	300–400	6.0–6.8	3.5–5.0
Optimal	34–32	41–44	270–299	5.4–5.9	3.2–3.4
Normal	33–27	34–40	228–269	4.5–5.3	2.6–3.1
Satisfactory	18–26	22–33	150–227	3.0–4.4	1.8–2.5
Unsatisfactory	< 18	< 23	< 150	< 3.0	< 1.8
dark grey podzolized					
Standard	35–45	150–200	170–220	5.3–6.0	1.6–2.6
Optimal	34–32	135–149	153–169	4.8–5.2	1.4–1.5
Normal	33–27	113–134	128–152	4.0–4.7	1.2–1.3
Satisfactory	18–26	75–112	85–127	2.7–3.9	0.8–1.1
Unsatisfactory	< 18	< 75	< 85	< 2.7	< 0.8
chernozem ordinary low-humus					
Standard	35–45	45–60	300–400	6.8–7.6	3.2–5.3
Optimal	34–32	41–44	270–299	6.1–6.7	2.9–3.1
Normal	33–27	34–43	228–269	5.1–6.0	2.4–2.8
Satisfactory	18–26	23–33	150–227	3.4–5.0	1.6–2.3
Unsatisfactory	< 18	< 23	< 150	< 3.4	< 1.6

and, consequently, the conditions of plant nutrition. The soil fertility indicators by long-term influence of the winter wheat growing technologies are presented in Table 3.

A comparison of the soil actual parameters with the optimum level allowed to establish general patterns of changes occurring in the soil component of agroecosystems, and to identify the main negative effects of technologies (Table 4):

nitrogen regime: there was no negative influence of the technologies on the content of nitrogen forms in typical, podzolized and dark gray podzolized soils. The exception was chernozem, in which the technology led to the decrease of nitrogen content. The effect varied from medium to strong. Negative phenomenon was not eliminated even with nitrogen application at the rates N_{15} - N_{75} kg ha^{-1};

- phosphorus regime: the technologies provided optimal soil parameters by the content of phosphorus form in all soils. The exception was observed in chernozem podzolized, where the basic technology implementation led to the depletion of mobile phosphorus

Table 3: Indicators of soil fertility for different technologies of winter wheat growing

Technology	Indicators of soil fertility				
	Nitrogen that is easily hydrolyzed	Labile phosphorus	Exchangeable potassium	pH_{KCl}	SOM, %
	mg kg^{-1}				
chernozem typical low-humus heavy loamy					
BT	102 ± 1.70	78 ± 3.40	65 ± 1.63	4.8 ± 0.09	5.4 ± 0.16
BT + manure 10 t ha^{-1}	109 ± 2.49	92 ± 0.94	103 ± 1.41	5.0 ± 0.09	5.5 ± 0.22
BT + $N_{52}P_{52}K_{52}$ + straw +N_{10}	112 ± 1.25	132 ± 2.83	112 ± 0.82	5.5 ± 0.21	5.3 ± 0.05
BT + manure 10 t ha^{-1} + $N_{52}P_{52}K_{52}$	119 ± 0.94	170 ± 2.62	153 ± 3.09	5.2 ± 0.09	5.5 ± 0.05
$LSD_{0.05}$	12.11	3.37	6.67	0.18	0.21
chernozem podzolized medium loamy slightly eroded					
BT	101.5 ± 0.26	78 ± 0.47	72 ± 0.94	5.0 ± 0.05	3.1 ± 0.05
BT + $N_{116}P_{60}K_{120}$	119.0 ± 1.89	146 ± 0.94	106 ± 0.29	3.5 ± 0.03	5.0 ± 0.22
BT + manure 16 t ha^{-1}	105.6 ± 0.19	246 ± 2.83	235 ± 0.31	3.8 ± 0.09	6.3 ± 0.16
BT + manure 8 t ha^{-1} + $N_{55}P_{30}K_{60}$	103.3 ± 0.14	490 ± 0.47	160 ± 0.12	3.5 ± 0.05	6.4 ± 0.09
$LSD_{0.05}$	22.12	14.05	17.23	0.86	1.16
chernozem typical low-humus medium loamy					
BT	84.2 ± 0.08	67 ± 0.38	230 ± 0.45	7.7 ± 0.02	2.9 ± 0.05
BT + $N_{60}P_{60}K_{60}$	94.5 ± 0.37	62 ± 0.05	240 ± 0.37	7.7 ± 0.03	3.2 ± 0.09
$LSD_{0.05}$	4.67	1.16	8.04	0.14	0.57
dark grey podzolized					
BT	75.4 ± 0.05	165 ± 0.12	53 ± 0.16	4.9 ± 0.09	1.3 ± 0.02
BT + $N_{90}P_{60}K_{60}$	87.5 ± 0.16	245 ± 0.14	65 ± 0.21	5.2 ± 0.05	1.5 ± 0.04
BT + manure 10 t ha^{-1}	91.0 ± 0.17	295 ± 0.08	85 ± 0.19	5.1 ± 0.16	1.5 ± 0.05
BT + $N_{90}P_{60}K_{60}$ + straw + green manures	91.0 ± 0.16	340 ± 0.09	91±0.26	5.6 ± 0.16	1.4 ± 0.05
$LSD_{0.05}$	2.96	15.11	6.12	0.92	0.58
chernozem ordinary low-humus					
BT	9.8 ± 0.09	100 ± 0.41	150 ± 0.16	7.15 ± 0.16	3.9 ± 0.02
BT + $N_{75}P_{50}K_{25}$	15.6 ± 0.34	243 ± 0.17	183 ± 0.17	7.00 ± 0.05	4.1 ± 0.05
BT + $N_{50}P_{50}K_{50}$	22.3 ± 0.17	280 ± 0.19	205 ± 0.21	6.72 ± 0.09	3.8 ± 0.02
BT + $N_{15}P_{10}K_{5}$	14.7 ± 0.08	199 ± 0.47	159 ± 0.37	6.98 ± 0.16	4.1 ± 0.09
BT+ manure 5.7 t ha^{-1}	11.4 ± 0.19	144 ± 0.19	219 ± 0.19	7.07 ± 0.08	3.8 ± 0.05
$LSD_{0.05}$	3.82	22.14	20.10	3.57	2.63

level and the influence of technology was characterized as strong;

potassium regime: there was a negative influence of the technologies on the content of exchangeable forms of potassium in all soil types, it varied from moderate to strong. The most dangerous impact of the technologies was recorded in dark gray podzolized soils (strong);

soil pH: the influence of technologies within the limits of the moderate and average in chernozem typical low-humus heavy-loamy and chernozem podzolized; in dark gray podzolized soil, the negative influence was observed only in the basic technology;

SOM content: technologies did not lead to negative changes in the SOM. The obtained results indicate the inappropriateness of using SOM status indicator as a diagnostic tool for assessing the influence of technologies on soil fertility.

Thus, it was established that in Polissya, Forest-steppe and Steppe of Ukraine the technologies of win-

Table 4: Influence of the winter wheat growing technologies on soil fertility indicators

Technology	Negative influence of technology on indicators of soil condition				
	Nitrogen that is easily hydrolyzed	Labile phosphorus	Exchangeable potassium	pH $_{KCl}$	SOM, %
chernozem typical low-humus heavy loamy					
BT	absent	absent	strong	moderate	absent
BT + manure 10 t ha^{-1}	absent	absent	strong	moderate	absent
BT + $N_{52}P_{52}K_{52}$ + straw + N_{10}	absent	absent	strong	moderate	absent
BT + manure 10 t ha^{-1} + $N_{52}P_{52}K_{52}$	absent	absent	strong	moderate	absent
chernozem podzolized medium loamy slightly eroded					
BT	absent	strong	strong	moderate	absent
BT + $N_{116}P_{60}K_{120}$	absent	absent	moderate	medium	absent
BT + manure 16 t ha^{-1}	absent	absent	absent	medium	absent
BT + manure 8 t ha^{-1}+ $N_{55}P_{30}K_{60}$	absent	absent	absent	medium	absent
chernozem typical low-humus medium loamy					
BT	absent	absent	moderate	absent	moderate
BT + $N_{60}P_{60}K_{60}$	absent	absent	moderate	absent	absent
dark grey podzolized					
BT	absent	absent	strong	moderate	moderate
BT + $N_{90}P_{60}K_{60}$	absent	absent	strong	absent	absent
BT + manure 10 t ha^{-1}	absent	absent	strong	absent	absent
BT + $N_{90}P_{60}K_{60}$ + straw + green manures	absent	absent	strong	absent	absent
chernozem ordinary low-humus					
BT	strong	absent	medium	absent	moderate
BT + $N_{75}P_{50}K_{25}$	strong	absent	medium	absent	absent
BT + $N_{50}P_{50}K_{50}$	medium	absent	medium	absent	moderate
BT + $N_{15}P_{10}K_{5}$	strong	absent	medium	absent	absent
BT + manure 5.7 t ha^{-1}	strong	absent	medium	absent	moderate

ter wheat growing negatively affected potassium regime of soils: - there was a depletion of mobile potassium forms; in chernozem typical low-humus and chernozem podzolized medium-loamy winter wheat growing led to deterioration of the acid-base conditions of the soil - there were processes of acidification of the soil solution. In Steppe, along with potassium regime, the technology negatively affected nitrogen regime of the soil - there were observed processes of depletion of stocks of nitrogen compounds. It is possible to predict that the indicated negative phenomena in the soil led to a violation of the interconnections in the soil-plant system. To confirm this, the productivity of winter wheat cultivation under various technologies was investigated.

It is known that productivity, that is the ability of ecosystem to form biomass over a certain period of time on a certain area, is an integral indicator of its state. In agroecosystems, it was decided to determine productivity of a main agricultural crop (a determinant). In our case, the determinant was winter wheat and, according to the level of its productivity, an assessment of the impact of technology on the agroecosystem was carried out. Potential productivity of a certain wheat variety was taken as a standard (etalon) since this indicator indicates the maximum possible biological potential the crop can use for the formation of biomass.

At the same time, in case of reduced wheat productivity relative to the standard, the limiting edaphic soil factors, that could negatively affect plant growth and development, were determined. In Forest-steppe zone based on Poltava Institute AIP named after M. Vavilov winter wheat of the Kosoch variety was studied. The potential yield of this variety is 5.93 t ha^{-1}. Climate of Poltava region is continental. The mean annual temperature is +8.2 ^{o}C. The mean annual precipitation is 580-480 mm. The productivity of wheat at the po-

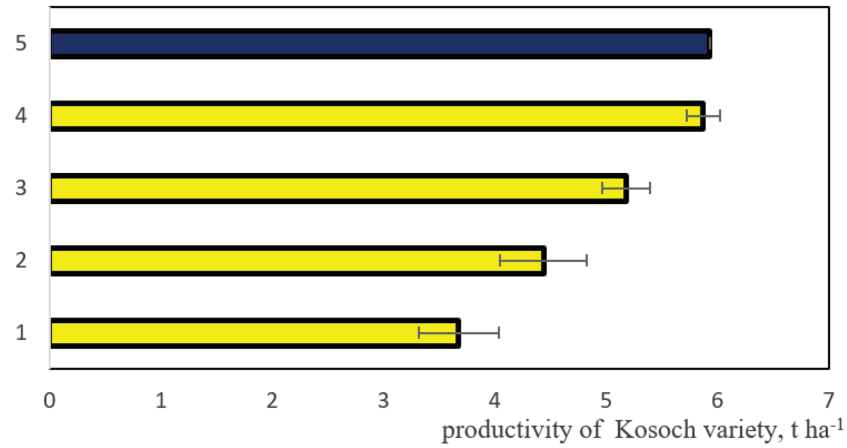

Figure 2: Influence of the technologies on productivity of winter wheat variety Kosoch (soil - chernozem typical low-humus heavy-loamy; 1. BT; 2. BT + manure 10 t ha^{-1}; 3. BT + $N_{52}P_{52}K_{52}$ + straw + N_{10}; 4. BT + manure 10 t ha^{-1} + $N_{52}P_{52}K_{52}$; 5. Standard)

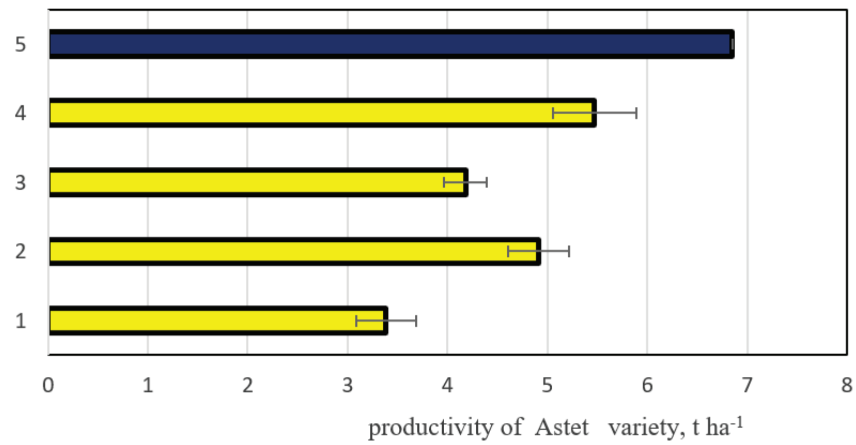

Figure 3: Influence of the technologies on productivity of winter wheat variety Astet (soil - chernozem podzolized medium loamy slightly eroded; 1. BT; 2. BT + $N_{116}P_{60}K_{120}$; 3. BT + manure 16 t ha^{-1}; 4. BT + manure 8 t ha^{-1} + $N_{55}P_{30}K_{60}$; 5. Standard)

tential level was ensured by the technology with integrated mineral and organic fertilizer application (BT + $N_{52}P_{52}K_{52}$ + manure of 10 t ha^{-1}). The basic technology, the technology with applied organic fertilizers did not allow achieving the standard level of crop productivity. The deviation from the standard was 26.4-35.5 %, that is, the negative impact of technology is estimated as medium (Figure 2). It can be assumed that one of the reasons for unsatisfactory growth and development of plants using these technologies was insufficient level of mobile potassium supply to plants. This effect was amplified by the discrepancy of the reaction of the soil environment with requirements of the crop.

In the conditions of Khmelnytskyi SARS (Forest-Steppe zone), winter wheat of the Astet variety was studied, whose potential yield is 6.85 t ha^{-1}. Climate of Khmelnytsky region is continental. The mean annual temperature is + 7.3°C. The mean annual precipitation is 530-670 mm. The basic technology, as well as the technologies of mineral fertilizers BT + $N_{116}P_{60}K_{120}$, BT + manure of 16 t ha^{-1} application did not provide the optimal conditions for plant growth and development. The productivity level of wheat was 27.3-52.8 % lower comparing the standard. The influence of the technologies on the state of agroecosystem by the level of wheat productivity can be estimated as strong and average (Figure 3). One of the reasons for this phenomenon could be the depletion of the mobile forms of soil phosphorus, potassium and the discrepancy of soil pH to requirements of the crop.

In the conditions of SS NULES of Ukraine "Agronomic Research Station" (Forest-Steppe zone) winter wheat of the Natsionalna variety was studied, the potential yield of which is 6 t ha^{-1}. Climate of Kyiv re-

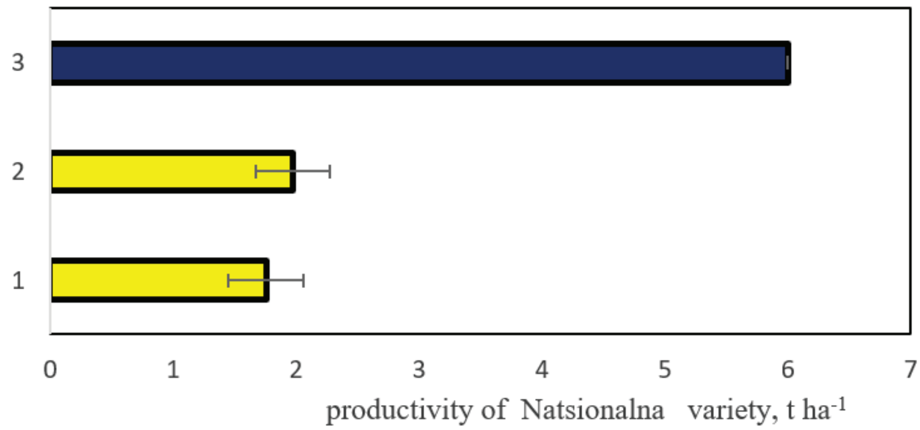

Figure 4: Influence of the technologies on productivity of winter wheat variety Natsionalna (soil - chernozem typical low-humus medium loamy; 1. BT; 2. BT + $N_{60}P_{60}K_{60}$; 3. Standard) (Note: Averages in groups are significantly different from each other, ANOVA with Bonferroni correction, except of those, marked by * $p < 0.05$)

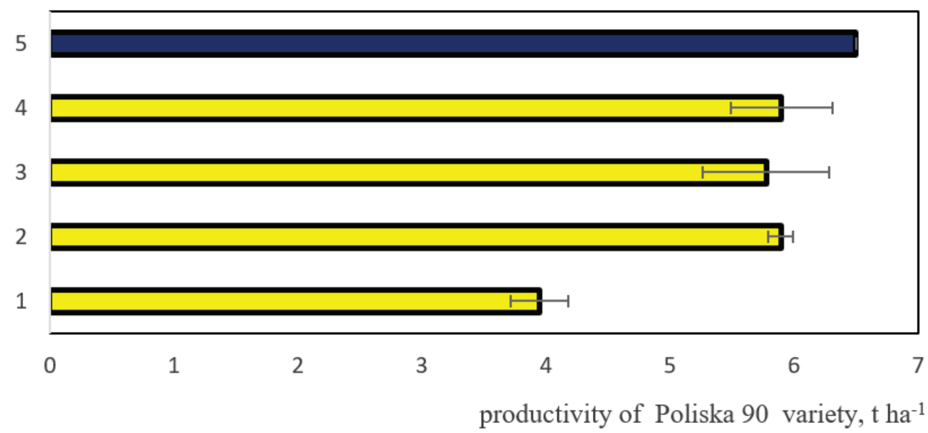

Figure 5: Influence of the technologies on productivity of winter wheat variety Poliska 90 (soil – dark grey podzolized; 1. BT; 2. BT + $N_{90}P_{60}K_{60}$; 3*. BT + manure 10 t ha^{-1}; 4*. BT + $N_{90}P_{60}K_{60}$+ straw + green manure; 5. Standard) (Note: Averages in groups are significantly different from each other, ANOVA with Bonferroni correction, except of those, marked by * $p < 0.05$)

gion is continental. The mean annual temperature is + 10.3 °C. The mean annual precipitation is 500-600 mm. The basic technology and the technology that envisaged mineral fertilizers application at the rate $N_{60}P_{60}K_{60}$ did not ensure the implementation of biopotential of the variety. The reduction of wheat productivity in relation to the standard was 62.3-65.2 %. Cosequently, the influence of the technology on the state of agroecosystem by wheat productivity can be estimated as strong (Figure 4). One of the reasons could be an insufficient level of plant's supply with mobile potassium forms.

In Polissya zone, based on Rivne SARS, winter wheat of the Poliska 90 variety was studied. According to the characteristics of the variety, the potential yield of this variety is 6.5 t ha^{-1}. Climate of Rivne region is continental. The mean annual temperature is + 7.8 °C. The mean annual precipitation is 600-700 mm. The actual yield level was close to the potential and achieved with the technology included mineral fertilizers application in combination with straw and green manure ($N_{90}P_{60}K_{60}$ + straw + green manure). The basic technology, as well as technologies that envisaged application of mineral and organic fertilizers separately, did not ensure the implementation of the variety biopotential. The deviation from the standard was 10.6-37.2 %, the impact of technology on the agroecosystem state was moderate and average (Figure 5). It can be assumed that one of the reasons was insufficient level of plant provision with mobile potassium forms.

In Steppe zone, in the conditions of Zaporizhzhya

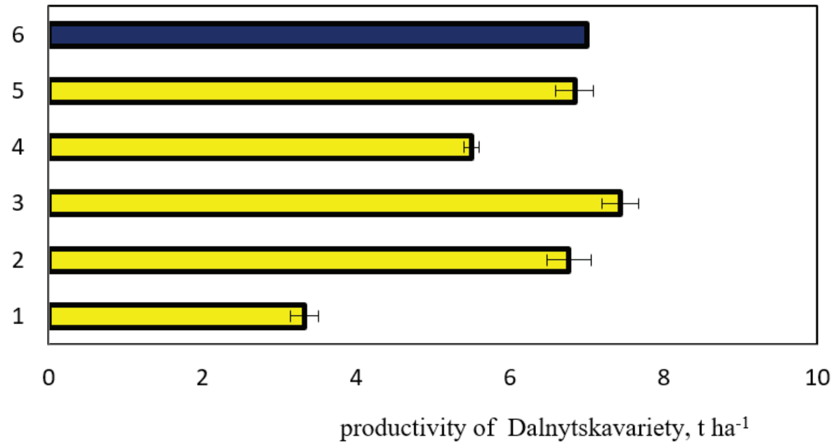

Figure 6: Influence of the technologies on productivity of winter wheat variety Dalnytska (soil – chernozem ordinary low-humus; 1. BT; 2. BT + $N_{75}P_{50}K_{25}$; 3. BT + $N_{50}P_{50}K_{50}$; 4. BT + $N_{15}P_{10}K_5$; 5. BT + manure 5.7 t ha^{-1}; 6. Standard)

RS IOS NAAS winter wheat of the Dalnytska variety was studied. According to characteristics of the variety, the potential yield is 7 t ha^{-1}. Climate of Zaporizhzhia region is continental. The mean annual temperature is + 12.8 °C. The mean annual precipitation is 430-475 mm. The technology with mineral fertilizers application provided optimum conditions for the growth and development of plants - wheat productivity exceeded the standard and amounted to 7.56 t ha^{-1}. Despite the fact that the average negative impact of this technology on nitrogen and potassium regimes of chernozem was noted, plant nutrition was balanced. At the same time, other combinations of fertilizers led to strong negative influence of the technologies on nitrogen regime of the soil and average influence on potassium regime. Consequently, the influence of these technologies can be estimated as strong and moderate according to wheat productivity (Figure 6).

Thus, it has been established that modern technologies of winter wheat growing, in most cases, do not provide plants with balanced mineral nutrition, which may be reason for the decreasing crop productivity if compared to its biological potential.

4 CONCLUSIONS

We recommend using a method based on identifying negative impacts of crop growing technologies on soil fertility in order to estimate ecological safety. Negative impacts of deviations from the optimum to the direction of deterioration are proposed to be grouped as follows: strong, which leads to an unsatisfactory ecological condition (> 50 %), average that provides a satisfactory state (> 25 %, but < 50 %), moderate, which provides a normal state (≤10 %, but < 25 %), absent, an optimal condition is provided (< 10 %).

It is revealed that technologies of winter wheat growing in Polissya, Forest-steppe and Steppe Zones of Ukraine can have a negative influence on potassium regime of soils; the influence by intensity can vary from moderate to strong. In Polissya and Forest-steppe, technologies of winter wheat growing can lead to deterioration of soil pH status. In the conditions of Steppe, along with potassium regime, the technologies can negatively influence nitrogen status and the effect may be characterized as strong.

It is shown that modern technologies of winter wheat growing, in most cases, do not provide plants with balanced nutrition, which may be one of the reasons for the low level of productivity of the crop relative to the biological potential of the variety. The decrease in productivity can fluctuate within 10.6-73.6 %.

Modern technologies of winter wheat growing need to be improved and revised taking into account the results of ecological evaluation of their impact on soil fertility indices.

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Influence of land configuration and fertilization techniques on soybean (*Glycine max* (L.) Merrill.) productivity, soil moisture and fertility

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Influence of land configuration and fertilization techniques on soybean (*Glycine max* (L.) Merrill.) productivity, soil moisture and fertility

Abstract: An experiment was conducted to investigate the impact of flatbed (FB), ridges and furrows (RF) and broad bed furrows (BBF) combined with recommended fertilizer dose $N_{30}P_{60}K_{30}$ kg ha^{-1} (F_1), 75 % NPK (F_2), 125 % NPK (F_3), 75 % NPK + 25 % N through farm yard manure (FYM)-F_4, 75 % NPK + 2 sprays of micro nutrient mixture (Fe, Zn, Cu, Mn, B and Mo) - 0.5 % at 35 and 60 days after sowing (DAS)-F_5 and 75 % NPK + 2 sprays of KNO_3 - 0.5 % at 35 and 1.0 % at 60 DAS (F_6) on the productivity of soybean in a split plot design. BBF stored 14.15 % more soil water and produced 1058.97 kg ha^{-1} more yield than FB. A significant 3.76 kg ha^{-1}-mm rain water use efficiency was notice in BBF compared to FB. The yield increment recorded under F_6 was 15.6 % higher than F_1. Grain nitrogen and oil contents were highest in F_3. The residual soil fertility was much improve by F_3 and F_5. Our result demonstrated that the combination of BBF and F_6 were the best technique to increase soybean yield in the Vertisol soil.

Key words: foliar fertilization; land configuration; soil moisture; soybean nutrition; yield

Vpliv priprave zemljišča in gnojilnih tehnik na pridelek soje (*Glycine max* (L.) Merrill.), na vlažnost in rodovitnost tal

Izvleček: Poskus z deljenkami je bil narejen za preučitev načinov priprave zemljišča kot so ravno zemljišče (FB), grebeni in brazde (RF) in široki grebeni (BBF) v kombinaciji s priporočenimi odmerki gnojenja: $N_{30}P_{60}K_{30}$ kg ha^{-1} (F_1), 75 % NPK (F_2), 125 % NPK (F_3), 75 % NPK + 25 % N kot hlevski gnoj (FYM) (F_4), 75 % NPK + 2 kratno pršenje z mešanico mikrohranil (Fe, Zn, Cu, Mn, B in Mo), 0,5 % 35 in 60 dni po setvi (DAS)(F_5) in 75 % NPK + 2 pršenja s KNO_3 - 0,5 % 35 in 1,0 % 60 dni po setvi DAS (F_6) na pridelek soje. Pri BBF se je ohranilo 14,15 % več talne vode in dalo za 1058,97 kg ha^{-1} več pridelka kot FB. Pri BBF je bila ugotovljena tudi značilno večja učinkovitost (3,76 kg ha^{-1}-mm) izrabe deževnice kot pri FB. Povečanje pridelka je bilo pri F_6 za 15,6 % večje kot pri F_1.Vsebnost dušika in olja v zrnih sta bili največji pri F_3. Rodovitnost tal se je znatno povečala pri F_3 in F_5. Rezultati so pokazali, da je bila kombinacija BBF in F_6 najboljša tehnika za povečanje pridelka soje v tleh na vertisolu.

Ključne besede: foliarno gnojenje; oblikovanost zemljišča; vlažnost tal; gnojenje soje; pridelek

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1 INTRODUCTION

Soybean (*Glycine max* (L.) Merrill.) is the most important oil seed crop in India, owing to its several domestic and industrial uses, besides its use in numerous food preparations and animal feed formulations. Soybean accounts for about 53 % of the world production share among the oilseed crops, and has therefore, occupied an important place in most farming systems in the Marathwada region of Maharashtra State, India (Talukdar & Shivakumar, 2016). However, under rain fed systems, soil moisture stress of 15-21 days at any growth phase of the soybean crop results in a significant yield loss in the Maharashtra State (Patil, 1992). These yield losses are especially severe in the early determinate genotypes (Chaturvedi et al., 2012). The drying and cracking nature of the vertisol soils in the region, coupled with their low fertility aggravates the situation (Keteku et al., 2016). To effectively manage the problem amidst irrigation difficulties, technologies targeted at soil moisture and nutrient conservation such as land layout are very crucial. Thus soil, water and nutrient conservation technologies are the key adaptation strategies to mitigate the rapid loss of moisture (Kurukulasuriya & Rosenthal, 2003); and help plants withstand the occurrence of short dry spells under rain fed farming. An earlier studies on soil management strategies, aimed to increase crop productivity revealed that, modification of land such as broad bed furrow, and ridges and furrows in vertisol soils were superior to flatbed under watershed development (Raut & Taware, 1997). It is noteworthy to also mention that, soybean is a high protein and energy crop, as such it has a high nutrient requirement. Unfortunately, the inadequate and imbalance fertilization practiced by farmers, also adds to the problem of decreasing yield (Chaturvedi et al., 2012).

Usually, the farmer's fertilizer programs focus solely on soil applied NPK, without plans for foliar application, however recent studies had shown the foliar fertilization enhance soybean yield (Gowthami & Rama, 2014; Chaturvedi et al., 2012). Others had also reported that micronutrients are essential for the optimum utilization of major nutrients, and also for the production of organic compounds (Gowthami & Rama, 2014; Intanon, 2013). Numerous previous studies had equally reported the impact of combine major and micro nutrients on crop yield (Keteku et al., 2018; Intanon, 2013; Salem & El-Gizawy 2012). Soybean is a focus crop for the realization of the Sustainable Development Goals (Shinde et al., 2009). Climate change threatens rainfall pattern and therefore, the achievement of the Sustainable Development Goals aimed at poverty and hunger reduction. Therefore our work is designed to investigate (i) the influence of land modification on soil moisture and soybean yield, and (ii) the effect of different fertilization techniques on soybean yield and soil properties. This is necessary to find the appropriate land configuration and techno-economic nutrient package for soybean production under such vertisol soil.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

The experiment was conducted at the experimental farms of the All India Coordinated Research Project on Dryland Agriculture (AICRP) station at Vasantrao Naik Marathwada Agriculture University (VNMKV), Marathwada, India, during rainy season, 2017. VNMKV is situated on a latitude and longitude of 19° 15' 28.0440" N and 76° 46' 25.4748" E respectively, and at 409 m above mean sea level. The average annual precipitation of the region is 963 mm, distributed in 48 rainy days, mostly during June – October. The mean maximum and minimum temperatures are 32.2 °C and 19.0 °C respectively. The total rainfall received during the trail was 308.4 mm, distributed in 35 rainy days. A total effective rainfall of 281.7 mm was recorded. Relative humidity was in the ranges of 81.8 % - 48.1 %, while that of mean bright sunshine was 6.7 hr. The mean evapotranspiration was 5.4 mm as well. The research soil was vertisol in classification (WRB), medium deep black, well drained, low in fertility, except K and levelled in topography. The soil nutrients and moisture contents at a depth of (0-20 cm) before the trial are shown in (Table 1). The pH of the soil was alkaline.

2.2 EXPERIMENTAL PLAN

The experiment was 2 factorial, conducted in split plot design with 3 replications. The treatments were

Table 1: Soil properties before the trial (sample size (n) = 3)

N	P	K	Fe	Zn	Cu	Mn	B	pH (1:1.5)	Moisture %
	%				mg/kg				
0.514	0.313	1.27	5.24	1.33	0.32	2.24	0.37	7.9	17.85

composed from 3 land configurations as main plots treatments and 6 fertilization strategies as subplots treatments, making a total of 18 treatment combinations. The 3 land configurations were; flatbed (FB), ridges & furrow (RF) and broad bed furrow (BBF), while the 6 fertilizers were a combination of urea, single super phosphate and murate of potash; recommended fertilizer dose $N_{30}P_{60}K_{30}$ kg ha^{-1} F_1 = 100 % by mass, 75 % NPK (F_2), 125 % NPK (F_3), 75 % NPK + 25 % N through 5 tons FYM ha^{-1} (F_4), 75 % NPK + 2 sprays of 0.5 % micro nutrient mixture (Fe, Zn, Cu, Mn, B and Mo)– 50 ml per 10 L water at 35 and 60 DAS (F_5) and 75 % NPK + 2 sprays of 0.5 % KNO_3 - 50 ml per 10 L water at 35 and 1.0 % - 100 ml per 10 L water at 60 DAS (F_6). Thus, 2500 ml for 0.5 % and 5000 ml for 1 % to 500 L water was used for ha.The KNO_3 contained 13 % and 45 % nitrogen and potassium, respectively. The gross and net plot sizes used were 5.4 x 6.0 m and 4.5 x 5.0 m, in length and width respectively.

The land was ploughed with a tractor drawn plough to a depth of 20 cm and harrowed twice before the preparation of the ridges and furrows, and broad bed furrows. The ridges measured 45 cm wide and 15 cm high while that of the broad bed furrows were 120 cm × 30 cm × 15 cm in width, length and height, respectively. The seeds of determinant soybean variety (‘MAUS-162’) were treated with *Rhizobium* culture *(Bradyrhizobium japonicum* (Kirchner 1896) Jordan 1982) and phosphate solubilizing bacteria (PSB), and sown at the recommended spacing of 45 × 5 cm^2. But on the broad bed furrows a planting distance of 37.5 × 5 cm^2 was used so as to obtain uniform plant population in all plots. A rate of 65 kg ha^{-1} was used, two seeds were dibbled per hill and thinned out after 14 DAS to maintain one seedling per hill. The solid fertilizers were applied by side placement method, 30 % was applied at sowing and the remaining 70 % applied at 30 DAS. The FYM was broadcasted and raked into the soil on the flatbed and broad bed furrows, but in the ridges and furrows, it was applied uniformly in the lines opened for sowing. The ‘MAUS-162’ seeds were sourced from the seed processing plant, VNMKV while the fertilizers, *Rhizobium* culture and PSB were obtained from AICRP, VNMKV. One spraying of Chloropyriphos 20 EC was performed to control leaf eating caterpillar. Two hand weeding and one hoeing were performed to control weeds and also loosen the soil for good aeration.

2.3 DATA COLLECTION

Before the trial, soil cores were collected from 12 spots on the research site at a depth of (0-20 cm) with the hand auger for the assessment of soil fertility and pH. The routine methods of Lu (1999) were followed for the determination of soil nutrients. Total nitrogen, phosphorus and potassium were determine by the Kjeldahl method, Bray’s no. II method and Neutral N ammonium method, respectively. The wet digestion (nitric-perchloric digestion) method was adopted for the analysis of iron, zinc, copper, manganese and boron. Soil pH was measured at 1:1.5 solution ratio, using the electrode (H19017 Microprocessor) pH meter. Another soil cores were also taken from a depth of (0-15 cm and 15-30 cm) for the determination of soil moisture content. Using the gravimetric method, the percentage soil moisture content were calculated for each depth and the mean worked out using the formula in (Equation 1). 50 g of the sample soil was oven dried at 105 °C ± 5 °C for 12 h.

$$\text{Moisture } \% = (m_2 - m_1)/(m_2) \times 100 \qquad \text{(Eqn 1)}$$

Where; m_1 = mass of wet soil sample, m_2 = mass of oven dried soil sample

Fifteen representative sampled plants were randomly selected in each plot and tagged for the measurement of vegetative growth. Plant height, number of leaves, number of branches, leaf area and total dry matter mass per plant were measured after 30 DAS at 15 days interval. At each periodic data collection, two representative plant were uprooted, processed and oven dried at 72 °C ± 2 °C for 12 h for total dry matter measurement. However on the harvest day, total dry matter weight was again measured from the 15 sampled plants in each plot. Leaf area was measured from the sampled plants uprooted for dry matter studies. The leaves were aerated into leaflets and grouped into three class viz., small, medium and big. The maximum length and diameter of five leaflets from each group were measured using the hand held laser leaf area meter (CID Bio-Science, Inc.), and the method of Pawar (1978) was used to calculate the leaf area/plant (Equation 2).

$$\text{Leaf area/plant (dm}^2) = \sum_{i=0}^{n=3}(L \times D)K \qquad \text{(Eqn 2)}$$

Where; L, D, n and K are leaf length, leaf diameter, number of leaves and leaf area constant for soybean (0.689), respectively. Only the final values were reported here. Also, yield components namely; number of pod plant^{-1}, pod mass plant^{-1}, grain mass plant^{-1} and 1000 seeds mass were measured from the fifteen sampled plants. After harvesting (120 DAS), grain mass plot^{-1} and straw mass plot^{-1} were measured, all the plants in the net plots were consider. The values were later converted to grain yield ha^{-1}. The biological yield produce was determined as the summation of grain mass plot^{-1} and straw mass plot^{-1} and again converted into ha. Harvest index (HI) was calculated as indicated in (Equation 3).

HI % = (Biological yield (kg))/(Grain yield (kg)) × 100 (Eqn 3)

The protein and oil content of the seeds were determined for quality assessment, grain nitrogen content was estimated by the micro Kjeldahal method (A.O.A.C., 1975), and was converted into crude protein percentage by multiplying the percent nitrogen with 6.25. Soxhlet ether extraction method was used to estimate the oil content.

After the trial, soil samples were again sampled and the properties estimated by the same methods above. Rain water use efficiency (RWUE) was computed by the formula (Equation 4) and expressed in kg/ha-mm.

RWUE (kg ha^{-1}-mm) = (Yield kg ha^{-1})/(Moisture use(effective rainfall) mm) (Eqn 4)

2.4 STATISTICAL ANALYSIS

The data recorded were subjected to Analysis of Variance (ANOVA) using SPSS 21 statistical package. The variation between treatments means were quantified at a probability of 5 %. Duncan's Multiple Range Test (DMRT) analysis was performed and presented in tables, in alphabets with 'a' depicting highest value. Interactions between factors were not significant, hence not presented. Regression analysis was used to show the relationship between some variables.

3 RESULTS AND DISCUSSION

3.1 INFLUENCE OF LAND CONFIGURATION ON SOIL MOISTURE CONTENT

The impact of land configuration on soil moisture content (0-30 cm) was significant ($p < 0.05$) as shown in Figure 1. Soil moisture content increased gradually from 30 to 90 DAS in the BBF and RF, compared to FB which recorded a decrease at 90 DAS. At 30, 60, 75 and 90 DAS, BBF conserved the highest significant soil moisture of 19.86, 27.30, 23.55 and 20.43 %, respectively when compared to RF and FB. Similarly, RF stored a significant amount of moisture on 60 and 90 DAS (27.29 % and 18.30 %, respectively) than FB. The furrows between the BBF and RF prevented the runoff of rain water and enhanced the infiltration of water into the soil. Probably the size of BBF also enhanced water conservation, as it has less surface area for evaporation when compare to the RF. In-situ land management strategies that reduces water lost caused by runoff and evaporation, and improves water infiltration and storage would lead to increase the amount of water retained in the soil for crops (Singh et al., 2014). BBF conserved 14.15 % more water than FB. Our results agrees with the previous findings of Shinde et al. (2009), Bharambe et al.(1999) and Patil et al.(1992) that BBF and RF conserved more water than FB. According to Kumar et al. (2010), furrow irrigated bed planting systems, on an average retained 40 % more water, compared to FB planting systems. Consistently, Selvarajua et al. (1999) also reported 17 % more soil moisture in BBF compare to FB. In contrasts to our findings, Singh et al. (2018) reported 28.54 % soil moisture in RF and 27.58 % in BBF, nevertheless they similarly reported the least soil moisture in FB. The principal aim of land configuration are; the preparation of a conducive seedbed for seed germination and seedling growth, conservation of soil moisture that influences the infiltration characteristics of the soil, and also, provides adequate soil depth for optimum root growth and proper fertilizers placement. The land configuration that stores enough moisture will reduce soil moisture tension, while improving nutrient flow and their availability for crop uptake (Singh & Kumar, 2009). Our findings has demonstrated that BBF and RF can conserve more moisture than FB.

3.2 INFLUENCE OF TREATMENTS ON SOYBEAN GROWTH

The results in Table 2 showed that soybean growth variables were significantly ($p < 0.05$) influenced by the various land configurations and fertilizers strategies. Interaction between factors were not significant and were therefore not discussed. Soybean height and number of branches (55.98 cm and 4.95) respectively, were superior in BBF compared to RF and FB. However, leaf area plant^{-1} was equal between BBF and RF, while that of total dry matter produced did not significantly differ among the land layouts. But the greatest dry matter mass of 24.20 g was produced by BBF. The high growth observed in BBF and RF could be related to the availability of optimum soil moisture at the key vegetative phase of the crop. When soil moisture tension is low, the ability of crops to absorb nutrients and that of the soil to supply nutrients are optimal, and so, nutrients availability are improved (Singh & Kumar 2009). In addition, the BBF and RF could also provide adequate aeration and a good soil depth for root expansion and nutrient exploration (Singh et al., 2014). From our re-

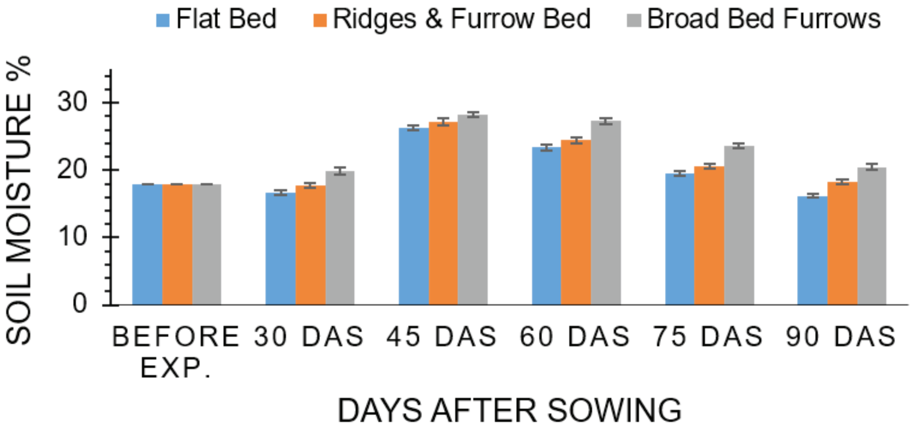

Figure 1: Effect of land configuration on soil moisture content (0-30 cm) during the trial

sults, it is evident that land layout had significant effect on growth.

Also, the application of F_6 (75 % NPK + 2 sprays of KNO_3- 0.5 % at 35 DAS and 1.0 % at 60 DAS) recorded the highest soybean height plant^{-1}, number of branches plant^{-1}, leaf area plant^{-1} and total dry matter mass plant^{-1} of 53.12 cm, 5.17, 22.10 dm^2 and 25.80 g respectively, but it was not significant when compared to F_3 and F_5 (dry matter). Increasing the NPK rate from F1 to F3 also increased soybean growth variables but did not give significant results. According to our result, F_1, F_2 and F_4 treatments had a similar effect on soybean growth. The performance of F_6 might probably be due to the rapid and efficient nutrients absorption resulting from the foliar spray of KNO_3 which contained 13 % N and 46 % K, while that of F_3 can be attributed to its higher NPK nutrients. Nitrogen particularly, is a principal constituent of protein, chlorophyll and the hormones which are essential for cell expansion and an increase in the vegetative apparatus of crops (Keteku et al., 2016; Nsoanya & Nweke, 2013). Besides having nitrogen which is an integral component of cell division in the fertilizers, the micronutrients do also influence cell division, chlorophyll construction and photosynthesis (Intanon 2013). This may probably explains why F_5 produced a similarly high total dry matter mass of 24.83 g, despite its low NPK content when compared to F_6 (25.80 g) and F_3 (24.50 g). It is noteworthy to indicate that the application of F_5 produced greater dry matter mass of 24.83 g when compared to F_3 (24.50); and a higher leaf area and dry matter mass when compare to F_1 (19.20 dm^2 and 23.30 g) respectively.

Previous studies by Khaliq et al. (2006) reported that, the sink capacity of a plant is mainly dependent on its vigorous vegetative growth; as such in our study, the treatments that recorded a large leaf area plant^{-1}, had more green areas available for the interception of active radiation during photosynthesis, for greater dry matter production (Azarpour et al., 2014). The regression analysis showed the impact of leaf area on dry matter produced by the fertilizers (R^2 = 0.8361) as shown in Figure 2.Dry matter production responded positively to an increase in leaf area plant^{-1}. Our results are also in agreement with Raj & Mallick (2017), in their studies the application of 80 kg N ha^{-1} + mixed spray of 0.203 % $Ca(NO_3)_2$ + 0.25 % KNO_3 produced the maximum leaf area index values (1.748 and 1.592), dry matter accumulation (1404.3 and 1288.8 g m^{-2}) and crop growth rate (27.87 and 25.68 g m^{-2} day^{-1}).

3.3 INFLUENCE OF TREATMENTS ON SOYBEAN YIELD COMPONENTS, YIELD, QUALITY AND WATER USE EFFICIENCY

The data in Table 3 showed a significant ($p < 0.05$) impact of land configuration and fertilizer on soybean yield components. Interactions between the factors were not significant. Pod mass plant^{-1}, grain mass plant^{-1} and 1000 seeds mass were significantly influenced by the different land configurations. The highest (12.32 g, 5.09 g and 85.56 g) respectively, were produced by BBF. This resulted to its greatest grain yield of 1058.97 kg ha^{-1} as well, but it was comparable to RF (1026.77 kg ha^{-1}) as shown in the Table 4. The soybean yield were in accordance with the vegetative growth record by the land configurations. BBF significantly increased grain yield by 8.8 %, when compared to FB. Straw yield, biological yield and harvest index did not vary significantly among the land configurations, nevertheless the greatest values were observed in BBF, and was followed by RF. A similar results had been previously reported in

Table 2: Influence of treatments on soybean growth

Treatments	Plant height plant^{-1} (cm)	Number of leaves plant^{-1}	Number of branches plant^{-1}	Leaf area plant^{-1} (dm^2)	Total dry matter mass plant^{-1} (g)
Land Configurations					
FB	46.83^{b}	16.68	3.82^{c}	17.98^{b}	23.20
RF	49.60^{b}	19.52	4.05^{b}	18.90^{ab}	23.72
BBF	55.98^{a}	19.53	4.95^{a}	21.47^{a}	24.20
CD @ 5 %	3.51	NS	0.19	2.63	NS
Fertilizers					
F_1	50.90^{ab}	18.43	4.10^{cd}	19.20^{bc}	23.30^{ab}
F_2	46.57^{b}	16.97	3.70^{e}	17.40^{c}	22.23^{b}
F_3	53.67^{a}	19.20	4.47^{b}	20.47^{ab}	24.50^{ab}
F_4	49.44^{ab}	17.60	3.93^{de}	18.03^{c}	21.57^{b}
F_5	51.13^{ab}	19.10	4.27^{bc}	19.50^{bc}	24.83^{ab}
F_6	53.12^{a}	20.17	5.17^{a}	22.10^{a}	25.80^{a}
CD @ 5 %	5.42	NS	0.32	2.22	2.72

Note: mean values with different superscript letter within each column denotes significance ($p < 0.05$) between different groups. CD = critical difference between means; NS = non-significant (n = 15)

other crops. In a study, Pramanik et al. (2009) reported a significant 16.8 % and 15.9 % rise in chickpea grain yield under raised bed planting over flatbed planting, in two seasons. According to Selvarajua et al. (1999) also, planting on BBF increased sorghum and pearl millet yields by 34 % and 33 % respectively, compared to FB. This they ascribed to the optimum water storage and safe disposal of excess rain water by BBF. Among all the in situ soil moisture conservation techniques, rain water use efficiency was the highest (3.76 kg ha^{-1}-mm) in BBF as well (Table 4). It is evident from the Table 4 that, an increase in water use efficiency corresponded to a greater soybean yield. When water utilization increased, nutrient uptake was enhanced by the mass flow process. Previous work of Lomte et al. (2006) also showed that, opening of furrows in every row recorded the highest water use efficiency of 3.15 kg ha^{-1}-mm than flat bed.

Among the fertilizers also, the application of F_6 recorded the greatest yield components; pod number plant^{-1} (30.65), pod mass plant^{-1} (13.40 g), grain mass plant^{-1} (5.36 g) and 1000 seeds mass (95.56 g). However, grain yield ha^{-1} was on a par between F_6 (1160.33 kg) and F_5 (1086.50 kg). A similar trend was noticed for straw yield and biological yield between the two treatments, and were followed by F_3. In addition, an increase in NPK rate from F1 to F3, also significantly increased grain yield by 2.3 % (Table 4). Additionally, the yield increment realized for F_6 and F_5 were 15.6 % and 9.9 % respectively, higher compared to the recommended fertilizer rate (F_1). The higher yield of F_6 was mainly due to its greater grain mass plant^{-1} and 1000 seed mass. The KNO_3 sprayed during the seed filling stages (60 DAS) might had increased the availability of N and K to the plants. N is central in organic compounds formation in plants (Intanon, 2013). Besides the beneficial functions of nitrate nitrogen, the prevalence of K^+ in KNO_3, may had also improved grain filling and phytomass production, and the translocation of assimilates to reproductive apparatus (Ravikiran et al., 2012; Waraich et al., 2011). The relationship between leaf area plant^{-1} and grain mass plant^{-1} ($R^2 = 0.8276$) showed that, the high vegetative growth produced affected grain yield positively (Figure 2).

Our result are in line with those of other previous experiments as well. Soil application of 80 kg N ha^{-1} + foliar spray of 0.25 % KNO_3 + 0.203 % Ca $(NO_3)_2$, led to an increased soybean yield of 1.68 t ha^{-1}, about 10.7 % increase over the 1.5 t ha^{-1} produced by 80 kg N ha^{-1} + water spray (Raj & Mallick, 2017).

Again, Vekaria et al. (2013) reported that foliar application of 0.4 % KNO_3 ha^{-1} significantly increased soybean yield by 18.4 % when compared to water spray only. Intanon (2013) did mentioned that, the micronutrient (Fe, Zn and Cu) are important for carbohydrate formation. The presence of this element in F_5 could partly account for its high yield output.

Table 3: Influence of treatments on soybean yield components

Treatments	Pods plant^{-1}	Pod mass plant^{-1} (g)	Grain mass plant^{-1} (g)	1000 seeds mass (g)
Land Configurations				
FB	27.71	11.45^{b}	3.76^{b}	81.77^{b}
RF	28.19	11.87^{ab}	4.55^{a}	82.72^{ab}
BBF	29.68	12.32^{a}	5.09^{a}	85.56^{a}
CD @ 5%	NS	0.48	0.58	3.24
Fertilizers				
F_1	28.28	11.41^{bc}	4.23^{bcd}	79.84^{b}
F_2	26.85	10.26^{c}	3.75^{d}	76.52^{b}
F_3	28.51	12.44^{ab}	4.50^{bc}	82.86^{b}
F_4	27.65	10.95^{c}	4.01^{cd}	77.35^{b}
F_5	29.20	12.83^{ab}	4.92^{b}	84.95^{b}
F_6	30.65	13.40^{a}	5.36^{a}	96.56^{a}
CD @ 5 %	NS	1.75	0.74	9.04

Note: mean values with different superscript letter within each column denotes significance (p <0.05) between different groups. CD = critical difference between means; NS = non-significant (n = 15).

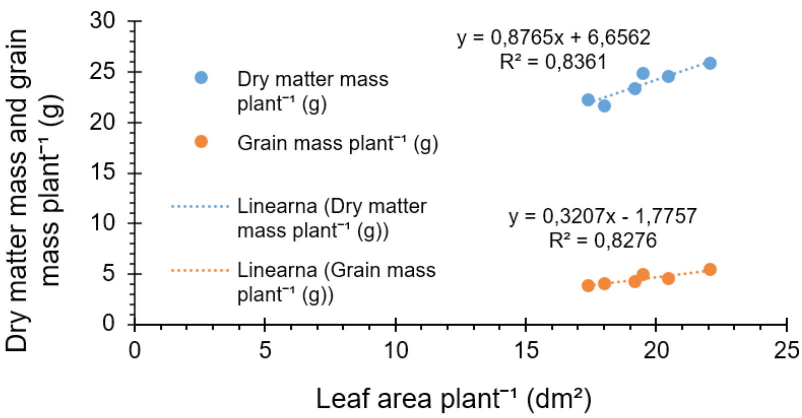

Figure 2: Regression analysis of leaf area plant^{-1} (dm^2) to total dry matter plant^{-1} and grain mass plant^{-1} (g)

The impact of boron (1 kg ha^{-1}) and molybdenum (0.5 kg ha^{-1}) on soybean yield had been demonstrated (Adkine et al., 2011); while the combination of NPK with 400 g Fe ha^{-1} and 20 g Mo ha^{-1} had also been reported by (Zahoor et al., 2013). In our work, land configuration had no significant effect on seed protein and oil content; likewise was the fertilizers on seed oil content, but numerically, the highest oil content of 19.65 % was obtained in F_5 and the lowest in F_2 (Figure 3). Seed protein content was significantly ($p < 0.05$) influenced by the fertilizers, with the highest (39.64 %, 39.38 %, 39.33 % and 39.18 %) realized in F_6, F_5, F_3, and F_4, respectively. The seed qualities observed in our study concur with those of (Kiran et al., 2008).

3.4 SOIL FERTILITY AFTER THE TRAIL

The different in situ soil moisture conservation techniques had no significant influence on soil fertility after the trial, though the best recordings were noticed in BBF (Table 5). The more soil water conserved in BBF, probably increased the crop residues added to the soil due to high vegetative growth (Selvarajua et al., 1999; Lal, 1995). The residual soil nitrogen (11.14 %), phosphorus (0.73 %) and potassium (1.85 %) contents were greatest in F_3 plots, probably due to the high NPK levels of this fertilizer formula. Phosphorous and potassium contents in particular, were significantly ($p < 0.05$) improved by F_3, when

Table 4: Influence of treatments on soybean yield, harvest index and rain water use efficiency.

Treatments	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Harvest index (HI)	RWUE (kg ha^{-1} mm^{-1})
Land Configurations					
FB	966.10^{b}	1913.00	2879.10	0.34	3.43^{b}
RF	1026.77^{ab}	1985.83	3012.60	0.34	3.64^{ab}
BBF	1058.97^{a}	2085.67	3144.63	0.34	3.76^{a}
CD @ 5 %	63.13	NS	NS	NS	0.22
Fertilizers					
F_1	979.03^{c}	1992.00^{abc}	2971.03^{bc}	0.33	3.48^{c}
F_2	916.37^{c}	1820.33^{c}	2736.70^{d}	0.34	3.25^{c}
F_3	1001.70^{b}	2019.33^{ab}	3021.03^{bc}	0.33	3.56^{bc}
F_4	959.73^{c}	1905.00^{bc}	2864.73^{cd}	0.34	3.41^{c}
F_5	1086.50^{ab}	2062.33^{ab}	3148.83^{ab}	0.34	3.86^{ab}
F_6	1160.33^{a}	2170.00^{a}	3330.33^{a}	0.35	4.12^{a}
CD @ 5 %	99.91	197.79	216.12	NS	0.35

Note: mean values with different superscript letter within each column denotes significance ($p < 0.05$) between different groups. CD = critical difference between means; NS = non-significant.

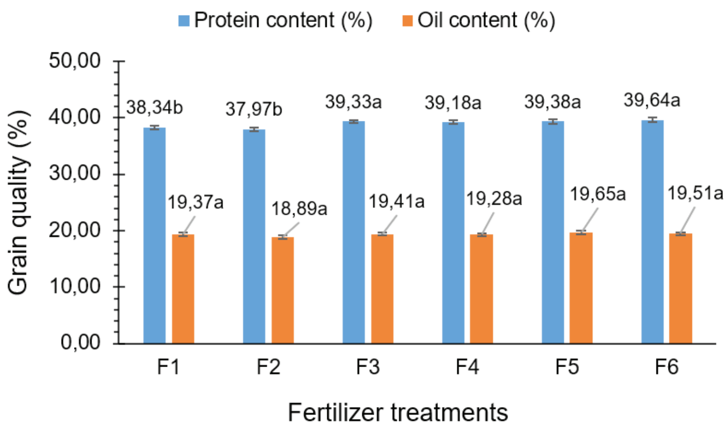

Figure 3: Influence of fertilizers on grain quality (n = 3)

compared to the other fertilizers. Generally, soil nitrogen and phosphorus content improve in all the plots, when compared to the levels before the trial. Our findings could be due to the *Rhizobium* culture (*Bradyrhizobium japonicum*) and phosphate solubilizing bacteria (PSB) treatment given to the seeds, as root nodules formations were generally observed on most roots. Also, the soil micronutrients; Fe, Zn, Cu, Mn and B contents were found the highest in F_5 with 8.75, 2.10, 0.60, 3.31 and 1.40 mg kg^{-1}, respectively. This could be due to the micronutrient spray given under this treatment. The lowest performance were observed in the F_2 plots.

4 CONCLUSION

Our result supports our hypothesis that (i) proper land configuration can promote soybean yield than flatbed; (ii) proper fertilization technique (foliar spray) can improve soybean yield and soil fertility. Our work has shown that BBF and RF can conserve more soil moisture for greater soybean growth and yield in vertisol soil. The application of F_6 also produced the highest soybean growth and yield. Therefore, for general soil improvement, the application of F_5 is recommended as it improved both the macro and micro soil nutrients elements, however for maximum yield, farmers in the

Table 5: Soil fertility after the trail

Treatments	N	P	K	Fe	Zn	Cu	Mn	B
			%			mg/kg		
Land Configurations		mg/kg						
FB	0.99	0.67	1.71	6.11	1.50	0.40	2.65	0.62
RF	0.98	0.66	1.71	6.23	1.53	0.39	2.66	0.58
BBF	0.99	0.69	1.73	6.43	1.57	0.42	2.68	0.62
CD @ 5 %	NS	NS	NS	NS	NS	NS	NS	NS
Fertilizers								
F_1	1.06[b]	0.71[ab]	1.79[b]	5.68[c]	1.50[b]	0.34[c]	2.50[bc]	0.40[d]
F_2	0.84[c]	0.60[d]	1.60[d]	5.59[c]	1.44[b]	0.35[c]	2.39[c]	0.41[d]
F_3	1.14[a]	0.73[a]	1.85[a]	5.77[c]	1.36[b]	0.37[b]	2.36[c]	0.43[cd]
F_4	0.89[c]	0.67[c]	1.71[c]	6.16[b]	1.43[b]	0.39[b]	2.88[b]	0.48[bc]
F_5	0.85[c]	0.64[cd]	1.61[d]	8.75[a]	2.10[a]	0.60[a]	3.31[a]	1.40[a]
F_6	1.13[ab]	0.68[bc]	1.74[bc]	5.58[c]	1.39[b]	0.36[b]	2.46[c]	0.54[b]
CD @ 5 %	0.07	0.04	0.05	0.29	0.22	0.03	0.38	0.06

Note: mean values with different superscript letter within each column denotes significance ($p < 0.05$) between different groups. CD = critical difference between means; NS = non-significant (n = 3).

Marathwada region of India are advise to adopt BBF + F_6 for soybean production.

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Dimensions of the perceived value for wine from the perspective of Slovenian wine consumers

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Dimensions of the perceived value for wine from the perspective of Slovenian wine consumers

Abstract: Perceived value is a subjective impression of the consumer about the value of a product or a service. There is a lack of research and understanding of Slovenian wine consumers preferences, while at the same time the importance of the Slovenian wine sector is increasing. 221 adults from the two biggest Slovenian winegrowing regions who at least occasionally consume and purchase wine were interviewed with a structured questionnaire. The Perval scale was used to measure and identify the factors (dimensions) of the perceived value for wine. Further, the relation between Slovenian winegrowing regions and the perceived value dimensions was investigated. It was shown that respondents from the two largest Slovenian winegrowing regions perceive three different dimensions of value for wine: quality-price, emotional-social, and a dimension where indicators for humane, environmental and region of origin factors highly correlated, and were therefore named terroir. We also found that respondents value Primorska winegrowing region the most, followed by Podravje and other wine regions. Both winegrowing regions correlate to terroir and quality-price value dimensions. It was also found which value dimensions are more important to different socio-demographic groups, which can give wine producers and wine-sellers some ideas on consumer segmentation and marketing strategy.

Key words: perceived value; wine; consumer; Perval scale; Slovenia; factor analysis

Dimenzije zaznane vrednosti vina s perspektive slovenskih kupcev vina

Izvleček: Zaznana vrednost je subjektivna ocena potrošnika o vrednosti izdelka oziroma storitve. Ker v Sloveniji primanjkuje raziskav, ki bi pripomogle k boljšemu razumevanju izbire potrošnikov vina, hkrati pa vinarski sektor pridobiva na pomembnosti, je bila med 221 potrošniki iz dveh največjih vinorodnih dežel v Sloveniji, ki vsaj občasno pijejo in kupijo vino, opravljena raziskava. Za ugotavljanje števila dimenzij zaznane vrednosti vina je bila uporabljena lestvica Perval, nato pa nas je zanimala povezava med dimenzijami in različnimi vinorodnimi deželami. Ugotovili smo, da vprašani potrošniki ločijo tri dimenzije zaznane vrednosti vina in sicer kakovostno-cenovno, emocionalno-socialno in tretjo dimenzijo, kjer so močno korelirali indikatorji okoljskih in človeških faktorjev, ter indikatorji, ki opisujejo regijo porekla vina; in smo jo poimenovali terroir. Anketiranci najbolj cenijo vinorodno deželo Primorska, sledi Podravje in nato ostale vinorodne dežele. Obe omenjeni vinorodni deželi korelirata s kakovostno-cenovno in terroir dimenzijo zaznane vrednosti vina. Ugotovili smo tudi, katere dimenzije zaznane vrednosti so pomembnejše respondentom z določenimi socio-demografskimi značilnostmi, kar bo vinskim pridelovalcem in prodajalcem lahko dobro izhodišče za segmentacijo potrošnikov in oblikovanje marketinške strategije.

Ključne besede: zaznana vrednost; vino; potrošnik; lestvica Perval; Slovenija; faktorska analiza

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1 INTRODUCTION

Perceived value is the perception about the product (or service) by the consumers, rather than something objectively determined by the seller and these perceptions involve a trade-off between what the consumer receives and what she or he gives up in order to acquire and/or use a product (Woodruff, 1997). It is a subjective value from the perspective of the consumer and is not equal to objective value of the product (Zeithaml, 1988). According to Woodruff (1997), consumer value is the source for competitive advantage and, as a concept, it is moving beyond the focus just on the quality. A promising direction for research toward an enhanced understanding of value is the dual perspective of value measurement, which includes assessing (intra-variable approach) and linking (inter-variable approach) value dimensions to other related measures, where the main flow of effects moves from perceived quality and perceived price to perceived value, satisfaction and to loyalty (Gallarza et al., 2011).

Olson and Jacobi (1972) defined consumer value as a sum of intrinsic and extrinsic information about a product. Intrinsic characteristics are physical part of the product (colour, taste, smell, sweetness, etc.), while extrinsic characteristics are connected with the product, but are not a physical part of it (label, cork, brand name, region of origin etc.). They showed, that for determining the quality of the product, intrinsic features are more important than extrinsic.

Intrinsic and extrinsic features are important for the perception and willingness to buy in the case of wine. Agnoli et al. (2016) found, that intrinsic features are more important for more regular consumers who are more familiar with wines. The concept of consumer value differs also with regard to circumstances within which a consumer thinks about value; when making a purchasing decision (when extrinsic features are more important) or when experiencing product performance during or after use, when intrinsic features come first (Woodruff, 1997). Price can be an indicator of the products quality when there is no other information to imply quality of the product, when there are many similar products on the market and when the consumer does not know the price (Zeithaml, 1988).

Perceived value is closely connected to value proposition. A brand's value proposition is a statement of the functional, emotional and self-expressive benefits delivered by the brand that provide value to the consumer (Aaker, 1996). Aaker (1996) considers price separately, as the price could reduce the value proposition of the product, however it can at the same time imply its higher quality.

There are different classifications of value found in the literature. Generally, value is defined as a two- or multidimensional construct, however Lin et al. (2005) claim that both theories are inadequate and contradictory at some points. They explain perceived value as a secondary indirect construct, where components of value and cost, both of which are manifested by numerous indicators, work as indirect indicators of perceived value. Most authors consider value as a multidimensional construct, but the agreement on the number of dimensions has not yet been reached (Gallarza et al., 2011). Number of dimensions most likely depends on the consumer and on the type of product in question.

Sheth-Newman-Gross model, also known as the theory of consumption values, is one of the analytical cognitive models of consumer behaviour. According to this model the consumer choice is a function of multiple consumption values, which make differential contributions in any given choice situation. Consumption values are independent, and are defined as: functional value, which is the perceived utility acquired from an alternative´s capacity for functional, utilitarian or physical performance and is presumed to be the primary driver of consumer choice; social value, which is the perceived utility acquired from an alternative´s association with one or more specific social groups; emotional value is the perceived utility acquired from an alternative´s capacity to arouse feelings or affective states; epistemic value is the perceived utility acquired from an alternative´s capacity to arouse curiosity, provide novelty, and/or satisfy a desire for knowledge; and conditional value, which is defined as the perceived utility acquired by an alternative as the result of the specific situation or set of circumstances facing the choice maker (Sheth et al., 1991). Even products, generally thought to be of high functional value are frequently selected on the basis of their social or emotional value (for example cars, kitchen appliances etc.).

On the basis of the Sheth-Newman-Gross model of consumer behaviour (Sheth et al., 1991), Sweeney & Soutar (2001) developed PERVAL, a multiple-item scale to assess consumers perception of value for different product types. The PERVAL scale consists of four dimensions, which are: functional/quality, emotional, social and price/value for money, and has been assessed for validity and reliability in assessing perceived value for different types of products (i.e. Orth et al., 2005; Sigala, 2006; Gill et al., 2007; Brown & Mazzarol, 2009; Walsh et al., 2014…). The PERVAL scale was used to assess the perceived value for wine in U.S. in 2005 and has been adapted due to the nature of the product. Adjustments included the addition of an environmental and a humane dimension and re-phrasing of few items

(Orth et al., 2005). The outcome was that the drivers of preference for wine in U.S. were: functional benefit/quality, price/value for money, social benefit, emotional benefit, environmental benefit and humane benefit. The PERVAL scale had been previously used also in Slovenian environment, where the author found that region of origin has a significant influence on the perceived value of chicken salami, which found to be at least as important as the four elements of the marketing mix (Vukasović, 2010).

Our study aimed to identify the dimensions of perceived value for wine from the perspective of Slovenian wine consumers and understand how they influence wine purchasing decisions. We also wanted to understand how the preferences of Slovenian wine regions differ depending on the dimensions of the perceived value for wine. Finally, we aimed to understand if the dimensions of the perceived value for wine differ depending on the socio-demographics of the respondents; and in the conclusion part of the paper also to give wine producers some guidance based on our findings, which could improve their marketing impact.

2 MATERIALS AND METHODS

2.1 QUALITATIVE RESEARCH

Prior to conducting the quantitative market research, a series of focus group meetings were conducted in order to understand all aspects of the perceived value of wine from the perspective of Slovenian wine consumers. A test focus group, represented by seven participants was held in June 2011 to prepare questions for other focus groups in a language understandable to average wine drinkers. In July 2011, three focus groups were conducted with a total of 20 Slovenian wine consumers (seven, five and eight participants). Each focus group consisted of participants from different socio-economic statuses and adult age groups. The main purpose was to obtain a set of indicators which would best describe the perceived value of wine from the perspective of Slovenian wine consumers so that, if required, we could upgrade the PERVAL scale for wine, which was previously used to study the perceived value of wine in a U.S. environment (Orth et al., 2005). Accordingly, the scale was updated by adding two indicators which were frequently mentioned during focus group discussions: »produced in a wine region that has the potential to produce high quality wines« and »produced in wine region with long wine-making tradition«. Focus group meetings were used as a basis to develop the questionnaire for the quantitative part of the study.

2.2 QUANTITATIVE RESEARCH

The data for the study were gathered in July and August 2016 from 221 Slovenian wine consumers, who were residents of two major Slovenian wine regions: 109 from the Primorska and 112 from the Podravje wine regions. The majority of high quality Slovenian wine is produced in the two biggest Slovenian wine regions, and while both have a long wine-making history and tradition, they belong to different wine zones ('Council regulation (EC) No 479/2008 on the common organization of the market in wine'), and they consequently produce different varieties and styles of wines. The online questionnaire used in this study was pretested online on a sample of 20 respondents from each studied wine regions to ensure that no semantic and measurement problems existed. Data were gathered through an online questionnaire using 1KA online software (University of Ljubljana, Faculty of Social Sciences), by means of convenience sampling with respect to balance in age, gender and place of residence. A specialized market research service provider was contracted to recruit their panel of respondents by email according to the sampling requirements. The screening section of the questionnaire asked four inclusion questions: the respondents had to (a) be born and reside in one of the two studied wine regions, (b) be above 18 years of age, (c) drink wine at least once per month and d) buy wine at least twice per year. Wine professionals, winemakers and sommeliers were excluded with a subsequent exclusion question. Those who were included were administered the remainder of the questionnaire, which was divided into three sections relevant for this paper: (1) wine purchasing and consumption habits; (2) PERVAL measures and wine region preferences; and (3) socio-demographic questions. The response rate of the contacted respondents was 48 %.

2.3 CONSTRUCTS MEASUREMENT

To assess wine purchasing, consumption habits and socio-demographic characteristics of the sample, closed questions with one possible answer were used. Standard socio-demographic questions were used. To assess preferences for wine regions, 7-point Likert scale was used. The main focus of the study was to identify the dimensions of the perceived value for wine. Consumer perceived value was measured using the PERVAL scale, which was developed by Sweeney & Soutar (2001) and adapted by Orth et al. (2005) to investigate the perceived value for wine, where it was validated in a U.S. environment as a six-factor scale. We adjusted the PERVAL scale by including two region of origin specific indicators, which were frequently mentioned during focus group discussions. We also re-phrased some of the claims due to semantic differences that were realized during focus group discussions to ensure the respondents would have a clear understanding of the claims. Our PERVAL scale consisted of 21 items, and a seven-point Likert scale ranging from 1 (strongly disagree) to 7 (strongly agree) was used to measure the intensity of all indicators.

2.4 DATA ANALYSIS

Analysis of wine purchasing and consumption habits were based on the type of variables and analyzed with t-test or chi-square test to understand the differences between the two studied wine regions. Exploratory factor analysis was used for the identification of number of factors in the PERVAL scale and confirmatory factor analysis was used to demonstrate the reliability and validity of the scale. To assess the connection between the dimensions of the perceived value for wine and socio-demographic data, Pearson's and Spearman's correlation coefficients were used. To calculate how the preferences towards different wine regions differ according to the dimensions of the perceived value for wine, multiple regression analysis was used. Preferences towards different winegrowing regions (measured on a Likert scale from 1 to 7) were independent variables, dependent variable was each dimension of the perceived value for wine.

3 RESULTS AND DISCUSSION

3.1 SAMPLE DESCRIPTION

Total sample consisted of 53 % male and 47 % female respondents. Average age of the respondent was close to 41 years of age, with most of them having at least secondary school education. 79 % of respondents were living in a small town or village. 21 % had below, 17 % above, and the rest average monthly income relative to the national average. More than half of respondents from both wine regions (53 %)

Table 1: Socio-demographic characteristics of the sample (n = 221)

		Primorska winegrowing region		Podravje winegrowing region		Total sample	
Socio-demographic characteristics		Count	Column N %	Count	Column N %	Count	Column N %
Gender	Male	62	57	56	50	118	53
	Female	47	43	56	50	103	47
Employment status	Student	8	7	13	12	21	10
	Unemployed	8	7	11	10	19	9
	Employed	81	74	78	70	159	72
	Retired	12	11	10	9	22	10
Size of place of residence	Town (above 100.000 inhabitants)	**9**	**8**	**37**	**33**	46	21
	Small town (10.000-100.000 inhabitants)	**34**	**32**	**20**	**18**	54	25
	Village (below 10.000 inhabitants)	64	60	54	49	118	54
Education status	High school and below	41	38	48	43	89	40
	Graduate degree	30	28	26	23	56	25
	Post-graduate degree	38	35	38	34	76	34
Monthly income	Under average	**14**	**13**	**32**	**29**	46	21
	Average	73	68	62	56	135	62
	Above average	20	19	17	15	37	17

Values in the same row and subtable marked with bolded fonts are significantly different at $p < .05$ in the two-sided test of equality for column proportions. Tests assume equal variances. Tests are adjusted for all pairwise comparisons within a row of each innermost subtable using the Bonferroni correction.

usually consumed white wines, 44 % reds and less than 3 % sparkling or rose wine. 48 % of respondents most commonly purchased wine at the supermarket stores and 35 % at winemakers. Average price of purchased wine was 8 EUR or below, highest number of respondents (35 %) in the last year usually bought wine for the price between 3 and 5 EUR. Looking at the preferences toward Slovenian winegrowing regions, we found that Primorska is the most preferred wine region (M = 6.15; SD = 1.28), followed by wine region Podravje (M = 5.48; SD = 1.45). Posavje and foreign wine regions were less preferred (M = 4.59; SD = 1.61; and M = 4.35; SD = 1.61, respectively). A comparison of socio-demographic characteristics of the respondents from the two winegrowing region samples indicates that samples were similar in relation to gender distribution, employment status and level of education. Some socio-demographic differences occurred due to demographic differences between the compared wine regions, namely size of place of residence and monthly income; significantly more respondents from winegrowing region Podravje had their monthly income below national average and more of them came from bigger town (table 1).

3.2 VALIDITY AND RELIABILITY OF THE PERVAL SCALE

We conducted exploratory factor analysis using SPSS software version 21 to verify the reliability and uni-dimensionality of the constructs. Statistically significant Bartlett test of sphericity and the Kaiser-Meyer-Olkin (KMO) above the 0.5 threshold confirmed suitability of data for factor analysis (Sharifpour et al., 2014). By use of the scree diagram, three factors for perceived value of wine were extracted: quality-price (quality and price indicators were highly correlated), emotional-social (high correlation of social and emotional indicators) and a factor we named terroir, where indicators for region of origin, humane and environmental indicators were highly correlated. The mean importance of quality-price was 5.3 (SD = 1.4), indicating that respondents paid most attention to the product's quality and price, followed by terroir (M = 5.1; SD = 1.6) and the factor explaining the emotional-social value of wine (M = 3.1; SD = 1.7). The factor loadings on all items were significant and exceeded the desired 0.7 threshold (Hulland, 1999) except for four items with loadings above 0.6; which were expected to reflect important information and were thus kept in for further

Table 2: Quality of the PERVAL scale (n = 221)

Constructs and items of the PERVAL scale (Crombach alpha)	Standardized loadings	% of Variance	CR	AVE
Factor Terroir (0.9)		24.1	0.95	0.74
…is produced in a wine region that has the potential…	0.8			
…is produced in an environmentlly friendly manner	0.8			
…is produced in wine region with long wine-making tradition	0.8			
…is crafted by dedicated individuals	0.8			
…is made from grapes under strictly controlled environment	0.8			
…is crafted by very special and unique experts	0.8			
…is made without polluting the environment	0.6			
Factor Emotional-social (0.8)		14.5	0.9	0.7
...improves the way I am perceived by others	0.7			
…gives its owner social approval	0.9			
…makes me feel good	0.9			
…would give me pleasure	0.6			
Factor Quality-price (0.8)		12.7	0.87	0.62
…has consistent quality	0.6			
…offers value for money	0.8			
…is reasonably priced	0.7			
... is a good product for the price	0.6			

Inexes of fit: $\chi 2(84) = 178.62$; $p < 0.001$; RMSEA = 0.07; NFI = 0.94; NNFI = 0.96; CFI = 0.97; IFI = 0.97; SRMR = 0.07
CR = composite reliability coefficient, AVE = average variance extracted.

analysis. The loadings for two indicators did not exceed 0.4 threshold and were excluded from further analysis. Cronbach's alpha for all factors was well above the desired 0.7 threshold. The variance explained by the three dimensions was satisfactory at 53 %. In addition to the reliability analyses, we used indexes to demonstrate the fit of model to the data, as demonstrated in Table 2. Confirmatory factor analysis was done with AMOS IBM 21 program with robust maximum likelihood method.

3.3 DIMENSIONS OF THE PERCEIVED VALUE FOR WINE WITH REGARD TO WINE REGIONS

Slovenian wine consumers perceive three different dimensions of value in wine: quality-price, emotional-social and a terroir dimension. In American paper from 2005 they found, that American consumers perceive six different dimensions of value for wine and those were: quality, price, emotional, social, environmental and humane dimension. In this research it was also shown that the consumers for which quality dimension is more important, prefer French wines, and those for which price is more important, prefer Californian and Australian wines (Orth et al., 2005). We were interested to find, which dimensions of the perceived value for wine Slovenian consumers link to which wine regions. The relationships between the dimensions of the perceived value for wine and preferences for different wine regions are depicted in Table 3.

We found that both wine regions, Primorska and Podravje, are associated with the terroir dimension of the perceived value for wine ($p < 0.001$ for both wine regions), and quality-price dimension ($p < 0.001$ for both regions), meaning that consumers, who value these two dimensions of the perceived value more, will rather choose wine from either of those two wine regions. Regression coefficient tells us, how much each of the perceived value dimension changes, if the preference for a wine region changes by one point. This means that wine region Podravje is more tightly connected with the terroir dimension of the perceived value for wine, than Primorska. Both wine regions are connected to the same extent also with quality-price dimension of the perceived value for wine. There is no connection between the preference for any wine region and emotional-social dimension and also, there is no connection between the wine region Posavje or foreign wine regions and any of the dimensions of the perceived value for wine. Despite the fact, that the overall preference is higher for Primorska wine region, the total sample believes that the same two value dimensions are important when purchasing wine from either Primorska or Podravje wine regions, namely terroir and quality-price. The respondents in our sample, who appreciate the terroir of wine more, would more likely choose wines from Podravje winegrowing region. It is possible that the respondents from our sample were not familiar enough with wines from region Posavje and foreign countries, and this could be the reason why these wines are not percieved as being connected with any of the studied value dimensions. Emotional-social was rated much lower than the other two value dimensions and it seems that to Slovenian consumers from our sample this dimension is so unimportant, that they do not consider it when choosing wine from any of the winegrowing regions. Another question is why American consumers consider five, and Slovenian consumers only three value dimensions while choosing wine. It is likely that because American consumers come from a more mature capitalist market, they are therefor more susceptible to a variety of marketing messages. On the other hand Slovenian wine consumers are more traditional, considering mainly the connection between the quality and price and the terroir of wine, meaning the region of origin and the tradition of the winegrowing region.

3.4 DIMENSIONS OF THE PERCEIVED VALUE FOR WINE WITH REGARD TO SOCIO-DEMOGRAPHIC CHARACTERISTICS

Lastly, we aimed to understand if the dimensions of the perceived value for wine differ depending on the

Table 3: Connection between wine region preferences and dimensions of the perceived value for wine

Perceived value dimension	Terroir		Emotional-social		Quality-price	
	B	P-value	B	P-value	B	P-value
Constant	2.58	< 0.001	3.22	< 0.001	2.98	< 0.001
Primorska	0.25	< 0.001	– 0.094	0.172	0.2	< 0.001
Podravje	0.28	< 0.001	0.105	0.133	0.2	< 0.001
Posavje	– 0.11	0.061	– 0.014	0.819	– 0.04	0.446
Foreign wine regions	– 0.03	0.637	0.027	0.639	0.02	0.615

B = regression coefficient.

Table 4: Relation between socio-demographics and perceived value for wine (n = 221)

Perceived value dimension		Terroir	Emotional-social	Quality-price
Age	Pearsons r	**0.15**	0.07	0.08
	P-value	**0.029**	0.276	0.245
Number of persons in the hausehold	Pearsons r	– 0.03	0.07	– 0.05
	P-value	0.646	0.335	0.448
Size of place of residence	Spearmans r	– 0.01	– 0.04	– 0.02
	P-value	0.914	0.543	0.819
Education	Spearmans r	– 0.04	–0.10	**– 0.17**
	P-value	0.589	0.124	**0.013**
Monthly income	Spearmans r	0.05	0.01	**– 0.14**
	P-value	0.468	0.908	**0.039**

socio-demographic characteristics of the respondents. We found that there is weak, but statistically significant connection between respondents age and terroir dimension of the perceived value for wine, meaning that older respondents value this dimension more than younger. We also found weak negative statistically significant link between education and monthly income and quality-price dimension of the perceived value for wine, meaning that for these consumers wine price or the ratio between quality and price is less important when purchasing wine. There were no differences in terms of consumers employment status and family status and the perceived value for wine (Table 4). These results are relevant when creating consumer segmentation, having in mind which are the most important consumer segments that are the basis for the creation of marketing strategy.

4 CONCLUSIONS

Our research demonstrated, that in the sample of Slovenian wine consumers from the two largest Slovenian winegrowing regions, who at least occasionally buy and drink wine, perceive three different dimensions of the perceived value for wine: quality-price, emotional-social and a dimension which we named "terroir", where the indicators for region of wine origin, humane and environmental claims highly correlated. Quality-price and terroir dimensions were rated above five on a seven-point Likert scale, indicating that both dimensions are very important to Slovenian wine consumers. Emotional-social dimension was rated just over three, meaning that marketing strategy from wine-makers and wine-sellers should focus on supporting superior terroir and quality-price ratio messages.

Both, quality-price and terroir dimensions correlated with Primorska and Podravje wine regions, but not with others. When making wine purchasing decisions, older respondents give greater value to the terroir dimension, while respondents with higher education and income are less concerned with the quality-price ratio. We propose that these information are taken into account in order to improve consumer segmentation and targeting.

According to the methodological requirements of this research, while measuring the constructs of consumer regiocentrism and regional identity, only consumers from wine regions Primorska and Podravje were included. It would be interesting to check if the perception of other (non-winegrowing regions and the third Slovenian winegrowing region-Posavje) consumers with regard to the perceived value would differ or confirm our findings. Also, we suggest that future research does not focus only on wine purchasing in terms of where the wine is bought, but also on the wine choice at the point of consumption (restaurants, social events etc.).

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Comparison of the toxicity and repellency of two conventional neonicotinoids and a coconut-derived insecticide soap toward the parasitoid wasp *Aphelinus mali* Haldeman, 1851

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Comparison of the toxicity and repellency of two conventional neonicotinoids and a coconut-derived insecticide soap toward the parasitoid wasp *Aphelinus mali* Haldeman, 1851

Abstract: The parasitoid wasp *Aphelinus mali* Haldeman, 1851 (Hymenoptera: Aphelinidae) is the most important biological control agent against the woolly apple aphid, *Eriosoma lanigerum* (Hausemann, 1802) (Hemiptera: Aphididae), which is an important apple orchards pest throughout the world. Based on the importance of using low-risk compounds to protect beneficial agents, the present study was carried out to evaluate the toxic and repellent effects of two conventional chemicals (imidacloprid and thiacloprid) and coconut-derived biopesticide soap (Palizin®) toward *A. mali*. The results of residual bioassays on apple leaf discs indicated that imidacloprid after 24 h and insecticide soap after 72 h exposure time categorized at the highest and no/little toxicity rates, respectively. Ingestion bioassays on filter papers revealed that imidacloprid and thiacloprid had moderate toxicity rate, while insecticide soap had a low-level toxicity rate. Repellency test at Y-tube olfactometer showed that the repellent effects of both chemicals were more than that of insecticide soap. It is concluded that coconut-derived soap was compatible with the parasitoid activity, and the caution should be paid when including the two neonicotinoid insecticides imidacloprid and thiacloprid for *E. lanigerum* management.

Key words: *Eriosoma lanigerum*; *Aphelinus mali*; insecticides; toxicity; repellency

Primerjava strupenosti in odvračalnega delovanja dveh konvencionalnih neonikotinoidov in insekticidnega mila iz kokosa na krvavkinega najezdnika (*Aphelinus mali* Haldeman, 1851)

Izvleček: Parazitoidna osica krvavkin najezdnik (*Aphelinus mali* Haldeman, 1851, Hymenoptera: Aphelinidae) je najpomembnejši naravni sovražnik krvave uši (*Eriosoma lanigerum* [Hausemann, 1802], Hemiptera: Aphididae), ki je pomemben škodljivec jablan širom po svetu. Zaradi vse večjega pomena okoljsko sprejemljivejših snovi pri zatiranju rastlinskih škodljivcev, saj na ta način varujemo tudi koristne žuželke, smo v raziskavi ovrednotili toksične in repelentne učinke dveh konvencionalnih kemičnih sredstev za varstvo rastlin (imidakloprid in tiakloprid) in bioinsekticidnega mila (Palizin®) iz kokosa na vrsto *A. mali*. Rezultati poskusa na diskastih izsečkih jabolčnih listov so pokazali, da sta imela imidakloprid 24 h po nanosu in insekticidno milo 72 h po nanosu največji učinek na uši in nič ali le malo strupenega učinka na parazitoida. Prehranjevalni poskus na filtrirnem papirju je pokazal, da sta imela imidakloprid in tiakloprid zmeren toksični učinek, medtem, ko je imelo insekticidno milo zelo majhnega. Preizkusi odvračalnega delovanja snovi v Y-cevastem olfaktometru so pokazali, da so bili odvračalni učinki obeh kemikalij večji kot pri insekticidnem milu. Zaključimo lahko, da je kokosovo milo kompatibilno s parazitoidom, več pozornosti pa je potrebno nameniti, kadar za zatiranje krvave uši uporabimo oba neonikotinoidna insekticida, imidakloprid in tiakloprid.

Ključne besede: *Eriosoma lanigerum*; *Aphelinus mali*; insekticidi; toksičnost; repelenca

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1 INTRODUCTION

The woolly apple aphid (*Eriosoma lanigerum* (Hausemann, 1802), Homoptera: Aphididae) is important pest in apple orchards. It can causes significant damages to the apple trees by sucking the sap from new shoots and branches (Rogers et al., 2011; Lordan et al., 2015). Its feeding activity leads to the formation of galls on woody tissue and young shoots. Buds can be destroyed and apple calyces may also be contaminated with the aphid honeydew and sooty molds (Beers et al., 2007). The woolly aphid parasitoid [*Aphelinus mali* Haldeman, 1851, (Hymenoptera: Aphelinidae)], is a specific and very efficient endoparasitoid of *E. lanigerum* that had been introduced from North America to other apple-growing regions of the world, such as in Iran (Heunis & Pringle, 2003, Su et al., 2017).

Although the application of synthetic insecticides is the conventional method for the management of *E. lanigerum*, such strategy can have various negative side effects, such as impact on non-target organisms, pest resistance and threats to human health (Desneux et al., 2007; Damalas & Eleftherohorinos, 2011). For example, organic phosphorus insecticides, which are widely applied to reduce populations of *E. lanigerum*, cause severe damage in its parasitoid wasp populations (Beers et al., 2007). Recent researches have shown that the chemical agents have lethal and sublethal effects on the hymenopterous parasitoids. For example, Desneux et al. (2004) indicated that lambda-cyhalothrin had a high toxicity on *Aphidius ervi* Haliday, 1834 (Aphidiinae). Further, low doses of this chemical weakened the orientation behavior of *A. ervi*. Toxic effects of the deltamethrin and its negative effects on recolonization capacities of *A. ervi* was also documented (Desneux et al., 2006). The adults of egg parasitoid wasp *Trichogramma chilonis* Ishii, 1941 (Trichogrammatidae) were very susceptible to chlorfenapyr, fipronil, spinosad, avermectins, ß-cypermethrin, and cartap (Wang et al., 2012). In other study, acute toxicity of mineral oil and pyriproxyfen (a juvenile hormone mimic) was tested against the parasitoid wasp *Aphytis melinus* DeBach, 1959 (Aphelinidae). Mineral oil had high mortality on the adults of *A. melinus*, but pyriproxyfen had neither lethal nor sublethal effects. However, parasitoid larvae were very susceptible to pyriproxyfen (Biondi et al., 2015).

Accordingly, the application of suitable chemical control tools that can reduce the side effects of pesticides and prevent damage to a pest's natural enemies is necessary. The aim of the present study was, therefore, to assess the non-target toxicity of two conventional neonicotinoids imidacloprid and thiacloprid and a novel coconut-derived bioinsecticide soap (Palizin®) on the parasitoid wasp *A. mali* adults in the experimental conditions.

2 MATERIALS AND METHODS

2.1 TEST INSECT

All bioassays were conducted using a parthenogenetic (all females) strain of *A. mali.* Apple branches ('Red Delicious') infested with *E. lanigerum* were collected from apple orchards at Miami County (Semnan province, Iran) (36°N 55°E), in June 2016, and placed in one-liter flasks until emergence of the parasitoid wasps. Prior to the experiment, the *A. mali* colony was reared for 3 to 4 generations in an incubator at 27 ± 1 ºC, 55 ± 5 % Relative Humidity (RH) and 14 L: 10 D photoperiod, using apple leaves infested with *E. lanigerum*. Adult parasitoids were kept in glass tubes and fed on 10 % honey-water solution smeared inside the test tubes' wall.

2.2 INSECTICIDES

Three commercial formulations of insecticides were used in the present study: imidacloprid concentrate suspension (Confidor®), provided as a commercial product by Paksame Iranian Company with 35 % active ingredient, thiacloprid (Calypso®) concentrate suspension, provided as a commercial product formulized by Bayer Company with 48 % active ingredient, and the coconut soap (Palizin®) as concentrate suspension was purchased from Iranian Kimia Sabzavar Company with 65 % active ingredient. For bioassays, maximum recommended concentrations were used: 0.5, 0.2 and 1.5 ml l^{-1} insecticide/distilled water imidacloprid, thiacloprid and the insecticide soap, respectively.

2.3. RESIDUAL CONTACT BIOASSAY ON APPLE LEAVES

Fresh apple leaves (*Malus pumila* Miller) were sampled from apple orchards, which had no history of chemical control in the past three years. Fresh apple leaves were sprayed by recommended concentrations of insecticides until runoff by using a hand sprayer (Williams et al., 2003). The leaves were put into a chemical hood for an hour and then transferred to Petri dishes, 9 cm of diameter. Twenty-five, one-day-old female parasitoids were placed in each Petri dish, and then the dishes were kept at room conditions at 27 ± 1 °C and 55

± 5 RH. In the untreated control groups, distilled water was used instead of insecticide concentrations. Each experiment was repeated four times and mortality was recorded 24, 48 and 72 h after the exposure beginning.

2.4 INGESTION BIOASSAY ON FILTER PAPER

To improve the feeding of the parasitoid adults and to mimic the field situation where they custom external nutrients, the same mentioned concentrations were prepared by adding 10 % of sugar. The filter papers (5 cm of diameter) was soaked in one ml of the solutions and were placed in the center of the 9cm Petri dishes (Rogers et al., 2011). Twenty-five parasitoid adults were transferred to each Petri dish under the laboratory conditions as described above. In the control groups, distilled water with 10 % sugar was also used instead of insecticides. The experiment was repeated 4 times and mortality rates was recorded 72 h post exposure.

2.5 REPELLENT EFFECT

The Y-tube olfactometer was assembled by 3 linked glass tubes (each 10 cm long and 1cm in diameter). The flow of air was established by an adjustable fan. To evaluate the repellency of the insecticides, infested apple leaves with 15 adult females of aphids were sprayed at the recommended concentrations and allowed to dry for one hour under test conditions. The insecticide-treated leaves were inserted in one of the openings of the Y-tube olfactometer and the other opening was considered as a control. The control groups consisted of leaves treated only with distilled water. Thirty adult parasitoids were released inside a one-liter glass container covered by a black cloth for stimulating the wasps to move from darkness to light, thus allowing the selection of one of the arms of the device. Number of insects were recorded on each side of the tube every 3 h for 18-hour duration. Y-tube olfactometer was kept in experimental conditions with 27 ± 1 °C and 55 ± 5 RH and tests were repeated 3 times (Sadeghi et al., 2014).

2.6 DATA ANALYSIS

Data of parasitoid mortality in the bioassays were corrected using Abbott's formula (Abbott, 1925) when mortality was observed in the control groups. Data analysis was carried out by SPSS software (Version 24). Data obtained after 24, 48 and 72 h of residual exposure and those from the ingestion bioassay were checked for normality using the Kolmogorov-Smirnov test. All data were normally distributed and thus the one-way analysis of variance (ANOVA) was run for each dataset. Mean separation was assessed using the Tukey's post hoc test at $p \leq 0.05$. Insecticide toxicity was rated according to IOBC toxicity categories (Sterk et al., 1999): (1) harmless, (2) slightly harmful, (3) moderately harmful, and (4) harmful (corresponding to reductions below 30 %, between 31-79 %, between 80-99 % and higher than 99 %, respectively). Percentage repellency (PR) was calculated as follows: $PR = [(C - T) / (C + T)] \times 100$, where C = number of wasps on the treated area with distilled water, and T = number of wasps on the area treated with insecticide (Nerio et al., 2009). Classification of PR values was done according to Juliana and Su (1983): class 0 (PR = 0.1 %), class I (PR = 0.1–20 %), class II (PR = 20.1–40 %), class III (PR = 40.1–60 %), class IV (PR = 60.1–80 %) and class V (PR = 80.1–100 %).

3 RESULTS

3.1 RESIDUAL CONTACT BIOASSAY

In the residual contact bioassay on apple, although all the insecticides caused significant mortality ($F = 75.044$, df = 2, 11, $p < 0.001$) after 24 h, imidacloprid caused the highest rate of parasitoid mortality (78.850 ± 4.703%); while insecticide soap caused the lowest mortality (28.351 ± 2.413%) at this duration. Mortality was also statistically significant after 48 ($F = 274.485$, df = 2, 11, $p < 0.001$) and 72 ($F = 209.515$, df = 2, 11, $p < 0.001$) h. In general, the mortality rates decreased as time pass due to reduction in residual concentrations. For example, mortality significantly decreased from 32.609 ± 2.413 % at the 24 hour exposure time to 16.304 ± 2.030 % after 72 h for thiacloprid (Figure 1 and Table 1). Observed mean mortality in the control groups were 5.00 ± 0.25 % after 24, 48 and 72 h. Toxicity ratings for insecticidal activity of imidacloprid, thiacloprid and insecticide soap in bioassays with apple leaves are also shown in Table 1. According to toxicity rating of IOBC, imidacloprid had moderately harmful effect after 24-hour and insecticide soap had lowest toxicity (harmless) after 72 hour exposure period (Table 1).

3.2 INGESTION BIOASSAY

All tested insecticides had significant effects on the parasitoid mortality ($F = 174.915$, df = 2, 11, $p < 0.001$) in the ingestion bioassays. After 72 h of exposure, imidacloprid caused the highest rate of parasitoid mortality (69.042 ± 1.011 %) and insecticide soap showed the lowest rate of mortality (29.875 ± 1.628%). However, insignificant differences were found between imidacloprid and thiaclo-

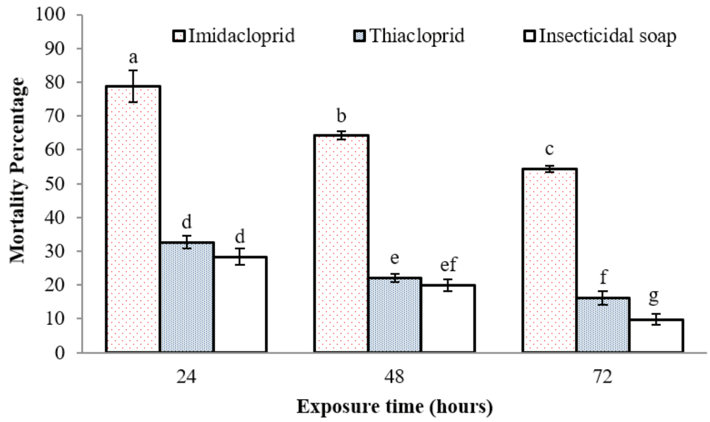

Figure 1: Mean percentage mortality (± SE) of *Aphelinus mali* females after exposure to dry residues of imidacloprid, thiacloprid and a coconut-derived insecticide soap on apple leaves. Mortalities (%) compared with Tukey's test at $p \leq 0.05$ in which same letters are not significantly different.

Table 1: International Organization for Biological Control (IOBC) toxicity classes for *Aphelinus mali* females exposed to dry residues in apple leaves or that have fed on sugary solutions contaminated with imidacloprid, thiacloprid and insecticide soap

Bioassay	Insecticide	Exposure Time (h)	Toxicity Rating
Residual Contact	imidacloprid	24	moderately harmful
		48	slightly harmful
		72	slightly harmful
	thiacloprid	24	slightly harmful
		48	harmless
		72	harmless
	insecticide soap	24	harmless
		48	harmless
		72	harmless
Ingestion	imidacloprid	72	slightly harmful
	thiacloprid	72	slightly harmless
	insecticide soap	72	harmless

Classification of PR values followed that cited in Juliana and Su (1983): Harmless, slightly harmful, moderately harmful, and harmful corresponding to mortality below 30 %, between 31 and 79 %, between 80 and 99 % and higher than 99 %, respectively.

prid (Table 1and Figure 2). Observed mean mortality in the control groups was 3.0 % after 72 h. Rating of insecticide toxicity indicated that imidacloprid and thiacloprid were slightly harmful while insecticide soap was harmless against *A. mali* (Table 1). In general, coconut-derived insecticide soap had lower toxicity against *A. mali* than the chemical insecticides: imidacloprid and thiacloprid in both bioassays with apple leaves and filter papers.

3.3 REPELLENT EFFECT

The repellent activity of the two neonicotinoids was more evident than that of the insecticide soap. Classification of the percentage of repellency (PR) values according to Juliana and Su (1983), indicated that insecticide soap ranked as class I (PR = 0.1–20 %) within an exposure time of 3 to 18 h. In contrast, the PR values of imidacloprid were classified as class III for all observation times and these values were in classes II and III with thiacloprid (Table 2).

4 DISCUSSION

Imidacloprid and thiacloprid as conventional neonicotinoid insecticides are effective against aphids and other

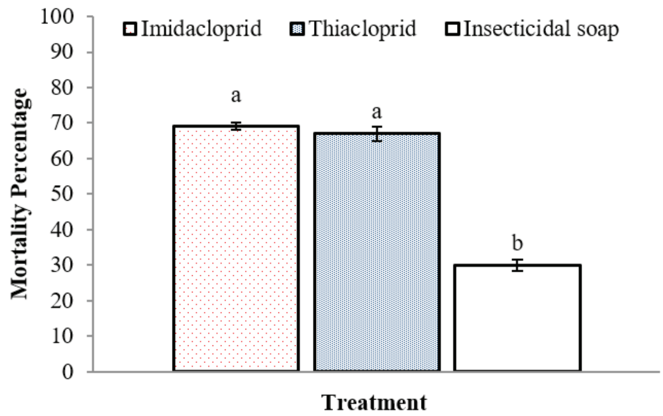

Figure 2: Mean percentage mortality (± SE) of *Aphelinus mali* exposed to imidacloprid, thiacloprid and a coconut-derived insecticide soap after feeding on contaminated sugary water in filter papers within 72 h. Mortalities (%) compared with Tukey's test at $p \leq 0.05$ in which same letters are not significantly different

Table 2: Percentage of repellency (PR) of imidacloprid, thiacloprid and coconut-derived insecticide soap against *Aphelinus mali* at different exposure times at the Y-tube olfactometer using sprayed apple leaves as volatile sources

	Time (h)					
Insecticide	3	6	9	12	15	18
Imidacloprid	49.340 (III)	48.564 (III)	52.625 (III)	47.965 (III)	36.833 (III)	53.118 (III)
Thiacloprid	43.593 (III)	33.359 (II)	38.121 (II)	35.034 (II)	40.551 (III)	38.039 (II)
Insecticide soap	14.268 (I)	10.69 (I)	15.992 (I)	17.417 (I)	14.276 (I)	16.685 (I)

Classification of PR values followed that cited in Juliana and Su (1983): class 0 (PR = 0.1 %), class I (PR = 0.1–20 %), class II (PR = 20.1–40 %), class III (PR = 40.1–60 %), class IV (PR = 60.1–80 %) and class V (PR = 80.1–100 %).

sap-sucking insects. However, insect natural enemies and pollinators such as honeybees, *Apis mellifera* L., are the later reported to be affected by the exposure to imidacloprid and thiacloprid (Schmuck et al., 2001; Blacquiere et al., 2012; Fischer et al., 2014). Toxicity of imidacloprid and thiacloprid to eggs, N1 and N5 nymphs and adults of the predatory bug, *Deraeocoris lutescens* (Schilling, 1837) (Hemiptera: Miridae), via residual contact was investigated and results showed that all stages were susceptible to these chemicals (Azimizadeh et al., 2012). Sublethal concentrations of imidacloprid reduced the longevity and the fecundity of females of the seven-spot ladybird beetle *Coccinella septempuntacta* L. (Coleoptera: Coccinellidae). Moreover, similar effects were noticed on the F1 generation, i.e., the progeny of the exposed individuals (Xiao et al., 2016). In the study of Martinou et al. (2014), the direct, residual and oral toxicity of thiacloprid were evaluated against *Macrolophus pygmaeus* (Rambur, 1839) (Hemiptera: Miridae) as a common generalist predator in Mediterranean agroecosystems. Along with lethal effects, thiacloprid caused an increase in resting and preening times of the predator. Based on the significant acute toxicity of imidacloprid and thiacloprid against *A. mali*, results of the mentioned studies are consistent with the results of the present work. Accordingly, the use of these chemicals cannot be compatible with the activity of this parasitoid and thus they should be used properly taking into consideration its effects on non-target organisms for successful integrated pest management (IPM) strategies. However, they clearly had less toxicity than some organophosphate insecticides such as chlorpyrifos, diazinon and carbaryl, which have been categorized as "highly toxic" compounds by Bradley et al. (1997). Further, Rogers et al. (2011) reported that thiacloprid had no or low-level toxicity on *A. mali* in the contact toxicity with filter papers, while in the present study it showed low and moderate levels of toxicity in the bioassays with apple leaves and filter papers, respectively. Different experimental conditions and materials might contribute to the variations in results. For example, contact toxicity in the study of Rogers et al. (2011) was carried out by filter paper but in the present study leaf discs were used. Furthermore, according to results of the present work, thiacloprid showed less toxicity in bioassays with leaf discs than that with filter papers. It is worthy to mention that leaf spray pesticide application would provide more realistic orchard conditions, resulting in lower toxicity against *A. mali*.

The insecticide soap (Palizin®), the coconut-derived pesticide, showed great potential in the management of insect pests and its toxicity and repellency against some insect pests have been recently acknowledged (Amiri-Esheli, 2009; Sadeghi et al., 2014; Sheibani & Hassani, 2014). The side effects of the Palizin® have not yet been reported before. Comparison of the Palizin® and imidacloprid toxicity was made against cotton aphid (*Aphis gossypii* Glover, 1877) and its natural enemy (*Aphidius colemani* Viereck, 1912) (Ketabi et al., 2014). In this research, imidacloprid had a higher level of toxicity against both the aphid and the wasp than Palizin® which consistent with the obtained findings.

5 CONCLUSION

Generally, the insecticide soap showed significantly less toxicity than tested chemicals in all bioassays. Furthermore, the repellent effects of insecticide soap were significantly lower than imidacloprid and thiacloprid applications. However, further studies should be conducted to assess the sublethal effects of Palizin® on the behavior and reproduction-related traits that could influence the population dynamics of the parasitoid in the field. These aspects are particularly true in the case of novel bioinsecticides that are *slower acting*, and for which the evaluation of the sole acute toxicity could not really represent the true risk for biological control agents (Biondi et al., 2012 and 2013). Obtained findings, need to be validated under realistic field conditions, considering that the novel bioinsecticide Palizin® is more suitable for the sustainable management of woolly apple aphid.

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Evaluation and comparison of drought tolerance in some wild diploid populations, tetraploid and hexaploid cultivars of wheat using stress tolerance indices

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Evaluation and comparison of drought tolerance in some wild diploid populations, tetraploid and hexaploid cultivars of wheat using stress tolerance indices

Abstract: This study was carried out on grain yield in wheat genotypes with the aim of assessing genetic potential of drought tolerance. The experiment was performed as split plot in the form of randomized complete block design with three replications under normal and drought stress conditions with 32 genotypes. Based on grain yield, and under the condition of non-stress and drought stress, 5 drought tolerance indices are estimated including Tolerance Index (TOL), Stress Tolerance (STI), Mean Productivity (MP), Geometric Mean (GMP) and, Harmonic Mean (HM) for all kinds of genotypes. The analysis of yield correlation and drought tolerance indices in two environments indicated that STI, MP, GMP, HM indices were the most suitable parameters for screening wheat genotypes. Principal components analysis exhibited that the 83 % of first principal component and the 15 % of second one justified the variation of the initial data. Drawing bi-plot diagram declared that Sabalan, Shabrang, Aria, Azar, Azadi, and T2 genotypes were highly functional and resistant to drought stress.

Key words: wheat; index; component analysis; cluster analysis

Ovrednotenje in primerjava tolerance na sušo nekaterih divjih diploidnih populacij, tetraploidnih in heksaploidnih sort pšenice z uporabo indeksov tolerance na stres

Izvleček: Raziskava je bila opravljena na pridelku zrnja med genotipi pšenice za oceno genetskega potenciala tolerance na sušo. Poskus je bil izveden kot popolni naključni bločni poskus z deljenkami s tremi ponovitvami na 32 genotipih pšenice v normalnih in sušnih razmerah. Na osnovi pridelka je bilo za vse genotype v nestresnih razmerah in v razmerah sušnega stresa določenih 5 indeksov tolerance na sušo in sice: indeks tolerance (TOL), stresna toleranca (STI), srednja produktivnost (MP), geometrična sredina (GMP) in harmonična sredina (HM). Analiza korelacije med pridelkom in indeksi tolerance na sušo je pokazala, da so bili v obeh okoljih za ovrednotenje genotipov pšenice primerni STI, MP, GMP, HM indeksi. Analiza glavnih komponent je pokazala, da je 83 % variabilnosti izvirnih podatkov pojasnila prva komponenta, 15 % pa druga. Bi-plot diagram je pokazal, da so genotipi pšenice Sabalan, Shabrang, Aria, Azar, Azadi, in T2 zelo produktivni in odporni na sušni stress.

Ključne besede: pšenica; indeks; analiza component; klasterska analiza

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1 INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important crop in the world (Sleper and Poehlman, 2006) and is cultivated in more than 250 million hectares of agricultural lands of all over the world (Royo and Di Fonzo, 2005). It is estimated that about 67 percent of the total Iranian wheat is produced in arid lands (Shamsi et al., 2011). So many breeding programs have been developed to improve drought tolerance of crop (Maazou et al., 2016). The crop rotation diversity is associated with a greater yield stability under abnormal conditions like drought stress (Gaudin et al., 2015). Selection of drought-resistant cultivars is the best solution to improve stability of crop yield in dry conditions (Farshadfar et al., 2012).

Drought tolerance is a quantitative trait that is controlled by many genes (Fleury et al., 2010). Thus, detecting of drought-tolerance genotypes is very difficult (Takeda and Matsuoka, 2008). Evaluation of relative yield of genotypes under drought stress and non-stress conditions is a starting point for identification of drought-tolerant mechanisms and for screening of drought-tolerant genotypes (Fernandez, 1992; Mitra, 2001). Based on tolerance index (TOL) as difference in the yield of two genotypes under both normal and stress conditions, the higher TOL indicates more sensitivity of plants to stress and thus, the selection was mainly based on the low amounts of TOL, but high mean productivity (MP), the mean yield of each genotype in two conditions, shows more tolerance to stress (Rosielle and Hamblin, 1981). In 1992, on the base of yield, mung bean's genotypes were divided into four groups under both normal and stress environments: (A) high-yield under both conditions; (B) high-yield under normal conditions; (C) good-yield under drought stress and (D) low-yield under both normal and drought stress condition (Fernandez, 1992). It showed that selections based on TOL and MP would be able to separate genotypes of B and D groups. By MP index, high yielding genotypes under both normal and stress conditions could be selected but this index is not able to distinguish between A and B groups. The MP index also leads to choose high yielding genotypes but with a low stress tolerance (Fernandez, 1992; Rosielle and Hamblin, 1981). When there is a significant relative difference between the yield under stress and non-stress conditions, MP index is oriented toward the yield under normal conditions so to obviate this problem, geometric mean productivity (GMP) index that is based on the geometric mean of genotypes yield under normal and stress conditions was proposed by Fernandez (1992). Since this index is less sensitive to different values in normal conditions and drought stress, Fernandez introduced another index called stress tolerance index (STI), in order to identify high-yield genotypes under two normal and stress conditions and also for identifying drought tolerance genotypes. This index considers the stress intensity (SI) that is estimated as mean ratio of genotypes yield in the stress environment to the normal environment from one (Fischer and Maurer, 1978; Schneider et al., 1997). High values of GMP and STI are subjected to determine stress tolerance genotypes. Harmonic mean index (HM) was also introduced by Fernandez. These indices are introduced as the best having a high correlation with grain yield in both normal and stress environments (Fernandez, 1992).

In 2006, STI, GMP, and MP indices were evaluated for selecting of wheat genotypes under water deficit conditions (Golabadi et al., 2006; Mardeh et al., 2006). Also, in 2009, STI, GMP, and MP were introduced as the most effective indicators for evaluating and selecting wheat genotypes under drought stress (Talebi et al., 2009).

The purpose of the present study is to determine the genotypes with yield stability under drought stress and non-stress conditions as well as introducing the appropriate indices for screening wheat genotypes under stress and non-stress conditions.

2 MATERIALS AND METHODS

In this experiment, 7 population of wild diploid wheat (*Triticum boeoticum* Boiss.) with AA genome, 5 population of wild diploid wheat (*Aegilops tauschii* Coss.) with DD genome, 10 cultivars of tetraploid wheat (*Triticum durum* Desf.) with AABB genome and 10 cultivars of hexaploid wheat (*Triticum aestivum* L.) with AABBDD genome, (Table 1) have been used. Wheat seeds were implanted in research farm of Graduate University of Advanced Technology in Kerman province in October 2016. The experiment was designed as split plot in a randomized complete block design (RCBD), with three replications, assigning normal and stress levels as main plots and population and wheat cultivars as sub-plots. Each plot consisted of three lines keeping 2 m length and 20 cm distance within which on each line 15 seeds had been implanted. All plots of the normal and stress experiments were well watered (once every 7 days) by using an installed pipeline system. The volume of water input for each plot was controlled by using adjustable counter. Since April (the grain-filling period), drought stress treatment, irrigation at 30-day intervals, was imposed on the plants until the beginning of June (time of harvesting). After harvesting each plot at crop maturity, grain yield was recorded in both normal and stress conditions.

Tolerance index, stress tolerance index, mean pro-

Table 1: Names of genotypes used in the study

Genotype	Species	Genome	Ploidy level	Source
B1	*Triticum. boeticum*	Wild Diploid Genome AA	2n = 2x = 14	Lorestan-10 km to Nurabad-On behalf of Alshatr
B2	*Triticum. boeticum*	Wild Diploid Genome AA	2n = 2x = 14	Lorestan - Firouzabad-Khorramabad Road
B3	*Triticum. boeticum*	Wild Diploid Genome AA	2n = 2x = 14	Kermanshah - Deh sefeed-Not-To-Dumbel Islamabad Kermanshah
B4	*Triticum. boeticum*	Wild Diploid Genome AA	2n = 2x = 14	Kermanshah - 20 km from Paveh to Ravensar
B6	*Triticum. boeticum*	Wild Diploid Genome AA	2n = 2x = 14	Kurdistan - Two Sanandaj Moths
B7	*Triticum. boeticum*	Wild Diploid Genome AA	2n = 2x = 14	Kurdistan - 10 km after Ganji to Ghorveh
B8	*Triticum. boeticum*	Wild Diploid Genome AA	2n = 2x = 14	Kurdistan – Bolban abad city
T1	*Aegilops tauschii*	Wild Diploid Genome DD	2n = 2x = 14	Gilan - Isfahan-Rasht Road
T2	*Aegilops tauschii*	Wild Diploid Genome DD	2n = 2x = 14	Mazandaran-Amol
T3	*Aegilops tauschii*	Wild Diploid Genome DD	2n = 2x = 14	Karaj Road - Chalous (50 km before Chalous)
T4	*Aegilops tauschii*	Wild Diploid Genome DD	2n = 2x = 14	Ardebil-Moghan-Parsabad plain
T5	*Aegilops tauschii*	Wild Diploid Genome DD	2n = 2x = 14	East Azarbaijan - 10 km from Ahar-Kalibar road
Aria	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
Behrang	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
Dehdasht	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
Dena	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
Shabrang	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
Karkhe	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
Yavarus	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
DW/95/4	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
DW/90/8	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
WS	*Triticum durum*	Tetraploid AABB	2n = 4x = 28	
Azar	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
Azadi	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
Pishtaz	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
Roshan	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
Sabalan	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
Superhead	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
Shiraz	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
Hirmand	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
Moghan	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	
mr-17	*Triticum durum*	Hexaploid AABBDD	2n = 6x = 42	

Table 2: Formulas drought tolerance indices

Index	Formula	Source
Tolerance Index (TOL)	Y_p - Y_s	(Rosielle and Hamblin, 1981)
Stress Tolerance Index (STI)	$(Y_p*Y_s) /(\bar{y}_P)^2$	(Fernandez, 1992)
Mean productivity (MP)	(Yp + Ys) / 2	(Rosielle and Hamblin, 1981)
Geometric Mean Productivity (GMP)	$(Y_P*Y_S)^{½}$	(Fernandez, 1992)
Harmonic Mean (HM)	[2(Yp × Ys)/ (Yp + Ys)]	(Fernandez, 1992)

ductivity (Rosielle and Hamblin, 1981) and the geometric mean productivity and harmonic mean (Fernandez, 1992) were calculated according to the following formulas (Table 2):

In which, YP and YS are the yield of each genotype under non-stress and stress conditions, respectively, and ȳP is the mean yield of all genotypes.

After calculating of different indices, correlation between grain yield under normal and stress conditions (YP, YS) and stress tolerance indices was calculated and the best index was determined. So that, the indices having high and significant correlation with grain yield under both conditions were introduced as the best indicators. Also, principal component analysis of the indices was performed for further evaluation of drought tolerant cultivars. To identify relatively tolerant cultivars, a biplot chart was drawn according to the relationship of the studied components and indices. Finally drought tolerant cultivars were identified for the weather condition of this experiment.

In order to obtain data analysis, SPSS ver. 24 and Excel 2017 software were used. Also, yield stability analysis was done using biplot software. In order to do this, each one of the drought stress treatment levels were considered as an environment and the mean yield (per unit surface) of the studied cultivars at different levels of the less-watering stress was analyzed for stability.

3 RESULTS AND DISCUSSION

For all genotypes, five indices of drought tolerance, yield potential (YP), and stress yield (YS) were calculated (Table 3). According to many of the researches these indices are the most applicant ones for selecting drought-tolerance genotypes (Mardeh et al., 2006; Pirayvatlou, 2001; Talebi et al., 2009). There was a significant difference among stress conditions for grain yield. The grain yield mean under normal and stress conditions was 2.36 g m^{-2} and 1.10 g m^{-2}, respectively. The stress intensity index (SI) would get value between 0 and 1. The larger value of stress intensity indicates more severe stress conditions (Raman et al., 2012), in this study SI was equal to 0/54, that showed yield reduction was about more than one-half under stress conditions in comparison to yield under normal conditions. Indices, that have strong correlation with the grain yield under normal and stress condition, are suitable for selecting stress tolerance genotypes (Farshadfar et al., 2012).

The T5 wild diploid and Sabalan hexaploid genotypes possessed the highest value for grain yield under normal and stress conditions, respectively. The genotypes of B2 and B8 in normal condition and Moghan and B7 in stress condition had the least value for grain yield (Table 3). The highest difference in yield (YS-YP) was found in the population T1, T3, T4, T5, and superhead.

Correlation coefficients between drought tolerance indices and seed yield in normal and stress conditions presented in Table 4. There was a significant positive correlation between YP and YS (r = 0.67, p < 0.01) showing that high yield under normal condition resulted in relatively high yield under stress conditions. Also, YP and YS with STI (r = 0.85 and 0.90), MP (r = 0.95 and 0.86), GMP (r = 0.89 and 0.92), and HM (0.83 and 0.95) have significant positive correlation (p < 0.01) (Table 4). These suggest that in selecting high yielding lines under stress and non-stress conditions, theses indices are very important. In fact, high correlation YS and YP with other indices is critical for selecting tolerance genotypes (Farshadfar et al., 2012). There is a significant positive correlation between YP and TOL (r = 0.82, p < 0.01) but for YS and TOL, this correlation is negative (r = 0.14) (Table 4). It has been concluded that selecting based on TOL, results in reduced yield in normal condition. Khalili et al. (2012) reported that MP, GMP and STI indices had a positive and significant correlation with grain yield under normal and stress conditions. Also, Naghavi (2013) indicated that there was a positive correlation between MP, GMP, YS and YP. Drought resistance indices such as MP and GMP could be appropriate for identifying tolerant genotypes (Farshadfar et al., 2012; Khalili et al., 2014; Mardeh et al., 2006; Mirzaei et al., 2014; Naghavi et al., 2013).

Principal component analysis (PCA) are represented in Table 5. Principal component analysis (PCA) showed that 83.77 % of variations resulted from YP, YS,

Table 3: Yield potential (YP), stress yield (YS), and five indices of drought tolerance for 32 wheat genotypes

Genotype	YP	YS	TOL	STI	MP	GMP	HM
B1	0.2	0.11	0.08	0	0.15	0.15	0.14
B2	0.18	0.15	0.03	0	0.16	0.16	0.16
B3	0.30	0.17	0.13	0.01	0.24	0.23	0.22
B4	0.44	0.17	0.26	0.01	0.30	0.27	0.25
B6	0.67	0.11	0.56	0.01	0.39	0.27	0.19
B7	0.20	0.1	0.1	0	0.15	0.14	0.13
B8	0.16	0.13	0.03	0	0.15	0.14	0.14
T1	4.6	1.4	3.20	1.25	3.03	2.57	2.18
T2	2.50	2.93	-0.4	1.39	2.71	2.70	2.70
T3	4.16	1.78	2.37	1.40	2.97	2.72	2.50
T4	5.14	1.81	3.23	1.76	3.47	3.05	2.68
T5	5.40	2.28	3.12	2.34	3.84	3.51	3.21
Dehdasht	2.15	0.55	1.6	0.22	1.35	1.08	0.87
Dena	1.97	0.43	1.54	0.16	1.20	0.92	0.70
DW/90/8	1.50	1.90	-0.3	0.54	1.70	1.69	1.68
DW/95/4	1.87	0.43	1.44	0.15	1.15	0.89	0.69
Karkhe	2.14	0.96	1.17	0.39	1.55	1.43	1.33
Yavarus	1.9	1.12	0.78	0.40	1.51	1.46	1.41
Shabrang	2.77	1.98	0.79	1.04	2.38	2.34	2.31
Behrang	3.17	0.23	2.94	0.13	1.70	0.85	0.42
WS	0.72	0.56	0.15	0.07	0.64	0.63	0.63
Aria	3.1	2.05	1.05	1.20	2.57	2.52	2.46
Azadi	3.09	1.53	1.56	0.89	2.31	2.17	2.04
Azar	3.58	1.90	1.68	1.29	2.74	2.61	2.48
Hirmand	3.98	1.68	2.30	1.26	2.83	2.58	2.36
Moghan	1.72	0.1	1.62	0.03	0.91	0.41	0.18
mr-17	1.14	1.16	-0.02	0.25	1.15	1.14	1.14
Pishtaz	2.60	0.53	2.07	0.26	1.57	1.17	0.88
Roshan	3.11	1.24	1.87	0.73	2.17	1.96	1/77
Sabalan	3.55	3.30	0.24	2.22	3.43	3.42	3.42
Shiraz	2.68	0.97	1.70	0.49	1.82	1.61	1.43
Superhead	4.75	1.42	3.33	1.27	3.08	2.59	2.18

STI, MP, GMP, and HM as first PCA (Table 5). Therefore, PCA 1, the first dimension, was named as a yield component and drought tolerance.

The second principal component analysis (PCA) indicated 15.20 % of total variations. The highest positive factor in the second PCA was YP and TOL, and the highest negative factor was YS. Therefore, this component can be named as sensitivity component to stress. The genotypes with low values of the second PCA have the least sensitivity to stress conditions. Thus, for both non-stress and stress environment, selection of genotypes with high PCA1 and low PCA2 are recommended. Therefore, genotypes B1 and B8 have high PCA1 and low PCA2 that are preferable genotypes. It's been also reported that genotypes with larger PCA1 and lower PCA2 scores gave high yields (stable genotypes), and genotypes with lower PCA1 and larger PCA2 scores had low yields (unstable genotypes) (Kaya et al., 2002). The principal component analysis (PCA) also was described by many of researchers (Parchin et al., 2013; Zabet et al., 2003). Shafazadeh

Table 4: Correlation coefficients between drought tolerance indices and seed yield in normal and stress conditions

	YP	YS	TOL	STI	MP	GMP	HM
YP	1						
YS	0.67**	1					
TOL	0.82**	0.14	1				
STI	0.85**	0.90**	0.45**	1			
MP	0.95**	0.86**	0.62**	0.94**	1		
GMP	0.89**	0.92**	0.94**	0.96**	0.98**	1	
HM	0.83**	0.95**	0.39*	0.96**	0.95**	0.99**	1

* $p < 0.05$, ** $p < 0.01$.

Table 5: Linear composition coefficients of the main components of stress tolerance indexes

Component	Eigen values	Variance	Study Indicators						
			YP	YS	TOL	STI	MP	GMP	HM
1	5.8	83.77	0.93	0.88	0.58	0.96	0.99	0.99	0.97
2	1.06	15.20	0.34	-0.45	0.81	-0.13	0.05	-0.10	-0.21

et al. (2004) in investigation of response of bread wheat genotypes to drought stress conditions, concluded that MP, GMP and STI indices in specification of drought tolerant genotypes provide an agreeable result than TOL and SSI indices.

For comparison among genotypes, many researchers have used of biplot analysis (Nazari and Pakniyat, 2010). Biplot analysis was used to study the relationship between drought tolerance indices and grain yield under both normal and drought stress conditions (Figure 1). This diagram can be divided into four zones. Zone A includes the best genotypes such as Sabalan, T2, Aria, Azar and Azadi which have high-yield and are resistant to stress. Zone B includes T1, T3, T4, T5, Hirmanad, Shiraz, Superhead and Roshan which have a high-yield but are sensitive to stress. Zone C does not possess desirable properties, since has low-yield and are sensitive to stress. Most of the genotypes of this group were wild diploids. The genotypes of zone D, unlike the low-yield, are resistant to drought stress. For soybean, applying genotype-trait (GT) biplot to the multiple trait data illustrated that, GT biplots graphically displayed the interrelationships among seed yield, oil content, protein content, plant height and days to maturity and facilitated visual cultivar comparisons and selection (Yan and Rajcan, 2002). Mollasadeghi et al. (2011) reported 4 zone with 12 wheat genotypes.

The cluster analyses based on yield under normal and stress conditions, and the 5 mentioned indices were carried out, and the results are shown in Figure 2. UPG-

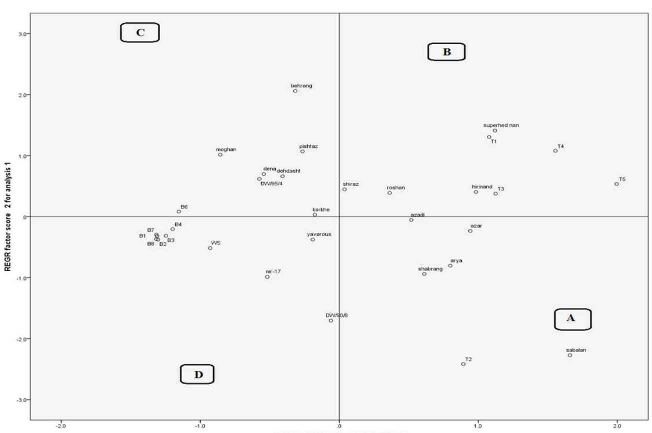

Figure 1: Biplot for drought tolerance indices in 32 genotypes wheat based on first two components.

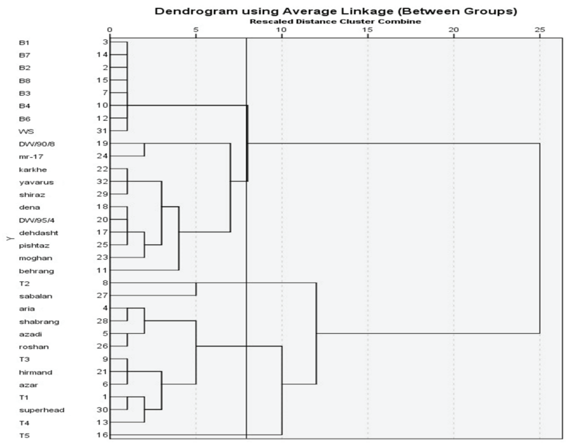

Figure 2: Dendrogram of cluster analysis of the wheat population tested based on TOL, STI, MP, GMP, HM indices using UPGMA method

MA dendrogram clustered the examined 32 wheat genotypes into two five clusters (Figure 2). Genotypes B1, B7, B2, B8, B3, B4, B6 and WS, which have the low yield and are resistant to drought stress, were put together. Second group are DW/90/8, mr-17, Karkheh, Yavarus, Shiraz, Dena, DW/95/4, Dehdasht, Pishtaz, Moghan, Behrang which have low yield. 'T2' and 'Sabalan' formed the third group with the highest yield, in normal and resistant to stress especially in stress conditions. Fourth group contains of genotypes are Aria, Shabrang, Azadi, Roshan, T3, Hirmand, Azar, T1, Superhead and T4, which are high in terms of yield and can be significant. 'T5' forms the 5th group and this genotype is high-yield and sensitive to stress.

The results of cluster analysis completely agreed by those of principal component analysis and biplot analysis. Based on biplot analysis, genetic variations were observed in studied genotype under drought stress. This is also approved by cluster analysis. Other researchers, using cluster analysis based on stress tolerance indices and genotypic classification, indicated that the results obtained from this method are consistent with the principal components analysis (Ahmadizadeh et al., 2012; Mohammadi et al., 2011; Rad and Abbasian, 2011). The clusteranalysis of 30 genotypes of wheat were placed into three separate groups (Parchin et al., 2013).

For tolerate adverse environmental conditions, there are different mechanisms. Sometimes, a combination of these mechanisms causes yield stability in stress conditions. The selection of genotype with such characteristics is not a simple task and is mainly difficult in the early stages of plant breeding. Thus, identifying and introducing new crop genotypes being resistant to water-limited conditions is one of the best methods for reducing the effects of drought stress. This method along with farm management are very effective strategies to reduce the negative effects of drought stress.

4 CONCLUSION

In the studied population, STI, MP, GMP and HM indices can be used to identify drought tolerant genotypes. Based on biplot chart, Sabalan, T2, Aria, Azar, Azadi genotypes retained both drought tolerance and high yield.

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Interactive effects of planting method and zeolite application on yield attributes of chickpea (*Cicer arietinum* L.) in dryland conditions

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Interactive effects of planting method and zeolite application on yield attributes of chickpea (*Cicer arietinum* L.) in dryland conditions

Abstract: Adopting an appropriate planting method and application of soil amendments such as zeolite in dryland conditions could be highly effective in improving growth and productivity of crop plants. Therefore the response of two chickpea (*Cicer arietinum* L.) genotypes (Azad and Adel) to two planting methods (flat and furrow patterns) and different rates of zeolite (0, 10 and 20 t ha^{-1}) was examined in a field study under dryland conditions. Zeolite application increased plant height, pods number/plant and seed yield, under flat planting pattern, while in furrow pattern, chickpea yield decreased with the application of zeolite. Under no zeolite condition, sowing in furrows improved plant height and pods number compared with flat sowing. Zeolite had no effect on Azad cultivar, but the pods number/plant of Adel was significantly increased by applying 20 t ha^{-1} zeolite compared with control. In general, the best result in terms of crop growth and yield was obtained from applying 20 t ha^{-1} zeolite in flat planting pattern. Positive response of the plant to zeolite application in flat planting pattern and, on the other hand, the yield reduction resulting from zeolite addition in furrow planting condition suggested that the influence of zeolite on the crop was dependent on planting method.

Key words: flat planting; furrow planting; dryland farming; zeolite

Medsebojni vpliv sejalnih metod in uporabe zeolita na komponente pridelka čičerke (*Cicer arietinum* L.) v sušnih razmerah

Izvleček: Uporaba primernih metod setve in dodatkov zeolita v tla bi lahko bili zelo učinkoviti ukrepi za izboljšanje rasti in povečanja pridelka poljščin v sušnih razmerah. V ta namen je bil ovrednoten odziv dveh genotipov čičerke (*Cicer arietinum* 'Azad', 'Adel') v poljskem poskusu z dvema načinoma setve (setev na ravno površino in setev v brazde)) ob različnih dodatkih zeolita (0, 10 in 20 t ha^{-1}) v sušnih razmerah. Uporaba zeolita je povečala višino rastlin, število strokov na rastlino in pridelek semena pri setvi na ravno površino medtem, ko se je pridelek čičerke pri setvi v brazde in dodatku zeolita zmanjšal. Pri dodatku zeolita se je pri setvi v brazde povečala višina rastlin in število strokov na rastlino v primerjavi s setvijo na ravno površino. Zeolit ni imel nobenega učinka na sorto Azad, pri sorti Adel pa se je število strokov na rastlino značilno povečalo pri dodatku zeolita 20 t ha^{-1} v primerjavi s kontrolo. Nasplošno so bili doseženi najboljši rezultati glede rasti in pridelka poljščine pri dodatku zeolita 20 t ha^{-1} in pri setvi na ravno površino. Pozitivni odziv rastlin ob dodatku zeolita pri setvi na ravno površino in upad pridelka ob setvi v brazde kažeta, da je vpliv zeolita na uspevanje poljščine odvisen od načina setve.

Ključne besede: setev na ravno površino; setev v brazde; kmetovanje na sušnih območjih; zeolit

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1 INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the second most important grain legume in the world with the harvest area of about 14.56 million ha, and in Iran is the most important food legume grown on 0.566 million ha with the annual production of 0.271 million tons (FAO, 2017). More than 97 % of chickpea in Iran is cultivated under rain-fed conditions and grown on stored soil moisture (Anonymous, 2017). Therefore, the application of any kind of technique or method that would increase the chickpea production efficiency in dryland conditions can be recommended. Selecting a proper planting pattern plays an important role in improving the growth and yield of crop plants especially in dryland farming and water deficit conditions.

Different cultivation techniques including two methods of flat and furrow planting have been studied and compared by many researchers. Results of a study on the effects of furrowing the soil surface on barley yield and soil water storage revealed that the application of deep furrow techniques had a positive effect on water storage and availability in soil leading to a significant increase in grain and straw yield of barley as compared with the natural (not furrowed) soil surface treatment (Abu-Awwad & Kharabsheh, 2000). Comparison of three planting systems of RP (ridge planting), FRP (flat planting with ridging at early jointing stage) and FP (flat planting without ridging) in rain-fed conditions showed that corn growth and yield in FRP system were significantly higher than those in RP and FP systems due to the improvement of soil moisture and temperature under FRP system (Song et al., 2013). Implementation of ridge-furrow planting system along with mulching techniques in semiarid conditions led to a significant increase in dry matter, grain yield and water use efficiency of corn compared with conventional flat farming (Ren et al., 2010). In an experiment the effects of three seed bed patterns including flat sowing (T1), raised bed with pair row of crop at 20 cm spacing on one bed and 40 cm furrow width (T2) and raised bed with pair row of crop at 30 cm spacing on one bed with 50 cm furrow width (T3) on chickpea were studied and the results showed that treatment T3 was superior to T1 and T2 in terms of pods number per plant, grain yield and growth parameters such as plant height and nodulation (Bhargav et al., 2018). Pourghasemian and Zahedi (2009) reported that the flat planting method compared to furrow method significantly increased seed yield, yield components, harvest index and seed oil content of safflower. Also, in a study carried out by Montazar et al. (2014), the planting method did not have a significant effect on any of the studied traits in wheat.

Preservation and storage of soil moisture in dryland farming conditions is of great importance. Zeolites are a large group of aluminosilicate mineral compounds with useful properties including absorbing and releasing water, high porosity and high cation exchange capacity, which are applied in agriculture to improve soil conditions (Mumpton, 1999; Eroglu et al., 2017). Zeolites improve the water use efficiency of plants by increasing the water holding capacity of soil and facilitating the transfer of water to plant roots (Sangeetha & Baskar, 2016). Zeolites can improve plant growth and increase its yield by storing and maintaining nutrients and releasing them at the right time and in a gradual manner (Polat et al., 2004). The beneficial effects of zeolite application on crops, especially under water-limited conditions have been reported in many studies (Mahmoodabadi et al., 2009; Aghaalikhani et al., 2012; Gholamhosseini et al., 2013; Najafinezhad et al., 2015; Ozbahce et al., 2015).

Although many studies have been done on the effects of planting techniques and zeolite application on different crops, there is no report on the interaction between planting method and application of zeolite in dryland conditions on chickpea. Therefore, the present study was conducted to investigate the growth and yield response of two chickpea cultivars to the application of different amounts of zeolite in two systems of flat and furrow planting under dryland conditions.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE DESCRIPTION

This experiment was carried out during 2015-2016 growing season in Kamyaran county, Kurdistan province, west of Iran. The experimental farm is located at a latitude of 34° 47' N and longitude of 46° 53' E with an elevation of 1425 m above sea level. The long-term annual precipitation and mean temperature of the region are 464.5 mm and 14.4 °C respectively. The monthly precipitation and temperature of the site in the growing season and the characteristics of farm soil are shown in Fig. 1 and Table 1 respectively.

2.2 EXPERIMENT LAYOUT AND MANAGEMENT

The experiment was conducted in a factorial arrangement with three factors based on a randomized complete block design in three replications under dryland conditions. The first factor was planting method including two patterns of flat and furrow, the second factor was zeolite application rate (0, 10 and 20 t ha^{-1} as Z0, Z10 and Z20 respectively) and two Kabuli type chickpea cul-

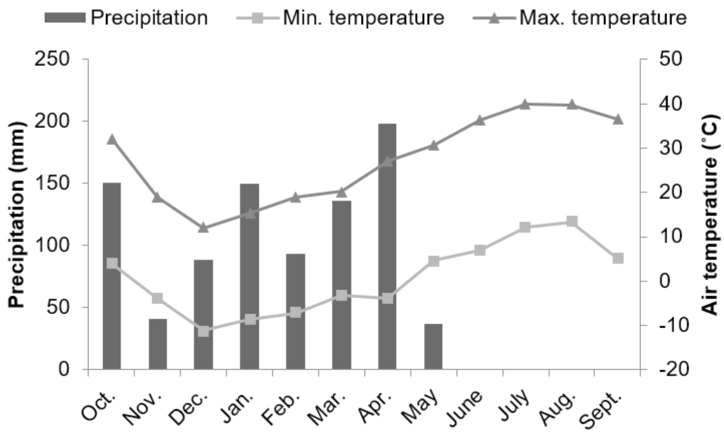

Figure 1: Precipitation and temperature during the growing season of 2015-2016 at the experimental site

Table 1: Soil properties of the experimental site

Clay (%)	Silt (%)	Sand (%)	OC (%)	TNV (%)	pH	EC (dS m^{-1})	N (%)	P (ppm)	K (ppm)
32.72	51.28	16	0.74	9.25	7.85	0.511	0.08	9.8	220

OC = Organic carbon, TNV = Total neutralizing value, EC = Electrical conductivity

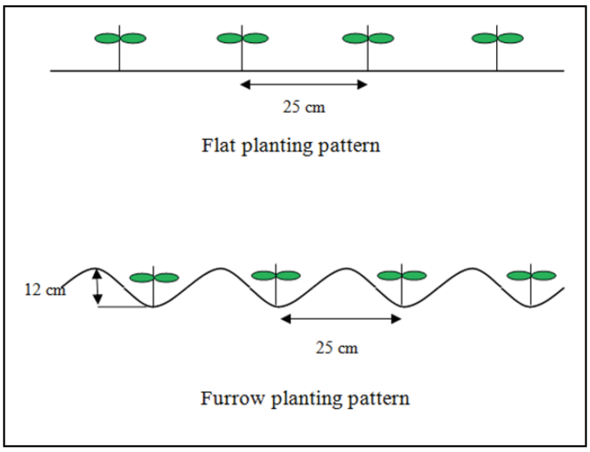

Figure 2: Schematic diagram of the two planting patterns

tivars of Azad and Adel were studied as the third factor items. Each experimental plot contained four rows, 3 m in length, 0.25 m apart and the space between the plants on each row was 10 cm. The fertilizers of urea, triple superphosphate and potassium sulfate were used before planting at the rates of 60, 35 and 25 kg ha^{-1} respectively according to the soil analysis results. The natural zeolite of clinoptilolite in relevant rates was applied in the seed bed prior to sowing. The seeds of chickpea were treated with benomyl fungicide before sowing to prevent soil-borne diseases. In flat planting pattern the seeds were sown at a depth of 3-5 cm and in furrow planting pattern were sown at the same depth in the middle of the furrows. The width and depth of furrows were 25 and 12 cm respectively (Fig. 2). The operation of sowing was performed manually in mid-March. The experiment was done under dryland conditions with no irrigation and hand weeding was performed at different growth stages of the plant.

2.3 DATA COLLECTING

Different crop traits including plant height, number of primary and secondary branches per plant, pods number per plant, seeds number per pod, 100-seed mass and seed yield were studied in the experiment. The morphological characteristics of the plant and yield components were measured using seven randomly harvested plants in each plot. The seed yield in each plot was determined by hand-harvesting the central two rows of the plot.

2.4 STATISTICAL ANALYSIS

The measured data were subjected to analysis of variance (ANOVA) and when the ANOVA result was significant, the least significant difference (LSD) test was done for means separation. The statistical operations were performed using SAS software (SAS Institute Inc., Cary, NC, USA).

3 RESULTS AND DISCUSSION

3.1 PLANT HEIGHT AND NUMBER OF BRANCHES

The plant height at maturity stage was significantly affected by the interaction of planting pattern and zeolite application but the number of primary and secondary branches per plant was not affected by the experimental factors and their interactions (Table 2). A significant increase in plant height was recorded by using 20 t ha^{-1} zeolite compared with Z0 (control) and Z10 treatments in flat planting pattern, whereas the plant did not respond to the application of zeolite in terms of plant height under furrow planting pattern (Fig. 3). Moreover, it can be seen that the plant height in furrow planting was higher than that in flat planting under the Z0 treatment (Fig. 3).

Table 3: Impact of planting pattern, zeolite application and cultivar on growth and yield parameters

Treatments	Plant height (cm)	Primary branches plant^{-1}	Secondary branches plant^{-1}	Pods no plant^{-1}	Seeds no pod^{-1}	100-seed mass (g)	Seed yield (kg ha^{-1})
Planting pattern							
Flat	35.0 ± 0.8	3.2 ± 0.09	9.9 ± 0.68	36.4 ± 1.8	1.19 ± 0.05	24.2 ± 0.9	1258.7 ± 48.4
Furrow	35.5 ± 0.4	3.1 ± 0.07	10.4 ± 0.56	33.6 ± 1.2	1.17 ± 0.03	24.4 ± 0.9	1052.4 ± 65.4
$LSD_{0.05}$	1.43	0.23	1.52	2.18	0.11	2.32	131.14
Zeolite rate							
Z0	34.4 ± 0.7	3.3 ± 0.13	10.3 ± 0.72	33.6 ± 1.9	1.18 ± 0.06	25.2 ± 1.5	1193.5 ± 82.8
Z10	34.9 ± 0.8	3.0 ± 0.08	9.8 ± 0.64	33.5 ± 1.0	1.18 ± 0.06	23.9 ± 0.8	1103.8 ± 54.4
Z20	36.5 ± 0.7	3.2 ± 0.09	10.3 ± 0.93	37.9 ± 2.4	1.18 ± 0.04	23.8 ± 0.6	1169.4 ± 89.6
$LSD_{0.05}$	1.75	0.28	1.86	2.67	0.14	2.84	160.62
Cultivar							
Azad	35.1 ± 0.6	3.2 ± 0.09	10.3 ± 0.75	36.2 ± 1.4	1.17 ± 0.03	25.7 ± 0.9	1237.1 ± 67.5
Adel	35.5 ± 0.6	3.1 ± 0.07	10.1 ± 0.46	33.8 ± 1.7	1.19 ± 0.05	22.9 ± 0.9	1074.1 ± 50.3
$LSD_{0.05}$	1.43	0.23	1.52	2.18	0.11	2.32	131.14
Source of variation							
Planting pattern (P)	ns	ns	ns	*	ns	ns	**
Zeolite (Z)	ns	ns	ns	**	ns	ns	ns
Cultivar (C)	ns	ns	ns	*	ns	*	*
P × Z	*	ns	ns	***	ns	ns	**
P × C	ns	ns	ns	**	ns	ns	ns
Z × C	ns	ns	ns	*	ns	ns	ns
P × Z × C	ns	ns	ns	ns	ns	ns	ns
CV%	5.86	10.50	21.85	9.01	13.70	13.82	16.42

ns: Not significant. *, ** and ***: Significant at the 0.05, 0.01 and 0.001 probability levels, respectively. Values are given as means ± standard errors.

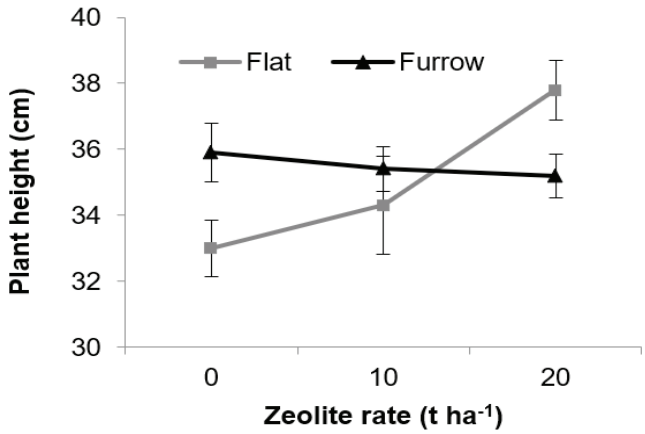

Figure 3: Interactive effect of planting pattern and zeolite application on plant height. Vertical bars indicate the standard error of the means

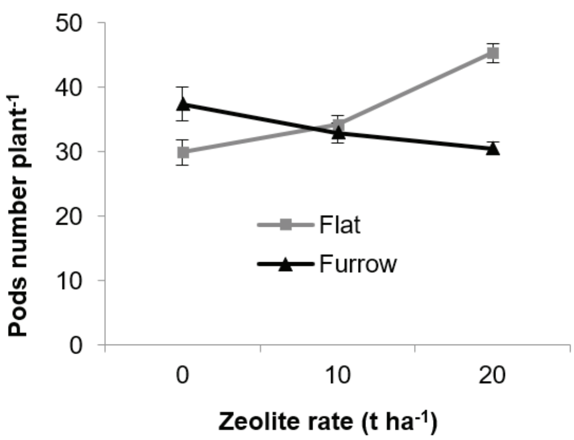

Figure 4: Interactive effect of planting pattern and zeolite application on pods number plant^{-1}. Vertical bars indicate the standard error of the means

An increased plant height in chickpea is a desirable trait for the ease of mechanical harvesting (Patil et al., 2014). According to the results of present study, plant height of chickpea can be increased through the application of zeolite in conventional flat planting system. The increase in plant growth due to zeolite application may be attributed to the positive effects of zeolite on the availability of water and essential nutrients for the plant (Rehakova et al., 2004; Mahmoodabadi et al., 2009; Zahedi et al., 2009).

3.2 YIELD COMPONENTS

The interaction between planting method and zeolite application had a significant effect on pods number plant^{-1} (Table 2). In the case of no zeolite application, the number of pods per plant in furrow system was higher than that in flat system (Fig. 4). Under flat planting pattern a significant increase in pods number was recorded by 14 and 52 % through the application of 10 and 20 t ha^{-1} zeolite respectively compared with Z0, while in furrow system a decreasing trend was shown in pods number with the application of zeolite (Fig. 4).

The interactive effect of planting pattern and cultivar on pods number demonstrated that under flat planting pattern there was no significant difference between two chickpea cultivars but in furrow system the number of pods per plant in Azad was significantly higher than that in Adel (Fig. 5).

The pods number per plant was significantly affected by the zeolite × cultivar interaction (Table 2). The effect of zeolite application on pods number in Azad cultivar was not significant while Adel positively responded to zeolite, so that the pods number/plant in this genotype

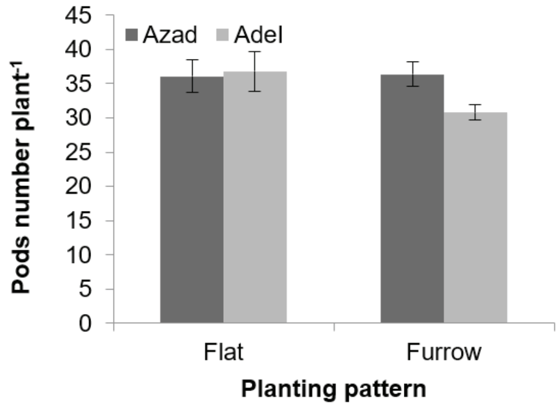

Figure 5: Interactive effect of planting pattern and cultivar on pods number plant^{-1}. Vertical bars indicate the standard error of the means

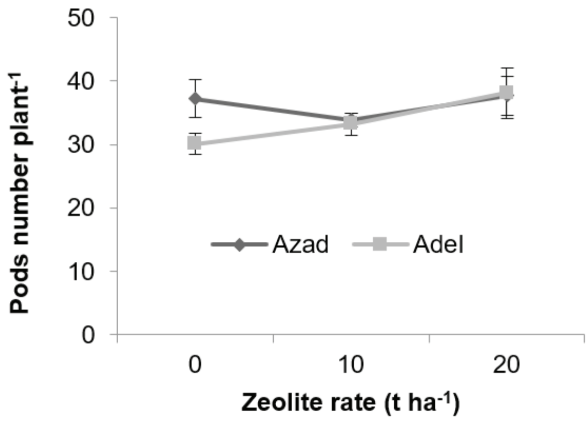

Figure 6: Interactive effect of zeolite application and cultivar on pods number plant^{-1}. Vertical bars indicate the standard error of the means

was increased by 10 and 27 % in Z10 and Z20 treatments, respectively, compared with control (Z0) treatment (Fig. 6). Our result regarding the different reaction of genotypes to zeolite application is consistent with that of Zahedi et al. (2009) who showed a significant interaction between zeolite and cultivar in canola. Different response of two chickpea cultivars to planting pattern and zeolite application in terms of pod production is an indication of different growth potential of genotypes under various agronomic practices.

Analysis of variance results indicated that number of seeds per pod was not affected by the experimental factors of planting pattern, zeolite, cultivar and their interactions (Table 2).

Planting method and zeolite application did not significantly affect the 100-seed mass, but it was significantly influenced by cultivar factor (Table 2). Azad cultivar was superior to Adel by 12 % in terms of 100-seed mass (Table 2). The non-significance of zeolite and planting method effects on seed mass was similarly reported by Sepaskhah and Barzegar (2010) and Bhargav et al. (2018).

3.3 SEED YIELD

Seed yield was affected by planting pattern, cultivar and the interaction between planting pattern and zeolite (Table 2). Azad cultivar with seed yield of 1237 kg ha^{-1} was significantly superior to Adel with 1074 kg ha^{-1} seed yield (Table 2). Seed yield response to the interaction of planting method × zeolite showed that under the flat planting pattern, the application of zeolite led to seed yield improvement. The highest seed yield (1407 kg ha^{-1}) was obtained in Z20 treatment under flat pattern which was 21 % higher than that of Z0 treatment (Fig. 7). On the other hand, under furrow method, seed yield decreased as the result of zeolite application (Fig. 7).

Our results regarding the positive effects of zeolite application on seed yield under conventional flat planting method are in line with those of previous studies. Zahedi et al. (2009) in a study on rapeseed showed that application of 10 t ha^{-1} zeolite under conditions that the crop is subjected to late season drought stress could improve the crop growth and yield. In another study, root and shoot dry mass and the content of micro and macro nutrients in roots and shoots of soybean were increased as the result of zeoilte application (Mahmoodabadi et al., 2009). The findings of Ozbahce et al. (2015) indicated that the application of zeolite significantly improved the concentration of different nutrients including nitrogen, potassium, zinc, manganese and copper in common bean leaves and increased the seed yield and yield components of the crop under water deficit stress conditions.

Seed yield reduction resulted from zeolite application under furrow planting pattern in current study may be related to the interaction between zeolite function and topology of the soil surface. In other words, the behavior of zeolite and its positive effects on crop growth can be affected by the shape of soil surface and the level of tillage operation. However, in order to better understand the performance of zeolite in each planting system, further studies, such as moisture and temperature measurements in different soil profiles and evaluation of soil nutrients availability in both planting patterns under the various amounts of zeolite are needed.

Under conditions of no zeolite application the response of chickpea to furrow sowing compared with flat planting pattern was more desirable in terms of plant height, pods number and seed yield (Fig. 3, 4 and 7), indicating the advantages of furrow planting over flat pattern with no zeolite application in dryland farming conditions. Several studies have shown that furrow sowing in dryland conditions has many benefits such as easily shedding of rain from ridges, accumulation of runoff in furrows, infiltration of rainwater below the furrow and better access of plant roots to sub-soil water (Yang et al., 1996; Feng et al., 2001; Ren et al., 2010; Roper et al., 2015) and improvement in growth and yield of various crops under furrow sowing compared with flat planting has been declared in many reports (Blackwell, 1993; Wang et al., 2011; Quanqi et al., 2012; Lian et al., 2016; Li et al., 2018).

4 CONCLUSIONS

Under flat planting pattern, plant height, pods number/plant and seed yield increased, whereas under furrow system, these traits decreased as the result of zeolite application. On the other hand, we found that under no zeolite condition, the furrow sowing had a positive effect on plant height and pods production. Genotypes comparison showed that Azad cultivar did not respond to zeolite application, while in Adel the pods number was increased due to zeolite application. In general, the findings of this study suggest that the effect of zeolite on chickpea growth and yield is dependent on seedbed configuration.

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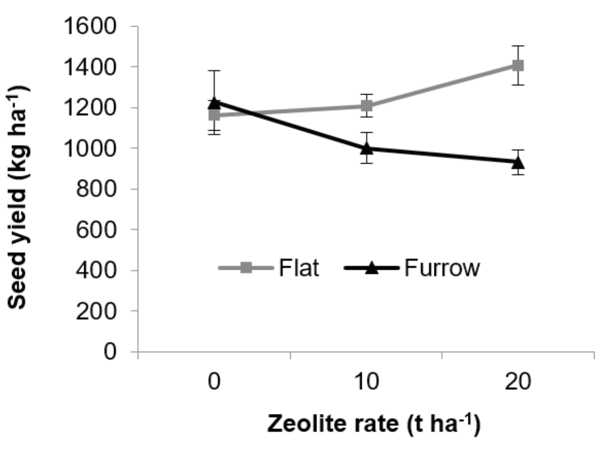

Figure 7: Interactive effect of planting pattern and zeolite application on seed yield. Vertical bars indicate the standard error of the means

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Allelopathic interference of *Sonchus oleraceus* L. with wheat and the associated weeds: a field study

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Allelopathic interference of *Sonchus oleraceus* L. with wheat and the associated weeds: a field study

Abstract: A field study was conducted to examine the allelopathic potential of *Sonchus oleraceus* L. residue against the weeds associated with wheat crop. Residue application was carried out under field conditions in two doses: 150 and 300 g m^{-2}. Weed richness, density and above-ground biomass were assessed at 6 and 12 weeks after application to evaluate the potential effect of *S. oleaceus* manure on weed control. Some growth criteria and the total yield of the cultivated wheat crop were also measured. The residue-containing quadrates attained lower weed richness, density and biomass. Unlikely, residue application reduced the grain yield of wheat. The available nitrogen and phosphorus were increased in soil at the higher application dose. These results suggest that *S. oleraceus* could interfere most of winter weeds, but affect productivity of wheat. Weed suppression could be attributed to the allelopathic potential of *S. oleraceus* residue. These results suggest also that the manure of this weed could be used successfully in the integrated weed management programs to reduce weed infestation in winter crops. However, another crop species may be selected.

Key words: organic agriculture; ecological weed management; allelopathy; *Sonchus oleraceus*

Alelopatični učinek navadne škrbinke (*Sonchus oleraceus* L.) na pšenico in njene plevele: poljski poskus

Izvleček: Za preučitev alelopatičnega potenciala ostankov navadne škrbinke (*Sonchus oleraceus* L.) na plevele povezane s pridelovanjem pšenice je bil izveden poljski poskus. Ostanki škrbinke so bili uporabljeni v dveh odmerkih, 150 in 300 g m^{-2}. Pestrost plevelov, njihova gostota in nadzemna biomasa so bili ocenjeni 6 in 12 tednov po nanosu ostankov škrbinke za oceenitev njihovega potenciala za nadzor plevelov. Izmerjeni so bili tudi nekateri parametri rasti in pridelka pšenice. Kvadranti polja, ki so vsebovali ostanke škrbinke so imeli manjšo pestrost, gostoto in biomaso plevelov. Uporaba ostankov škrbinke je zmanjšala pridelek zrnja pšenice. Na območjih z večjim odmerkom ostankov škrbinke sta se povečali razpoložljivost dušika in fosforja v tleh. Rezultati kažejo, da ostanki škrbinke lahko vplivajo na večino ozimnih plevelov a hkrati vplivajo tudi na pridelek pšenice. Zaviranje rasti plevelov lahko torej pripišemo alelopatičnem učinku ostankov škrbinke. Ti rezultati tudi nakazujejo, da bi se ostanki škrbinke lahko uspešno uporabljali kot gnojilo pri integriranem upravljanju s pleveli pri ozimnih poljščinah, vendar bi v tem primeru namesto pšenice morali izbrati drugo vrsto poljščine.

Ključne besede: organsko kmetijstvo; ekološko upravljanje s pleveli; alelopatija; *Sonchus oleraceus*

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1 INTRODUCTION

As common practices in modern agriculture, large quantities of synthetic fertilizers and agrochemicals are used to enhance crop productivity (Rüegg et al., 2007). The continuous use of synthetic herbicides in agriculture produces the emergence of herbicide-resistant weeds and leads to environmental pollution with impacts on both human health and ecosystems (Qasem, 2013). Thus, there is an increasing demand for alternative and sustainable practices and, thereby, the research institutions are currently applying innovative approaches to improve agriculture without synthetic herbicides (Khanh et al., 2007; Chauhan and Gill, 2014). The increased incidence of herbicide-resistant weed species, and the related biological consequences, poses a major threat to the potential sustainability of crop production. Natural weed management practices are truly looking for solutions to minimize environmental impacts related to the input of synthetic herbicides into the agroecosystems. Now, organic farming is a substantial aspect that gives the environmentally safe practices with respect to weed control and crop productivity.

Organic agriculture provides several merits as an agriculture practice if compared with the conventional one. Briefly, organic agriculture represents an approach to land management that emphasizes preservation of the immediate environment, improves employment opportunities in the local communities as a social benefit, and finally has been positively correlated with economic growth (Luttikholt et al., 2007; Vaarst, 2010). Furthermore, it is considered as an environmentally safe tool for weed control (Lemessa & Wakjira, 2015). Within this context, there is an existing trend to incorporate ecological practices to the agroecosystems so as to design alternative and sustainable cropping systems (Hassan et al., 2018) either for weed management or crop safety.

Allelopathic interactions generally involve the release of chemical compounds (i.e. allelochemicals) from living or dead plant parts in sufficient quantities that may suppress germination and/or establishment of weed seedlings in the agroecosystem (Qasem & Foy, 2001; Hassan et al., 2014a). Used as cover crops, some plants produce relevant amounts of allelochemicals which are released from living or dead plant tissues that can exert a strong influence on the target weeds (Cheng & Cheng, 2015). In this regard, some ecologists pay attention to the use of allelochemicals as 'bioherbicides' in weed control, providing environmentally safe agriculture (Gomaa & Abd El-Gawad, 2012). Application of plant residues is a common practice that is frequently recurring in the agroecosystems, and it mostly offers a strategy for weed control (Campiglia et al., 2010). In this regard, the use of phytotoxic or allelopathic plants is gaining attention due to positive results in the potential weed management. Potential use of annual sowthistle residue as a weed species in this regard was still unknown. In this article, I used the residues of such common weed for this purpose.

Annual sowthistle (*Sonchus oleraceus* L.) is an annual weed species native to Eurasia and North Africa. It has been introduced to a wide range of countries around the world and become a common weed causing a major problem in the agroecological systems (Peerzada et al., 2019; Widderick et al., 2010). It also dominates weed communities in winter crops and other urban areas where water is available (Gomaa et al., 2012; Hassan & Hassan, 2019). Therefore, this weed may give heavy biomass. Moreover, it may provide a cheapest, highly available and environmentally safe material to be used in the agroecology in terms of potential weed management due to its allelopathic capacity (Gomaa et al., 2014; Hassan et al., 2014b) and natural biofertilizer to enhance crop yield due to its fertilizing agency (Hassan et al., 2018).

During hoeing practices in cultivated fields, the farmers uproot this plant and mix it with the soil during ploughing, the phenomenon that may affect the incoming crops and its associated weed species due to the potential release of some phytotoxins from the plant residue. *S. oleraceus* was found to be allelopathic against some common weeds (Gomaa et al., 2014; Hassan et al., 2014a). Besides, the allelopathic compounds released from its residue were long persistent in soil (Hassan et al., 2014b). Nevertheless, the previous studies were performed under greenhouse conditions, and a field application to evaluate the allelopathic potential of this weed is still lacking. It was therefore necessary for wide-ranged researchers interested and specialized in this field to fill this gap. On the other hand, the amounts of residue application of such weed that were similar to those applied the current study stimulated growth and productivity of kidney bean crop (Hassan et al., 2018). In the view of these statements, I tested the hypotheses that (i) the strongly allelopathic *S. oleraceus* can display adverse effects on emergence and growth of the common weeds associated with wheat under field conditions and (ii) a potential stimulatory or, at least, no effect on the crop tested (i.e. wheat) could be obtained. The main objective of this field study was to assess the allelopathic potential of *S. oleraceus* residue against some common weeds associated with wheat (*Triticum aestivum* L.), i.e. its ability to suppress weeds, and the potent to use *S. oleraceus* as a bioherbicide.

2 MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIAL

Fresh shoots of *S. oleraceus* L. were collected during the growing season, from different locations in the agroecosystems of Beni-Suef governorate, Egypt, (from January to April 2015). Plant collection was carried out during flowering-early fruiting stage in order to facilitate the distinction of *S. oleraceous* from *S. asper* (L.) Hill. Furthermore, at this stage, plants mostly pose the maximum amounts of bioactive metabolites. Plant material was placed in polyethylene bags and immediately carried to the laboratory for further processing. In the lab, fruits and inflorescences were completely removed and the plant material was air dried and stored in refrigerator at 2 °C until use.

2.2 THE SELECTED CROP

Common wheat (*Triticum aestivum* L.) is one of the most important cereals in the world in terms of production and use for human and animal feeding under a wide range of climatic conditions and in many geographic regions (Feldman, 1995; Shewry and Hey, 2015). In addition, it was proved to be a major source of carbohydrates and energy, and it also provides substantial amounts of ingredients which are important or beneficial for health such as proteins, vitamins, dietary fibers, and other phytochemicals (Shewry and Hey, 2015). Due to considerable land extension dedicated to wheat production through the world, there were substantial amounts of economic funds invested in weed management. It was necessary to provide a potential safe weed management strategy in wheat fields as weeds threaten the production of wheat worldwide (Oerke, 2006). Consequently, increasing the wheat crop yield will be expected.

2.3 FIELD WORK

Field experiment was conducted at a crop farm land in Beni-Suef governorate; about 17 km north west of Beni-Suef University (lat. 29° 09.13 N, long. 031° 08.36 E, alt. 29 m a.s.l.), Egypt, in the period from the beginning of January to the mid of May 2016. This period was synchronized with the time of cultivation of this crop. Before cultivation soil surface was ploughed twice to homogenate the soil and simultaneously provide a high potential for the equal distribution for the weed seeds in the seed bank. The soil characteristics

Table 1: Soil physicochemical properties (mean ± S.D.) of study field prior to cultivation

	Soil type	Sandy clay loam
	Field capacity	39.08 ± 0.038
	pH	7.97 ± 0.04
	EC	0.42 ± 3.13
	OC	1.69 ± 0.052
	OM	2.975 ± 0.096
Available	N (mg kg^{-1} soil)	99.0 ± 9.31
	P (mg kg^{-1} soil)	2.83 ± 0.61
	K (mg kg^{-1} soil)	607.5 ± 92.78
	Zn (mg kg^{-1} soil)	5.9775 ±1.07

Table 2: Mean average meteorological data of Beni-Suef governorate during the growing season

Parameter	Jan.	Feb.	Mar.	Apr.	May
Mean average high temperature (°C)	23	21.5	25.5	29.5	32.5
Mean average low temperature (°C)	10	11.5	14.5	16.5	19.5
Rainfall (mm)	7.5	355.5	14.5	1	0
Relative Humidity (%)	61.5	53.5	48.5	40.5	37.5

and the surrounding climatic conditions of the study site are well illustrated in Table 1 and 2, respectively. Thereafter, under field conditions, *S. oleaceus* residues were amended in the study area previously divided as quadrate (2 × 2 m^2 each) at the rates 150 and 300 g m^{-2}, whereas the residue-free quadrates were left as control. The experiments were conducted in a complete randomized design (CRD) involving four replicates for each treatment.

Weed control assessment was carried out twice: six and twenty weeks after residue application, during which I determined the emerging weed species, species richness, density and biomass of the total as well as individual weed species from each quadrat. Identification and nomenclature of the weed species detected were obtained using Boulos (1999, 2002 & 2005). The aboveground parts of the detected weeds were carefully cut and oven-dried at 70 °C till constant dry weight to obtain the biomass.

The grains of wheat were immediately seeded after residue incorporation via manual spraying, as usual in Egyptian wheat fields, obtaining quantities around 200 grains/m^2. After 3 weeks, the emerging individuals were thinned to the most similar 100 ones. No fertilization or herbicide regimes were applied, and irrigation process was carried out when need. At harvest, growth parameters of such crop were measured using ten randomly selected plants per quadrate. These parameters comprised shoot height, above-ground biomass, leaf area and grain yield (expressed as total dry weight m^{-2}). Besides, three soil samples were collected from each quadrate at 0–30 cm depth to form a single composite for each treatment. Soil properties comprising pH, soil electrical conductivity (EC), organic carbon, organic matter, available nitrogen, phosphorus, potassium, and zinc were determined using the standard methods (Allen, 1989).

2.4 STATISTICAL ANALYSIS

The obtained field data were first tested for their normality and homogeneity of variances using Kolmogorov-Smirnov and Levene's tests, respectively. If the data were normal and homogeneous, the data were analysed through one-way ANOVA followed by Tukey´s test ($p \leq 0.05$) for post hoc multiple comparison of means. When the data exhibited non-normal distribution and heteroscedasticity of variances, Kruskal-Wallis H test ($p \leq 0.05$) was performed. A correlation analysis was performed between the total weed richness, density and biomass vs. the dose of the residue applied. All statistical analyses were carried out using the IBM SPSS Statistics 20.0 software package (IBM SPSS Inc., Chicago, IL, USA).

3 RESULTS

3.1 WEED COMPOSITION

The weed species with their corresponding families detected in wheat field are listed in Table 3. A total of eight weed species belonging to five families were detected. Four of these species were monocots, whilst the remaining ones were dicot.

3.2 EFFECT OF *S. OLERACEUS* RESIDUE ON ON WEED DENSITY, RICHNESS AND BIOMASS

The effects of *S. oleraceus* residue depended mostly upon the amount of residue applied, weed species detected and time of harvest (Table 3). Amongst the weed species, the density of *Convolvulus arvensis* L. was not affected at all under the influence of *Sonchus* residue. However, the highest dose significantly reduced the biomass of such weed at both harvests. On converse, the highest dose significantly inhibited the emergence of *Coronopus niloticus* (Del.) Spreng. at both harvests. The biomass of this species was not affected in the treated quadrates. With respect to the first harvest, three weed species, namely: *Phalaris minor* Retz., *Polypogon monspeliensis* (L.) Desf. and *P. vridis* (Gouan) Breistr., were totally absent. Simultaneously, the density of *Matricaria chamomilla* L. was significantly reduced at the highest dose. Besides, its biomass was significantly supressed at both doses. The density of *Chenopodium murale* L. and *Poa annua* L. showed gradual decline with increasing the dose applied. At the end of the experiment (i.e. the second harvest), the density of *Phalaris minor* and *Poa annua* was substantially reduced. Additionally, *Polypogon monspeliensis* was completely absent at the higher application rate. On the contrary, the emergence and biomass of *Polypogon viridis* was significantly stimulated. Furthermore, the biomass of *Phalaris minor* was gradually increasing with the amount of residue applied.

As a whole, the total number of weeds (weed richness) was maintained in the treated plots at the first harvest only. However, density and biomass of the weeds observed were significantly decreased at both harvests in the residue-amended plots. Furthermore, the reduction in these criteria increased gradually with the increment in the dose applied (Table 4).

The correlation analysis between the measured criteria; weed richness, density and the above-ground biomass, with the amount of manure applied is shown in Table 5. Significant negative correlation was observed between the total weed density and biomass with the amount of residue applied at both harvest intervals. Besides, such correlation was also manifested between the weed richness and the dose applied at the second harvest.

3.3 EFFECT OF *S. OLERACEUS* MANURE ON YIELD COMPONENTS OF WHEAT

In general, the application of *S. oleraceus* manure had not affected shoot length and the above-ground biomass of wheat crop (Table 6). However, the leaf area showed significant reduction in wheat plants at the higher dose (10 %, $p \leq 0.05$). Furthermore, the grain output of wheat was significantly reduced at both treatments (Table 6).

3.4 SOIL ANALYSIS

The measured soil criteria after application of *S. oleraceus* residue are shown in Table 7. Clearly, most of the measured soil parameters were not affected on addition of the plant residue. However, the available soil nitrogen

Table 3: Effect of *Sonchus oleraceus* residue at the rates 150 and 300 g m^{-2} on density (no. quadrate^{-1}) and aboveground biomass (g quadrate^{-1}) (Mean ± SE) of the weed species detected in wheat crop at 6 and 12 weeks after application (WAA)

Weed species	Time after application	weed density at the dose applied			weed biomass at the dose applied		
		0	150	300	0	150	300
Chenopodium murale L. (Chenopodiaceae)	6 WAA	6.33^{a} ± 0.28	4.33^{b} ± 0.27	2.0^{c} ± 0.0	1.74^{a} ± 0.05	1.27^{b} ± 0.052	1.07^{c} ± 0.0
	12 WAA	-	-	-	-	-	-
Convulvulus arvensis L. (Convolvulaceae)	6 WAA	2.33^{a} ± 0.29	2.66^{a} ± 0.67	3.33^{a} ± 0.27	1.53^{a} ± 0.16	1.55^{a} ± 0.21	0.92^{b} ± 0.07
	12 WAA	2.33^{a} ± 0.28	2.33^{a} ± 0.27	3.66^{a} ± 0.29	3.42^{a} ± 0.18	2.93^{ab} ± 0.21	2.40^{b} ± 0.19
Coronopus niloticus (Delile) Spreng.(Brassicaceae)	6 WAA	4.33^{a} ± 0.29	4.33^{a} ± 0.29	2.0^{b} ± 0.0	1.01^{a} ± 0.04	0.96^{a} ± 0.1	0.84^{a} ± 0.03
	12 WAA	4.0^{a} ± 0.29	3.33^{ab} ± 0.52	2.33^{b} ± 0.29	1.12^{a} ± 0.10	1.01^{a} ± 0.2	0.95^{a} ± 0.03
Matricaria chamomilla L. (Astraceae)	6 WAA	4.33^{a} ± 0.27	3.33^{ab} ± 0.26	2.33^{b} ± 0.27	4.71^{a} ± 0.28	3.63^{b} ± 0.16	2.47^{b} ± 0.14
	12 WAA	3.66^{a} ± 0.0	3.33^{a} ± 0.77	2.0^{a} ± 0.27	1.83^{a} ± 0.01	5.1^{b} ± 0.061	3.33^{c} ± 0.006
Phalaris minor Retz. (Poaceae)	6 WAA	-	-	-	-	-	-
	12 WAA	9.0^{a} ± 0.56	4.33^{b} ± 0.28	1.67^{c} ± 0.26	1.67^{a} ± 0.01	2.67^{a} ± 0.007	6.70^{b} ± 0.006
Poa annua L. (Poaceae)	6 WAA	7.33^{a} ± 0.28	5.67^{b} ± 0.27	4.33^{c} ± 0.28	8.60^{a} ± 0.48	7.88^{a} ± 0.38	5.43^{b} ± 0.16
	12 WAA	6.33^{a} ± 0.30	4.33^{b} ± 0.29	2.33^{c} ± 0.28	6.70^{a} ± 0.36	2.67^{a} ± 0.46	1.67^{b} ± 0.27
Polypogon monspeliensis (L.) Desf. (Poaceae)	6 WAA	-	-	-	-	-	-
	12 WAA	10.33^{a} ± 0.77	3.33^{b} ± 0.27	0.0^{c} ± 0.0	10.37^{a} ± 0.78	4.50^{b} ± 0.28	0.0^{c} ± 0.0
Polypogon viridis (Gouan) Breistr.(Poaceae)	6 WAA	-	-	-	-	-	-
	12 WAA	2.33^{a} ± 0.29	8.0^{b} ± 0.51	8.0^{c} ± 0.51	8.4^{a} ± 0.42	13.67^{b} ± 0.44	19.34^{c} ± 0.38

Values in each row within the same parameter sharing the same letter are not significantly different at the 0.05 probability level according to Tukey's test. - = not detected.

Table 4: Effect of *Sonchus oleraceus* residue at the rates 150 and 300 g m^{-2} on the species richness, total weed density and above-ground biomass (Mean ± SE) of the weed species detected in the wheat field at 6 and 12 weeks after application (WAA)

Parameter	Time after application	Dose applied (g m^{-2}) 0	150	300
Species richness (No. m^{-2})	6 WAA	5.0^{a} ± 0.0	5.0^{a} ± 0.0	4.66^{a} ± 0.29
	12 WAA	6.67^{a} ± 0.28	6.0^{b} ± 0.0	5.67^{b} ± 0. 29
Total weed density (No. m^{-2})	6 WAA	24.67^{a} ± 0.76	21.0^{b} ± 0.88	13.0^{c} ± 0.29
	12 WAA	37.0^{a} ± 1.34	31.0^{b} ± 0.88	20.0^{c} ± 1.04
Total weed biomass (g m^{-2})	6 WAA	17.58^{a} ± 0.76	15.3^{b} ± 0.38	10.99^{c} ± 0.066
	12 WAA	40.6^{a} ± 1.04	37.69^{a} ± 1.30	30.02^{b} ± 0.43

Values in each row within the same crop sharing the same letter are not significantly different at the 0.05 probability level according to Tukey's test.

and phosphorus were significantly induced at the higher dose.

4 DISCUSSION

The results of this study indicated that *S. oleraceus* residue significantly reduced the richness, emergence and biomass of most of the detected weeds associated with the studied wheat field. This observation was consistent with that of Hassan et al. (2014b) who indicated that *Sonchus* residue reduced emergence and growth of some tested weeds under greenhouse conditions. This result was also consistent with field observations monitored by Hassan et al. (2018) who indicated that the same amounts of the residues applied suppressed weed density, richness and biomass in a kidney bean field (unpublished data). This result substantially obeys the first hypothesis of this study. This result suggests also that the residue added had a role in weed interference and declining species richness. In general, the decaying plant residues release phenolic compounds into the rhizosphere part of the soil (Djurdjević et al., 2011). Furthermore, these phenolic allelochemicals were found in the soils amended with *S. oleraceus* (Hassan et al., 2014b). Accumulation of phytotoxins in soil leads to inhibition of seed germination, seedling growth and uptake of mineral nutrients (Rice, 1984), the circumstances that decrease density of individual plant species from the plant communities as suggested by several authors (Barritt and Facelli, 2001; Djurdjević et al., 2011). Therefore, weed suppression could be related to the phenolic compounds released from the plant residues, i.e. allelopathic interaction. This notion may be consistent with that of Foy and Inderjit (2001) who reported that allelopathy had an important role in weed interference and declining weed diversity. Within this side, the dry matter of *S. oleraceus* shoots has been reported to be rich in saponins, alkaloids and total phenols (Gomaa et al., 2014). On the other hand, most of the measured soil criteria were not affected by the residue applied. However, the higher dose-amounts enhanced the available soil nitrogen and phosphorus. The increase in the available N and P could be attributed to the residue applied. May be, *Sonchus* residue is rich in nitrogenous and phosphorus compounds. This result obeys that obtained by Hassan et al. (2014b) and Hassan et al. (2018) who indicated that the residue of this species induced soil nutrients. Therefore, it was so difficult to claim that the reduction in weed diversity and growth was attributed to the change in soil criteria. This result confirms the phytotoxic effect of the residue applied.

Table 5: Correlation coefficients (r) between the measured weed criteria and the amount of *Sonchus oleraceus* residue applied in wheat field at both time intervals after residue application

Parameter	Time after application (weeks)	r value
Total weed richness	6	- 0.55
	12	- 0.71*
Total weed density	6	- 0.96**
	12	- 0.96**
Total weed biomass	6	- 0.96**
	12	- 0.91**

* Correlation is significant at $p \leq 0.05$.
**Correlation is significant at $p \leq 0.01$.

The field observations obtained by Hassan et al. (2018) showed that the same amounts of the incorporated residue had a stimulatory effect of on growth, productivity and several metabolites of the kidney beans. However, the effect of these amounts on wheat was contradictory. *S. oleraceus* produced a negative effect on the total seed yield of wheat. This result substantially does not obey the second hypothesis of this study. Such effect could be also attributed to the smaller seed size of wheat when compared with kidney bean. In this regard, larger seed-sized species were more resistant/tolerant for the released allelochemicals from plant litter (Hassan, 2018).

Table 6: Growth and yield parameters (mean ± SE) of wheat in response to *Sonchus oleraceus* residue at the rates 150 and 300 g m^{-2}

	Dose applied (g m^{-2})		
Parameter	Control	150	300
Shoot length (cm)	85.0^{a} ± 3.85	82.6^{a} ± 3.60	86.0^{a} ± 2.62
Shoot biomass (g individual^{-1})	14.4^{a} ± 0.70	13.44^{a} ± 0.59	13.28^{a} ± 0.64
Leaf area individual^{-1} (cm^{2})	162.92^{a} ± 5.50	153.15^{ab} ± 5.95	146.31^{b} ± 5.24
Number of spikes tiller^{-1}	4.27^{a} ± 0.32	4.43^{a} ± 0.27	4.20^{a} ± 0.20
Spike length (cm)	8.60^{a} ± 0.47	6.65^{a} ± 0.41	8.72^{a} ± 0.51
Seed/Grain yield (g m^{-2})	703.0^{a} ± 37.23	548.8^{b} ± 22.61	570.6^{b} ± 26.36

Values in each row within the same crop sharing the same letter are not significantly different at the 0.05 probability level according to Tukey's test.

Table 6: Influence of *Sonchus oleraceus* residue at the rates 150 and 300 g m^{-2} on the measured soil properties (Mean ± SE) at harvest of wheat

		Dose applied (g m^{-2})		
	Soil Properties	control	150	300
	pH	7.95^{a} ± 0.015	7.95^{a} ± 0.017	7.88^{a} ± 0.035
	EC (mS cm^{-1})	0.38^{a} ± 0.025	0.39^{a} ± 0.004	0.46^{a} ± 0.027
	OC (%)	1.54^{a} ± 0.028	1.77^{a} ± 0.095	1.48^{a} ± 0.16
	OM (%)	2.7^{a} ± 0.048	3.08^{a} ± 0.16	2.60^{a} ± 0.28
	N (mg kg^{-1} soil)	91.0^{a} ± 7.50	113.0^{a} ± 6.80	139.0^{b} ± 2.20
Available	P (mg kg^{-1} soil)	1.77^{a} ± 0.11	2.25^{ab} ± 0.13	3.09^{b} ± 0.07
	K (mg kg^{-1} soil)	590.0^{a} ± 50.68	647.0^{a} ± 45.53	562.0^{a} ± 82.53
	Zn (mg kg^{-1} soil)	4.75^{a} ± 0.46	4.71^{a} ± 0.43	4.38^{a} ± 0.76

Values in each row within the same crop sharing the same letter are not significantly different at the 0.05 probability level according to Tukey's test.

Besides, wheat germination and early growth displayed a degree of sensitivity to phytotoxic species (Tamak et al., 1994; Al-Sherif et al., 2013). Therefore, the dose of the applied residue should be further explored and adjusted to add benefits in weed control and, at the same time, avoid undesirable phytotoxicity on the selected crop.

As proved by Hassan et al. (2014b), the phenolic compounds released from *S. oleraceus* residues have been detected during 60 days after residue incorporation into the soil, the result that may explain the extending effect of the residue. In this study, the bioactivity of *S. oleraceus* manure seems to be extended in time since herbicidal effects were still evident 12 weeks after manure application. Progressive and long-term effects collected suggest that *S. oleraceus* manure gradually releases phytotoxic compounds, showing a strong suppressive effect on weed emergence. The extending bioactivity of the phytotoxic residue applied is highly needed because weeds in the soil seed bank are not synchronized, but germinate gradually all along the establishment of the crop (Mohler et al., 2001; Puig et al., 2013).

In this study, as expected, the weed suppression seemed to be related to the dose of *S. oleraceus* manure applied. In general, the magnitude of weed suppression is quantitatively proportional to the applied dose in the studies related to phytotoxicity (Hassan et al., 2014b; Hassan, 2018).

While the emergence and biomass of *Phalaris minor*, *Poa annua*, *Polypogon monspeliensis* was significantly reduced through the phytotoxicity of *S. oleraceus* manure, *Polypogon viridis* was notably increasing with respect to its density and biomass in the long term. Different responses to chemical compounds can be associated to the amplitude of weed sensitivity to phytotoxicity (Latif et al., 2017), even those weed species are closely related. Moreover, selective inhibition of weed growth may be related to concentration and distribution of allelochemicals in the rhizosphere soil (Blum et al., 1999; Aslam et al., 2017). However, rather than indicating phytotoxic resistance, the emergence of *P. viridis* could be also associated with seed germination dynamics or better competitive abilities that allows this weed to rapidly occupy its own niche after the decline of other monocot species.

The results also indicate a punctual weed increase in the case of *Matricaria chamomilla* at the second harvest and mainly *Polypogon viridis* at both. None of them was considered as a highly problematic weed. In this sense, weed stimulation can be related with differential responses as plant residues are able to exhibit positive or negative effects on target species based on chemical concentration or environmental conditions (Cheng and Cheng, 2015). Also, punctual weed stimulation could be related to the nutritional benefits provided by the residue incorporated (Hassan et al., 2014b).

5 CONCLUSION

The present study represents evidence that *S. oleraceus* residue showed remarkable weed suppression. This investigation had the merit of field application. Apparently, reduction of richness, density and biomass of the weeds associated with wheat crop was manifested. The inhibitory effect of the residue was not attributed to soil properties, but it could be related to some phytotoxins released from the residue that reduce or, perhaps, completely inhibit the emergence and growth of the detected weeds. The undesirable result obtained in this study was the reduction of grain yield of wheat. Therefore, application of such residue in another crop species, probably with larger seeds, may be recommended. Moreover, the dose of the manure applied should be adjusted in order to attain the desired weed management with a potential stimulatory or, at least, no effect on the cultivated crop. The experimental approach described was adequate to demonstrate the efficacy of *S. oleraceus* residues as a bioactive green manure for future weed management practices.

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Effects of *Boerhavia diffusa* L. nom. cons. and *Chromolaena odorata* (L.) R. M. King & H. Rob. extracts on some field insect pests of okra (*Aabelmoschus esculentus* (L.) Moench)

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Effects of *Boerhavia diffusa* L. nom. cons. and *Chromolaena odorata* (L.) R. M. King & H. Rob. extracts on some field insect pests of okra (*Aabelmoschus esculentus* (L.) Moench)

Abstract: A field study was carried out to assess the efficacy of some botanicals in the control of field insect pests in two varieties of okra. Dry leaf extract of *Chromolaena odorata* (Siam weed) and fresh and dry root extracts of *Boerhavia diffusa* served as treatments which were compared with Lambda-cyhalothrin and a control. The experiment was fitted into a randomized complete block design with three replications. Data collected were subjected to Spearman's correlation analysis and a two-way analysis of variance and significant different means were separated using Fishers Least Significant Difference (LSD) test at 5 % level of probability. The results revealed that treated plants generally performed better than the untreated plants as they were taller, had more leaves, branches, wider stem girths and gave higher fruit yield. Fresh root extract of *B. diffusa* performed better in most parameters measured. A significant positive correlation was observed between insect pest populations, and between growth and yield parameters, while there was a significant negative correlation between overall yield and insect pest population. NHAe 47-4 okra variety performed better than F1-Lucky variety. Fresh root extract of *B. diffusa* is recommended for management of *B. tabaci* (Genadius, 1889) and *Amrasca biguttula* Ishida, 1912 in okra.

Key words: botanicals; *Boerhavia diffusa*; *Chromolaena odorata*; *Bemisia tabaci*; *Amrasca biguttula*; sticky traps

Učinki izvlečkov iz rastlin *Boerhavia diffusa* L. nom. cons. in *Chromolaena odorata* (L.) R.M. King & H. Rob. na nekatere škodljive žuželke na jedilnem oslezu (*Aabelmoschus esculentus* (L.) Moench)

Izvleček: Za ocenitev učinkovitosti izvlečkov izbranih rastlin pri uravnavanju škodljivih žuželk na dveh sortah jedilnega osleza (okre, bamije) je bil izveden poljski poskus. Suhi listni izvlečki vrste *Chromolaena odorata* (L.) R.M. King & H. Rob. in sveži ter suhi izvlečki korenin vrste *Boerhavia diffusa* L. nom. cons. so bili uporabljeni za obravnavanja v primerjavi z lamda cihalotrinom in kontrolo. Poskus je bil izveden kot popolni naključni bločni poskus s tremi ponovitvami. Zbrani podatki so bili analizirani s Spearmanovo korelacijo in dvosmerno analizo variance, značilno različna poprečja so bila ločena s Fisherjevim testom najmanjše značilne razlike (LSD) pri 5 % verjetnosti. Rezultati so pokazali, da so tretirane rastline na splošno rastle bolje kot netretirane, bile so višje, imele so več listov in stranskih poganjkov, večji obseg stebla in večji pridelek plodov. Sveži izvlečki korenin vrste *B. diffusa* so se izkazali kot boljši v vseh merjenih parametrih. Opažena je bila značilno večja pozitivna korelacija med populacijami škodljivih žuželk in med parametri rasti in pridelka medtem, ko je bila korelacija med celokupnim pridelkom in populacijami škodljivih žuželk značilno negativna. Sorta okre NHAe 47-4 je uspevala bolje kot sorta F1-Lucky. Za uravnavanje tobakovega ščitkarja (*B. tabaci* (Genadius, 1889)) in vrste *Amrasca biguttula* Ishida, 1912 na jedilnem oslezu priporočamo izvlečke svežih korenin vrste *B. diffusa.*

Ključne besede: botanična sredstva za zatiranje škodljivcev; *Boerhavia diffusa*; *Chromolaena odorata*; *Bemisia tabaci*; *Amrasca biguttula*; lepljive pasti sticky traps

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1 INTRODUCTION

Okra, *Abelmoschus esculentus* (L.) Moench, is a commercially grown vegetable crop widely cultivated in Africa and Asia. Within 2009 and 2010, a global area of 0.43 million hectares was cultivated with total production standing at 4.54 million tons (Varmudy, 2011). India is the largest producer (67.1 %), with Nigeria following at (15.4 %) and Sudan (9.3 %) (Varmudy, 2011).

Okra plays a vital role in human diet (Kahlon et al. 2007, Saifullah and Rabbani 2009) providing phosphorus, calcium, sulphur, iron, fibre, fats, proteins, carbohydrates and vitamins (Lamont 1999, Owolarafe and Shotonde, 2004, Gopalan et al., 2007, Arapitsas 2008, Dilruba et al, 2009). Okra fruit is normally boiled in water to give slimy soup sauces that is relished. The seeds are nutritious, can be dried roasted and ground for use as additive or coffee substitute (Moekchantuk and Kumar 2004) while the fruits are also used to thicken soups and prepare vegetable curds.

In industries, mucilage from okra is often used to produce glace papers and confectioneries, also serving as a replacement for blood plasma as well as blood volume expander (Lengsfeld et al., 2004, Adetuyi et al., 2008, Kumar et al., 2010), it is also a useful remedy against genito-urinary problems, chronic dysentery and spermatorrhoea (Nadkarni, 1927). Okra has also been reported to cure ulcers and hemorrhoids (Adams, 1975). Results of tests conducted in China holds that alcoholic extracts from okra leaves have a potential to remove free radicals, reduce proteinuria, and generally improve renal functions (Liu et al., 2005, Kumar et al., 2009).

The cultivation of okra in Nigeria has indeed known success, however, it has also been greeted with a handful of constraints which include but may not be limited to insect pest infestations, disease incidence and poor soil nutrient level (Onunkun, 2012). In response to the constraints from insect pests, man has continued to search out eco-friendly strategies and methods for the management of these setbacks to okra production and agriculture in general (Praveen and Dhandapani, 2001). Some of these methods are; cultural, physical and biological control (Praveen and Dhandapani, 2001). Chemical control is also used and has become the most commonly used control method among farmers because of its almost immediate rate of success in the eradication of these insects, there has however been serious concerns about the long-term negative effect of continued or excessive use of synthetic formulations (Praveen and Dhandapani, 2001). More so, there is a serious need to enlighten farmers on safer, yet effective measures which are useful with almost no side effects or residual negative effects as those experienced while using chemicals. This study was therefore carried out to evaluate the effects of *Boerhavia diffusa* and *Chromolaena odorata* in control of insect pest infestations of two varieties of okra.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE

The experimental site was located behind the Faculty of Agriculture greenhouse, University of llorin, Kwara State, Nigeria, located in the Southern Guinea Savannah agro-ecological zone of Nigeria, between Latitude 8°29'N and Longitude 4°35'E. The experiment was carried out in 2018 cropping season. The climate of Ilorin includes rainy season which starts in April and ends in September while the dry season commences in October and ends in March. The dry spell is experienced in August of every year. The site has an average temperature of 35 ^{0}C and is characterized by sandy loam soil.

2.2 FIELD PREPARATION, AND LAYOUT OF EXPERIMENTAL PLOT

The land was cleared using cutlass and then mapped out into a plot size of 30 m x 15 m. Ridges were made at intervals of 50 cm spacing within plots. The field already mapped out for the experiment was set out using a Randomized Complete Block Design (RCBD). A spacing of 3 m was maintained between the two subplots of varieties, there were three replicates per treatment and 1 m was maintained between replicates, and 2 m from the boundaries of the plot. Each plot measured 2m x 3m (6 m^2) with three (3) ridges in each plot.

2.3 OKRA VARIETIES AND PLANTING

Two varieties of okra common to the farmers in Kwara State were used for the study, they are F1 Lucky and NHAe 47-4 varieties purchased from an Agro-input store in Ilorin. Seeds were planted at five (5) seeds per hole and 50 cm intra-row spacing on 6th October 2018. Two weeks after emergence, thinning was carried out leaving two plants per stand. There were 24 plants in each plot.

2.4 TREATMENTS, PREPARATION, AND APPLICATION

Treatments used for the study were; ethanol leaf extracts of Siam weed, *Chromolaena odorata* (L.) R.M. King & H. Rob**.** ethanol extracts of fresh and dried Red Spiderling, *Boerhavia diffusa* L. nom. cons. Laraforce ® (a.i. lambda-cyhalothrin) as standard check; and untreated control. *Chromolaena odorata* leaf and *B. diffusa* root were sourced from the forest of University of Ilorin. These plant parts were harvested and air dried in the shade for three (3) weeks, the roots were crushed using mortar and pestle while the leaves were ground using a kitchen size electric blender. They were further sieved using a sieve of 2 mm mesh to obtain a uniform powder.

Plant extracts were prepared by maceration. 50 g of each powder were measured into 400 ml of 90 % ethanol and allowed to stand for twenty-four hours while shaking from time to time to ease extraction. The resulting suspension was filtered using muslin cloth, and the filtrate was made up to 2 litres (1:40 or 2.5 % w/v ratio) by adding distilled water, 2 ml of liquid soap per litre of solution was used as surfactant (Anjarwalla, 2016). Lambda-cyhalothrin 2.5 % emulsifiable concentrate was used as a standard check at 0.5 g per litre.

Treatment application commenced four (4) weeks after planting and was done at one-week interval to the 7th week after planting (WAP).

2.5 CULTURAL PRACTICES

Plots were weeded using hoe at 3 WAP (Adigun, 2005). Selective spraying using herbicide (Glyphosate) was done at 6 WAP at the recommended rate.

2.6 INSTALLATION OF YELLOW STICKY TRAPS

One (1) yellow sticky trap was installed per plot, they were placed at the center of the plots at 4 WAP. They were 50 cm above the ground and were left on the plots for two weeks after which they were collected and taken to the Crop Protection Department Laboratory for insect identification and counting. Insects identified include whiteflies (Homoptera) and leafhoppers (Hemiptera). Physical counting was done using hand lens of 10 x magnification and Gordon's dichotomous key to the order of insects (Gordon, 2019) as aid.

2.7 DATA COLLECTION

Five plants per plot were selected at random and tagged. Collection of data commenced at 5 WAP and continued at a week interval to fruiting and harvest. Data were collected on plant height, number of branches, whitefly and leafhopper population, number of days to flowering, number of fruits, and mass of fruits.

2.8 DATA ANALYSIS

Data collected were subjected to a two-way analysis of variance (ANOVA) and Spearman's Correlation Analysis using Genstat 17th edition, and significantly different means were separated using Fishers Least Significant Difference (LSD) test at 5 % level of significance.

3 RESULTS AND DISCUSSION

The results of the study revealed that the various treatments used in the experiment had significant effects on both growth and yield parameters of F1-Lucky and NHAe 47-4 varieties of okra. Plants treated with fresh root extracts of *B. diffusa* generally performed better than untreated plants. The treated plots produced plants which were taller and had more branches compared to those of untreated plots (Tables 1 and 2). This may be because treated plants suffered less infestation from whitefly (*Bemisia tabaci* (Gennadius, 1889) and leafhopper (*Amrasca biguttula* Ishida, 1912) compared to untreated plots (Table 4). This corroborates the findings of Bindhu et al. (2015) who reported that *B. diffusa* and some other plants contained various compounds which are known to have repellent and toxic effects on insects. It also confirms the reports from several studies which have shown *C. odorata* to have pesticidal properties, among these reports are Owolabi et al. (2010) who reported that *C. odorata* has been used to manage *Sitophilus zeamais* (Motschulsky), 1855 which belongs to the order (Coleoptera). Studies have revealed that *B. diffusa* and *C. odorata* contain phytochemicals that are effective in insect pest control (Afolabi et al., 2007 and Deepti et al., 2013).

The better growth performance recorded in treated plants may also be because the plants had lesser infection from diseases vectored by the insect pests identified during the study. Okra is susceptible to at least nineteen plant viruses (Brunt et al., 1990; Swanson and Harrison, 1993). These viruses seriously affect its growth and yield. In several parts of Africa, okra leaf curl disease (OLCD) is considered to be the most serious disease threatening okra production (N'Guessan et al., 1992; Swanson and Harrison, 1993; Bigarré et al., 2001). OLCD is transmitted by whitefly (*Bemisia tabaci*). The incidence and abundance of whiteflies will always be directly proportional to the incidence and severity of OLCD (Bigarré et al., 2001). Therefore, the treated plants facing lesser infestation of whiteflies may have also had lesser incidence of

Table 1: Main effect of treatments and variety on the plant height of okra

	Plant height (cm) WAP				
Treatments	5	6	7	8	9
Fresh Root extract of *B. diffusa*	25.29	39.92	54.53[a]	59.70[a]	70.70[a]
Dry root extract of *B. diffusa*	23.15	35.33	47.83[ab]	55.75[ab]	62.71[ab]
Dry leaf extract of *C. odorata*	20.74	28.61	38.00[b]	43.68[ab]	49.10[b]
Lambda-cyhalothrin	21.82	31.66	41.22[ab]	47.14[ab]	53.76[ab]
Control	19.90	28.47	36.79[b]	41.33[b]	46.47[b]
SEM	2.67	4.39	5.47	6.00	7.07
LSD	7.81	12.84	15.99	17.57	20.69
Variety					
F1-Lucky (kousko)	18.55[b]	28.40[b]	39.10	45.00	51.50
NHAe 47-4	25.81[a]	37.10[a]	48.20	54.00	61.50
SEM	1.34	2.61	3.52	3.88	4.69
LSD	3.88	7.59	10.22	11.27	13.63

Values in the same column followed by the same letter(s) are not significantly different at $p = 0.05$ according to Fisher's protected Least Significant Difference (LSD) Test
Key: WAP: Weeks after Planting

Table 2: Main effect of treatments and variety on number of branches of okra

	Number of branches (WAP)			
Treatments	6	7	8	9
Fresh root extract of *B. diffusa*	4.18[a]	5.43[a]	6.48[a]	7.48[a]
Dry root extract of *B. diffusa*	3.02[b]	4.27[b]	5.24[b]	6.24[b]
Dry leaf extract of *C. odorata*	2.28[b]	3.53[b]	4.96[b]	5.96[b]
Lambda-cyhalothrin	3.35[ab]	4.60[ab]	5.40[ab]	6.40[ab]
Control	2.75[b]	4.00[b]	4.48[b]	5.48[b]
SEM	0.50	0.50	0.50	0.50
LSD	1.10	1.10	1.10	0.70
Variety				
F1-Lucky (kousko)	2.00[b]	4.00[b]	5.00[b]	6.00[b]
NHAe 47-4	4.00[a]	5.00[a]	6.00[a]	7.00[a]
SEM	0.30	0.30	0.30	0.30
LSD	0.70	0.70	0.70	0.70

Values in the same column followed by the same letter(s) are not significantly different at $p = 0.05$ according to Fisher's protected Least Significant Difference (LSD) Test
Key: WAP: Weeks after Planting

Okra Leaf Curl Virus which translated to better growth and yield (Table 6). Fidèle (2010) reported a significant decrease in plant height and some other yield-contributing parameters in four accessions of okra which had symptoms of OLCD.

The NHAe 47-4 variety flowered earlier than the F1-Lucky variety (Table 3). Furthermore, treated plants in the plots for fresh root extracts of *B. diffusa* gave higher fruit yield in both varieties studied (Table 6). This may be because treated plants which were less infested were able to carry out photosynthesis without disruption. Edward and Abdelaziz (2007) reported that any impairment of photosynthetic efficiency by insect pests such as leafhoppers is irreversible and hopper-burn affects plant biomass

Table 3: Main effect of treatments and variety on days to flowering of okra

Treatment	Days to flowering
Fresh root extract of *B. diffusa*	45.76
Dry root extract of *B. diffusa*	46.37
Dry leaf extract of *C. odorata*	46.97
Lambda-cyhalothrin	48.07
Control	48.70
SEM	1.69
LSD	4.17
Variety	
F1-Lucky (kousko)	49.38[b]
NHAe 47-4	44.96[a]
SEM	0.85
LSD	5.90

Values followed by the same letter(s) are not significantly different at p = 0.05 according to Fisher's protected Least Significant Difference (LSD)

Table 4: Main effect of treatments and variety on the population of adult whitefly and leafhopper per yellow sticky trap

Treatment	Whitefly population	Leafhopper population
Fresh root extract of *B. diffusa*	298.20[b]	2.33[a]
Dry root extract of *B. diffusa*	404.20[d]	9.00[d]
Dry leaf extract of *C. odorata*	289.30[a]	5.00[b]
Lambda-cyhalothrin	301.50[c]	5.50[bc]
Control	653.30[e]	7.00[c]
SEM	1.12	0.66
LSD	3.32	1.96
Variety		
F1-Lucky (kousko)	416.00[b]	6.00
NHAe 47-4	363.00[a]	5.53
SEM	0.71	0.42
LSD	2.10	1.24

Values in the same column followed by the same letter(s) are not significantly different at p = 0.05 according to Fisher's protected Least Significant Difference (LSD) Test

accumulation by reducing green leaf area and efficiency of solar radiation capture. Aishwarya (2018) also submitted that both nymph and adults cause damage to okra by sucking the cell sap from the lower surface of leaf, twigs and other tender parts of the plant, causing curling of leaves and leading to stunted growth of the plants. They further reported that leafhoppers excrete honey dew which causes the black sooty mold which has an adverse effect on the photosynthetic ability of the plants. Several reports including Kedar et al. (2014) and Aishwarya Ray (2018) have revealed that whiteflies and their nymphs suck cell sap from plant leaves and affected leaves curl and dry with the affected plants showing a stunted growth. Whiteflies are also responsible for transmitting yellow vein mosaic virus (YVMV), an economically important disease of okra, the symptoms being interwoven networks of yellow veins surrounded by islands of green tissues on the leaves which also turn yellow with time (Kedar et al., 2014; Aishwarya, 2018). The control of incidence of these insect pests and the consequent reduction or elimination of the occurrence of diseases mediated by the insect pests identified on the plots may have translated to the better yield obtained from the treated plots.

The study also revealed a strong positive correlation exists between leafhopper and whitefly population, plant height and number of fruits, plant height and overall yield, and number of fruits and overall yield (Table 5). This suggests that the leafhopper population increased with the whitefly population, while the yield was directly proportional to the growth of the plants.

4 CONCLUSION

The findings of this study revealed that ethanol extracts of *Boerhavia diffusa* and *Chromolaena odorata* had significant positive effects on growth and yield of okra and were also effective in reducing the population of leafhoppers and whiteflies in treated plots. There was a significant positive correlation between leafhopper and whitefly population, plant height and number of fruits, plant height and overall yield, and number of fruits and overall yield. While there was a significant negative correlation between overall yield and whitefly population. There was a non-significant negative correlation between whitefly population and plant height, whitefly population and number of fruits, leafhopper population and plant height, leafhopper population and number of fruits, and leafhopper population and overall yield.

Of the two botanicals evaluated fresh root extract of *B. diffusa* on NHAe 47-4 okra variety performed better than other treatments from germination and emergence, through growth parameters to yield.

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Table 5: Spearman's correlation matrix for various parameters in the study

	Whitefly population	Leafhopper population	Plant height	Number of fruits/ha
Leafhopper population	0.469**			
Plant height	-0.287	-0.191		
Number of fruits/ha	-0.249	-0.117	0.688**	
Overall yield (kg ha^{-1})	-0.364*	-0.215	0.746**	0.966**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 6: Main effect of treatments and variety on number of fruits per plant, number of fruits per plot, and overall yield (kg ha^{-1}) of okra

Treatments	Number of fruits/plant	Number of fruits/plot	Fruit yield (kg ha^{-1})
Fresh root extract of *B. diffusa*	13.76	165.10	2029.00^{a}
Dry root extract of *B. diffusa*	9.48	113.80	1231.00^{ab}
Dry leaf extract of *C. odorata*	9.50	114.10	1304.00^{ab}
Lambda-cyhalothrin	8.81	106.10	1223.00^{ab}
Control	7.66	91.20	980.00^{b}
SEM	2.20	26.30	334.20
LSD	6.40	76.90	977.60
Variety			
F1-Lucky (kousko)	7.00^{b}	80.00^{b}	878.00^{b}
NHAe 47-4	13.00^{a}	155.90^{a}	1829.00^{a}
SEM	1.10	13.50	178.30
LSD	3.30	39.25	518.40

Values in the same column followed by the same letter(s) are not significantly different at p = 0.05 according to Fisher's protected Least Significant Difference (LSD) Test

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Agro-biološka raznolikost slovenskih ekotipov in standardnih sort navadne pasje trave (*Dactylis glomerata* L.): primerjava in agronomska vrednost

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Agro-biološka raznolikost slovenskih ekotipov in standardnih sort navadne pasje trave (*Dactylis glomerata* L.): primerjava in agronomska vrednost

Izvleček: Opisovanje in vrednotenje akcesij, na primer ekotipov kulturnih rastlin, je temeljna naloga kmetijske rastlinske genske banke. S poljskim poskusom smo v letih 2015-2019 raziskovali agro-biološko raznolikost 15 slovenskih ekotipov (skupina 1) in 7 standardnih sort (skupina 2) navadne pasje trave (*Dactylis glomerata* L.) s poudarkom na primerjavi teh dveh skupin. Ocenili smo tudi agronomsko vrednost ekotipov glede na sorte. Zasnova poskusa je bila naključni blok s 3 ponovitvami. Vsak ekotip oziroma sorta je bila zastopana z 20 posameznimi rastlinami na ponovitev. Med obravnavanimi entitetami so bile ugotovljene značilne razlike v vseh preučevanih agro-bioloških lastnostih ($p < 0{,}001$). Značilne razlike so bile ugotovljene tudi pri primerjavi skupin ($p < 0{,}001$) razen v okuženosti z listnimi boleznimi ($p = 0{,}113$). Pri tem so bile sorte z agronomskega vidika boljše kot ekotipi. Raznolikost znotraj populacij je bila pri slovenskih ekotipih večja kot pri standardnih sortah, na njo je odpadel tudi večji del skupne variance ekotipov. Analiza glavnih komponent (AGK) na vseh preučevanih lastnostih je pokazala izrazito razlikovanje ekotipov od sort in večjo podobnost med sortami kot med ekotipi. Pri slednjih se kot rezultat AGK kažeta dve podskupini, kar pa ne moremo razložiti z izvorom ekotipov.

Ključne besede: genska banka; populacije; opisovanje; vrednotenje

Agro-biological diversity of Slovene ecotypes and standard varieties of cocksfoot (*Dactylis glomerata* L.): comparison and agronomic value

Abstract: Characterisation and evaluation of accessions, e.g. ecotypes of cultivated plants, are the primary task of each agricultural gene bank. In a field experiment, agro-biological diversity and agronomic value of 15 Slovene ecotypes (group 1) and 7 standard varieties (group 2) of cocksfoot (*Dactylis glomerata* L.) were investigated during the 2015-2019 period. A particular emphasis was given to the comparison of the two groups. The spaced plant experiment with 20 single plants of each treatment entity per replicate was arranged in a randomized complete block design with three replicates. Significant differences among investigated entities were confirmed for all agro-biological traits ($p < 0.001$). The same holds when the groups were compared ($p < 0.001$) except for the infection with leaf fungal diseases ($p = 0.113$).Considering these differences varieties possessed higher agronomic value than ecotypes. In general, the intra-population diversity of Slovene ecotypes was higher than that of standard varieties and represented a higher portion of the ecotype complete variance. Principal component analysis (PCA) of all investigated traits showed a distinctive difference between ecotypes and varieties and higher similarity within the variety group than within the ecotype group. PCA also showed that the ecotypes can be separated into two subgroups, which however cannot be explained by the characteristics of ecotype origin.

Key words: gene bank; populations; characterization; evaluation

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1 UVOD

Navadna pasja trava (*Dactylis glomerata* L.) je trajnica zmernega podnebnega pasu, ki jo sestavlja več različno ploidnih podvrst. Na travinju v Evropi in širše prevladuje antropofilna, tetraploidna (2n = 4x = 28) oblika (Lindner in Garcia, 1997; Tuna in sod., 2004). Enako velja tudi za situacijo z navadno pasjo travo v Sloveniji (Martinčič in sod., 2007).

Navadna pasja trava je konkurenčna in na stres tolerantna vrsta, zato je pomembna za pridelavo krme na rodovitnih travniških tleh, v travno-deteljnih mešanicah na njivah in na manj rodovitnih travniških tleh, kjer se redno pojavlja suša. Priporoča se za setev v dveletnih do štiriletnih mešanicah z ljuljkami, ker pripomore k boljši in zanesljivejši pridelavi krme v drugi polovici pridelovalnega obdobja (Suter in sod., 2013; AGFF, 2017).

Krmna vrednost navadne pasje trave je v zgodnjih razvojnih fazah zelo velika. V primerjavi z drugimi kakovostnimi travami izstopa z veliko vsebnostjo surovih beljakovin (DLG, 1997). Tako je na primer v zadnji dekadi aprila povprečna dveletna vsebnost surovih beljakovin v zelinju navadne pasje trave znašala 219 g kg^{-1} sušine, pri trpežni ljuljki pa le 171 g kg^{-1} sušine (Čop in sod., 2009). Slabšanje kakovosti zelinja s staranjem poganjkov je agronomska pomanjkljivost navadne pasje trave, ki se ji lahko delno izognemo s primerno zgodnjo rabo. Uspešnost pridelave krme z navadno pasjo travo, tako v smislu prilagajanja okoljskim in pridelovalnim razmeram kot zagotavljanja ustrezne kakovosti krme, je v veliki meri odvisna od sort, pa tudi od ekotipov, ki se uporabljajo za žlahtnjenje.

Agro-biološke lastnosti trav, s katerimi se ukvarjamo pri ocenjevanju ekotipov, sort v potrjevanju (test razločljivosti, izenačenosti in nespremenljivosti – RIN-test) in žlahtnjenju, so pomembne tako s stališča količine in kakovosti pridelka kot trajanja pridelave (trpežnosti). Ocenjujemo agro-biološke lastnosti, kot na primer višina stebel, pokončnost rasti (habitus), začetek latenja, tvorba socvetij med rastno sezono, regeneracija po defoliaciji, okuženost z boleznimi, trpežnost idr. (IPGRI, 1985; UPOV, 2002). Kakovostna sorta navadne pasje trave ne sme biti previsoka. Mora biti pokončne rasti in pozna v razvoju, imeti mora slabo izraženo determinantno rast ter dobro regeneracijo, odpornost proti boleznim in trpežnost.

Z raziskavo smo želeli ugotoviti: (1) kakšno je variiranje preučevanih agro-bioloških lastnosti med in znotraj ekotipov in standardnih sort, (2) kakšna je agronomska vrednost ekotipov glede na preučevane lastnosti v primerjavi s standardnimi sortami in (3) ali izvor ekotipov (geografska lega, talne lastnosti, pridelovalne razmere) oziroma žlahtnjenje sort povzroči njihovo grupiranje ob upoštevanju vseh preučevanih lastnosti obenem.

2 MATERIALI IN METODE

2.1 EKOTIPI IN STANDARDNE SORTE

V raziskavo je bilo vključenih 15 slovenskih ekotipov in 7 standardnih sort navadne pasje trave. Ekotipe smo nabirali v obliki semena na trajnih travnikih v nižinskih, gričevnatih in hribovitih predelih Slovenije v letih od 2007 do 2014. Vsi so vključeni v rastlinsko gensko banko Slovenije. Sorte smo pridobili neposredno od žlahtniteljev oziroma pooblaščenih semenarskih hiš. Pri izboru sort smo upoštevali priporočili inštitucij, ki izvajata RIN-teste, tj. Centralnega inštituta za kontrolo in testiranje v Bratislavi (Ústredný kontrolný a skúšobný ústav poľnohospodársky v Bratislave, Slovaška) in Raziskovalnega centra za testiranje sort v Słupia Wielka (Centralny Osrodek Badania Odmian Roslin Uprawnych, Poljska). Upoštevali smo tudi pridelovalno vrednost in ekološke lastnosti sort. Podatki o izvoru ekotipov in sort so v Preglednici 1.

2.2 OPIS POSKUSA

Poljski poskus je potekal po standardiziranem postopku na poskusnem polju Oddelka za agronomijo Biotehniške fakultete v Ljubljani od leta 2015 do leta 2019. Zasnovan je bil v naključnem bloku s tremi ponovitvami. Vsak ekotip oziroma sorto v posamezni ponovitvi je sestavljalo 20 rastlin, ločeno posajenih na razdaljo 50 × 70 cm. Vseh rastlin v poskusu je bilo 1320. Letna oskrba poskusa je zajemala spomladansko gnojenje z NPK 15:15:15, dve dognojevanji s kalcijevim amonijevim nitratom (KAN, 27 % N), tri košnje in dve okopavanji. Letni odmerek hranil je znašal 200 kg N v treh obrokih, 80 kg P_2O_5 in 80 kg K_2O ha^{-1}. Višina rezi ob košnji je bila od 5 do 7 cm.

Na poskusnem polju se nahajajo rjava aluvialna tla na karbonatnem pesku in produ. V zgornji 30 cm plasti je meljasta ilovica, pod njo je do globine 110 cm zmerno oglejena meljasto glinasta ilovica. Na poskusnem polju je postavljena cevna drenaža, po kateri odteka padavinska voda, ki pronica skozi tla. Na začetku poskusa so bila tla v zgornji 22 cm plasti nevtralna (pH 6,9 v $CaCl_2$) ter dobro preskrbljena s fosforjem (137 mg P_2O_5 kg^{-1} tal) in srednje dobro s kalijem (172 mg K_2O kg^{-1} tal).

Vremenske razmere so bile v poskusnem obdobju razmeroma ugodne za rast krmnih trav. Poletna suša, ki je običajna v pretežnem delu Slovenije, je bila izrazitejša

Preglednica 1: Podatki o izvoru ekotipov in standardnih sort navadne pasje trave, vključenih v raziskavo. Dodani so podatki o rastišču in rabi za ekotipe in podatki o zgodnosti za sorte.
Table 1: Data on the origin of ecotypes and standard varieties of cocksfoot included in the research. Data on the habitat and number of harvests for ecotypes and development earliness for the varieties are added.

Ekotip/ Sorta	Pokrajina/ Država	Lokacija/ Žlahtnitelj	Zemljepisna širina (S)	Zemljepisna dolžina (V)	Nadmorska višina (m)	Vlažnost rastišča	Košnja (število)
Dg 01/14	Bela krajina	Špeharji	45°26′	15°8′	278	suho	1
Dg 02/14	Bela krajina	Marindol	45°30′	15°19′	238	suho	2
Dg 03/14	Bela krajina	Semič	45°39′	15°6′	446	polsuho	1-2
Dg 04/14	Zasavje	Padež	46°3′	15°0′	748	suho	1
Dg 01/13	Dolenjska	Veliko Mlačevo	45°56′	14°40′	328	sr. vlažno	2-3
Dg 04/13	Dolenjska	Zdenska vas	45°49′	14°42′	441	polsuho	1-2
Dg 05/13	Dolenjska	Kompolje	45°48′	14°44′	463	polsuho	1-2
Dg 08/13	Dolenjska	Ambrus	45°49′	14°48′	355	sr. vlažno	2-3
Dg 09/13	Zasavje	Ribče	46°5′	14°46′	272	polsuho	1-2
Dg 13/13	Gorenjska	Jamnik	46°16′	14°12′	830	polsuho	1-2
Dg 14/13	Osrednja Slovenija	Črnuče	46°56′	14°26′	298	sr. vlažno	2-3
Dg 16/13	Osrednja Slovenija	Gorenja Brezovica	45°56′	14°26′	481	suho	1
Dg 19/13	Zasavje	Podkum	46°4′	15°2′	750	suho	1
Dc 06/07	Notranjska	Travnik	45°41′	14°35′	703	sr. vlažno	2
Dg 03/07	Notranjska	Travna Gora	45°44′	14°38′	891	polsuho	2
Trerano	Nemčija	Feldsaaten Freudenberger	zelo zgodna sorta				
Beluga	Švica	DSP AG	zelo pozna sorta				
Reda	Švica	DSP AG	zgodna sorta				
Intensiv	Nizozemska	Barenbrug	srednje zgodna do pozna sorta				
Dascada	Nizozemska	Barenbrug	zgodna do srednje zgodna sorta				
Barlegro	Nizozemska	Barenbrug	zelo pozna sorta				
Padania	Italija	CREA-ZA Lodi	srednje zgodna sorta				

v letih 2016, 2017 in 2019. Najbolj je na to vplivala neugodna časovna razporeditev padavin med rastno sezono, manj pa tudi povečana temperatura zraka. Tako je bilo v skrajnih primerih 151 mm manj padavin med majem in avgustom 2017 in 4,4 °C višja povprečna temperatura zraka v juniju 2019 kot v referenčnem obdobju 1981-2010.

2.3 MERITVE IN OCENJEVANJE AGRO-BIOLOŠKIH LASTNOSTI

V posameznih letih smo opravili meritve višine rastlin in šest ocenjevanj agro-bioloških lastnosti. Pri tem smo upoštevali navodila Mednarodnega inštituta za rastlinske genske vire (IPGRI, 1985) in Mednarodne zveze za zaščito novih rastlinskih sort (UPOV, 2002). Višino poganjkov po posameznih šopih smo merili v maju, ko je bila večina poganjkov v fazi zorenja plodov. Upoštevali smo dolžino od osnove do vrha socvetja večine poganjkov v šopu. Ocenjevali smo ob pomoči petstopenjske lestvice, razen pri prisotnosti generativnih poganjkov v jeseni in okuženosti z listnimi boleznimi. V prvem primeru smo uporabili dvostopenjsko lestvico, v drugem pa devetstopenjsko. Habitus pomeni izraženost pokončne rasti poganjkov in sicer je 1 ležeča rast (prostratum) in

5 pokončna rast (erektum). Začetek latenja je razvojna faza trav, ki jo je najlažje določiti in se uporablja za oceno zgodnosti. To ocenjevanje smo izvedli v časovnem obdobju dveh mesecev v petih terminih (pred 15. aprilom, 15. in 22. aprila, 9. in 23. maja). Faza začetka latenja je nastopila, ko so se v šopu pojavili trije poganjki s socvetjem. Regeneracijo po košnji smo ocenili v juliju, ko visoka temperatura zraka in pogosto tudi suša poslabšata tvorbo novih listov, ki pomeni začetek nove rasti [ocene: 1 (slaba) do 5 (zelo dobra regeneracija)]. Tendenco tvorbe generativnih poganjkov izven sezone generativne rasti, smo ocenili konec septembra z binarnim popisom prisotnosti oziroma odsotnosti le-teh po posameznih šopih. Vigor rastlin smo ocenili konec pete rastne sezone, s čimer smo dobili posredno oceno trpežnosti [ocene: 1 (slab) do 5 (zelo dober vigor)]. Okuženost listov z glivičnimi boleznimi, med katerimi so prevladovale okužbe z glivami rodu *Puccinia*, smo ocenili v začetku avgusta [ocene: 1 (< 10 %) do 9 (75-100 % okuženost)].

2.4 STATISTIČNA OBDELAVA

Raznolikost preučevanih agro-bioloških lastnosti med in znotraj ekotipov in standardnih sort je prikazana z grafom za razpršenost oziroma z grafi za frekvenčno porazdelitev podatkov. Okvir z ročaji je bil uporabljen pri zvezni spremenljivki za višino rastlin, stolpčni grafi pa pri ordinalnih spremenljivkah za vse druge lastnosti. Razlika v višini rastlin med posameznimi obravnavanimi entitetami (ekotipi in standardne sorte) ter med skupino ekotipov in skupino sort je bila testirana z analizo variance, razlike v drugih lastnostih pa s Kruskal-Wallisovim H testom. Primerjava ekotipov in standardnih sort po vseh parametrih hkrati je bila narejena z analizo glavnih komponent, pri čemer smo zaradi večinoma ordinalnih spremenljivk uporabili polihorično korelacijo med spremenljivkami. Analize smo opravili v statističnem programskem okolju R (R Core Team, 2019). Za izris stolpčnih grafov ordinalnih spremenljivk smo uporabili knjižnico »likert«.

3 REZULTATI Z RAZPRAVO

3.1 AGRO-BIOLOŠKA RAZNOLIKOST EKOTIPOV IN STANDARDNIH SORT

Primerjava obravnavanih entitet navadne pasje trave je pokazala, da so med njimi statistično značilne razlike v vseh preučevanih agro-bioloških lastnostih ($p < 0,001$). Enako velja za primerjavo skupine slovenskih ekotipov s skupino standardnih sort ($p < 0,001$) razen pri okuženosti listov z boleznimi, kjer razlika ni značilna ($p = 0,113$).

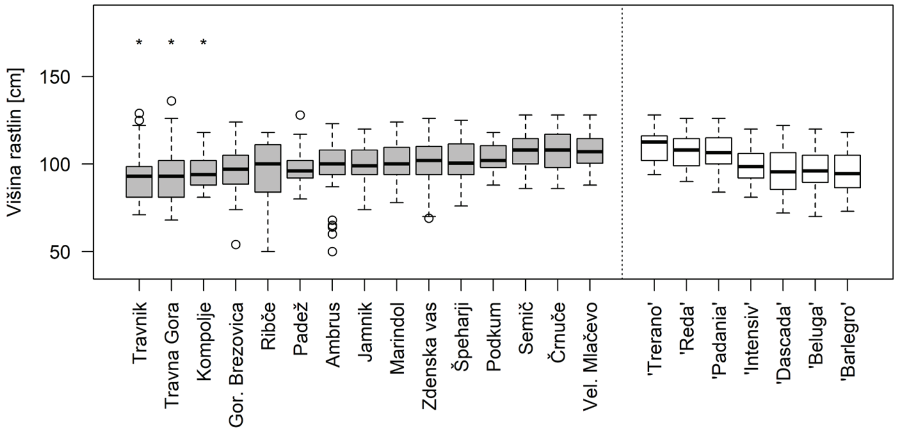
V agronomskem smislu so bile sorte izrazito boljše od ekotipov v treh lastnostih, ki so zelo pomembne za količino in kakovost pridelka ter trajanje pridelave. V primerjavi z ekotipi so sorte rastle izrazito bolj pokončno, se počasneje razvijale in dalj časa ohranjale rastno kondicijo (Slike 2, 3 in 6). Rastline standardnih sort so tudi bolje regenerirale po defoliaciji in razvile manj generativnih poganjkov (Sliki 4 in 5), vendar prednost sort pred ekotipi v teh dveh lastnostih ni bila tako izrazita kot pri prvih treh. V preostalih dveh lastnostih – višini generativnih poganjkov in okuženosti listov z glivičnimi boleznimi – so si bile sorte in ekotipi podobni (Sliki 1 in 7). Za višino poganjkov to velja kljub značilni razliki med skupinama, ki je posledica zgolj treh značilno manjših ekotipov od povprečne višine sort. Navedene razlike med ekotipi in sortami so pričakovane, zlasti zato, ker smo za standard izbrali najboljše sorte. Te pa so praviloma boljše od ekotipov oziroma izvornega selekcijskega materiala (Fehr, 1991).

Pri naši primerjavi ekotipov in standardnih sort ni bilo nobenega izjemnega ekotipa pri katerikoli lastnosti, čeprav tak primer navajajo Boller in sod. (2009). Pri mnogocvetni ljuljki so namreč ugotovili večjo pridelovalno vrednost izbranih švicarskih ekotipov v primerjavi s sortami.

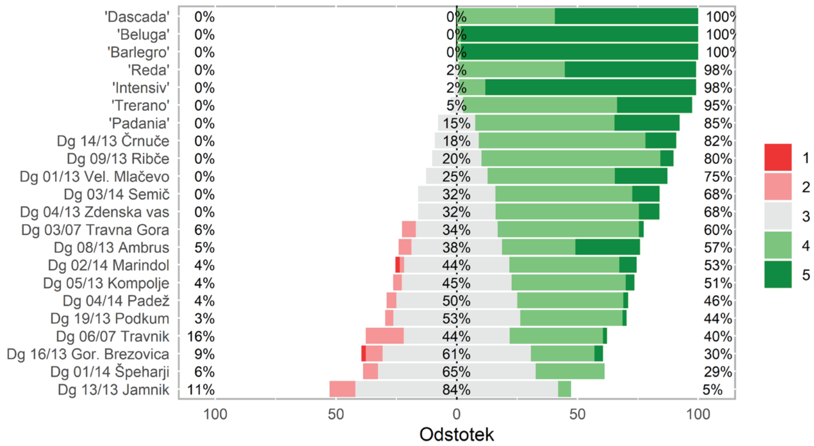
3.2 ZNOTRAJ POPULACIJSKA RAZNOLIKOST EKOTIPOV IN STANDARDNIH SORT

Znotraj populacijska raznolikost v preučevanih lastnostih je bila izražena tako pri ekotipih kot sortah. Vendar so bile populacije ekotipov izrazito bolj raznolike glede habitusa, regeneracije po defoliaciji, izvensezonske tvorbe generativnih poganjkov in vigorja (Slike 2, 4, 5 in 6). V primerjavi z večino sort je bila znotrajpopulacijska raznolikost ekotipov tudi večja glede zgodnosti razvoja in okuženosti z listnimi boleznimi (Sliki 3 in 7). Znotrajpopulacijska raznolikost v višini generativnih poganjkov je bila podobna pri obeh skupinah (Slika 1).

Ker je navadna pasja trava alogamna vrsta, so populacije ekotipov in sort genetsko – s tem pa tudi fenotipsko – precej raznolike. Vendar je ta raznolikost izrazitejša pri ekotipih, ker naravna selekcija poteka manj usmerjeno kot odbira pri žlahtnjenju. Raznolikost v preučevanih lastnostih znotraj ekotipov je bila na splošno tudi večja od raznolikosti med samimi ekotipi. Enake ugotovitve glede genetske in fenotipske raznolikosti navajajo Last in sod. (2011) ter Xie in sod. (2012) za ekotipe navadne pasje trave, Bolaric in sod. (2005) za ekotipe trpežne ljuljke ter Pagnotta in sod. (2011) za ekotipe črne detelje. Ve-



Slika 1: Razpršenost višine 20 rastlin navadne pasje trave po posameznih ekotipih (označeni so z lokacijo) in standardnih sortah, prikazana z okvirji z ročaji. Meritev je bila opravljena 30. maja 2017. Opomba: Simbol * označuje ekotipe, ki se značilno razlikujejo od povprečja standardnih sort.
Figure 1: Box-plot for the height distribution of 20 cocksfoot plants by individual ecotypes (denoted by location name) and standard varieties. The measurement was conducted on May 30 2017. Note: Symbol * marks ecotypes which differed significantly from the average height of varieties.



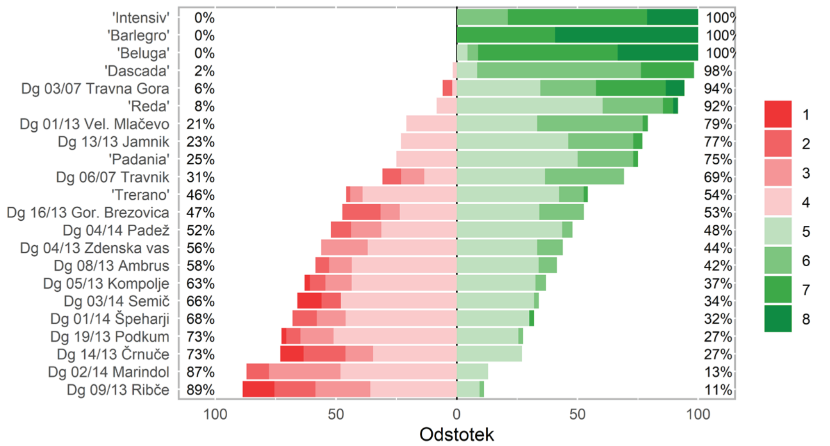
Slika 2: Pogostnostna porazdelitev ocen habitusa po posameznih ekotipih in standardnih sortah navadne pasje trave (n = 20). Ocenjevanje je bilo opravljeno 23. maja 2016. Ocene: 1 (ležeča rast) do 5 (pokončna rast).
Figure 2: Frequency distribution of the habitus estimates by individual ecotypes and standard varieties of cocksfoot (n = 20). The estimation was conducted on May 23 2016. Estimates: 1 (prostrate growth) to 5 (erect growth).

lika genetska raznolikost med ekotipi je bolj redka kot znotraj ekotipov in je predvsem pogojena z geografsko oddaljenostjo izvora ekotipov (McGrath, 2008; Last in sod., 2013), kar povečuje razlike v rastnih razmerah in zmanjšuje prenašanje genov med oddaljenimi skupinami rastlin.

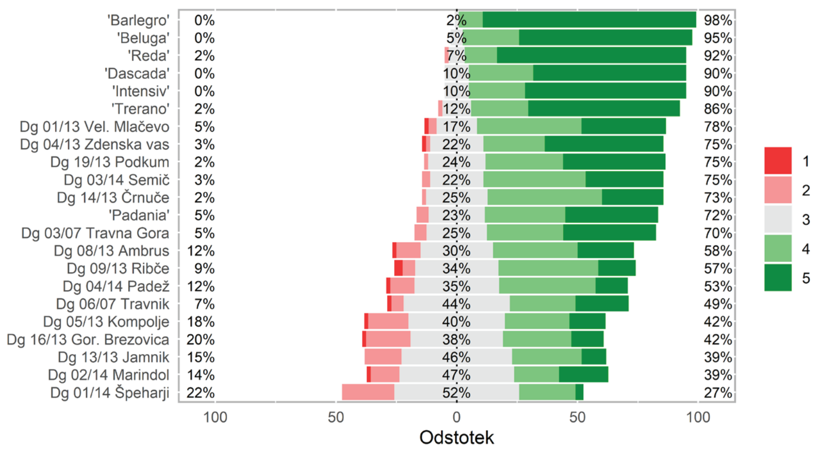
Genetska raznolikost vrst, ki se na travinju izraža tudi v raznolikosti ekotipov, je bistvenega pomena za vitalnost ekosistemov, pri kulturnih rastlinah pa tudi za žlahtniteljski napredek pri vzgoji novih sort. Pri sortah namreč hitro pride do izgube redkih alelov, prisotnih v naravnih ali polnaravnih populacijah, zato vključevanje samo teh v žlahtnjenje zmanjša možnosti za uspeh (Boller in Greene, 2010).

3.3 MULTIVARIATNA PRIMERJAVA EKOTIPOV IN STANDARDNIH SORT

Analiza glavnih komponent, v katero so bile vključene vse preučevane spremenljivke, je pokazala, da prvi dve glavni komponenti pojasnjujeta večino (79,8 %) variabilnosti obravnavanih entitet. Pokazala je tudi, da obstaja zelo jasno razlikovanje med populacijami ekotipov in



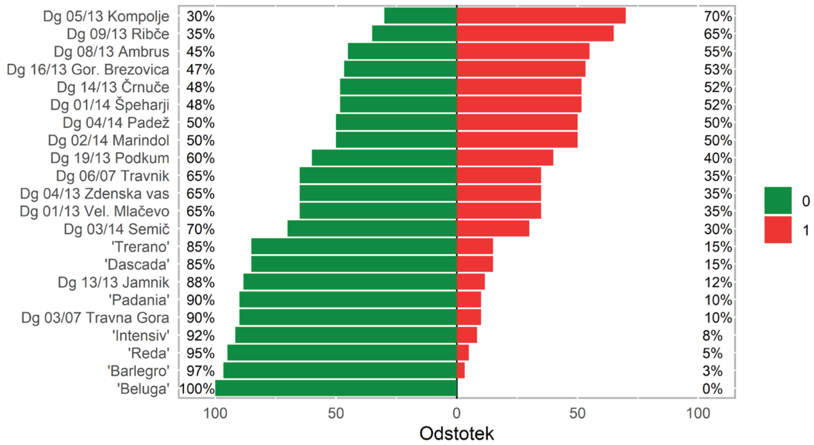
Slika 3: Pogostnostna porazdelitev ocen zgodnosti po posameznih ekotipih in standardnih sortah navadne pasje trave (n = 20). Zgodnost je bila ocenjena z razvojno fazo »začetek latenja«, pomladi 2016. Ocene: 1 (zelo zgodnja) do 8 (zelo pozna).
Figure 3: Frequency distribution of the earliness estimates by individual ecotypes and standard varieties of cocksfoot (n = 20). The development earliness was estimated by means of development stage »ear emergence« in spring 2016. Estimates: 1 (very early) to 8 (very late).



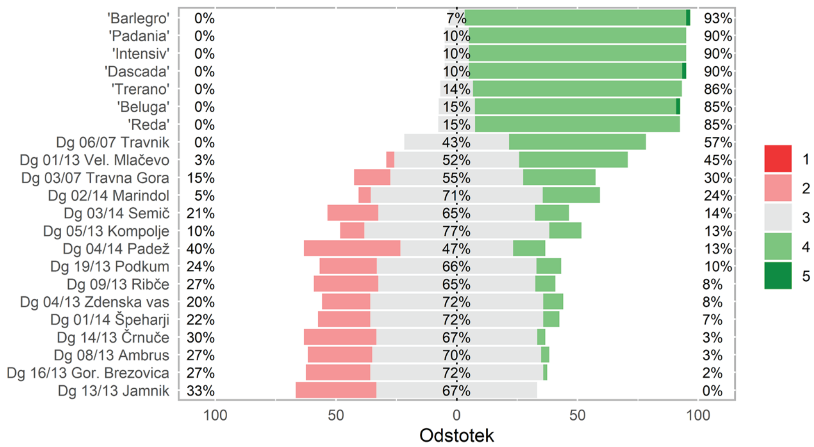
Slika 4: Pogostnostna porazdelitev ocen regeneracije po posameznih ekotipih in standardnih sortah navadne pasje trave (n = 20). Ocenjevanje regeneracije rastlin po poletni defoliaciji je bilo opravljeno 10. julija 2017. Ocene: 1 (zelo slaba) do 5 (zelo dobra regeneracija).
Figure 4: Frequency distribution of the regeneration estimates by individual ecotypes and standard varieties of cocksfoot (n = 20). The estimation of plant regeneration after summer harvest was conducted on July 10 2017. Estimates: 1 (very poor) to 5 (very good regeneration).

sortami (Slika 8), kar smo glede na rezultate preučevanih lastnosti tudi pričakovali. Velika podobnost sort je posledica žlahtnjenja, katerega cilji so bili v vseh primerih enaki – vzgoja sort, primernih za zmerno celinsko podnebje in srednje intenzivno rabo. Podobnost ekotipov je znatno manjša od podobnosti sort. Predvsem odstopajo v smeri druge glavne komponente štirje ekotipi, za katere pa ni bila ugotovljena neka skupna posebnost glede izvora. Za to skupino, ki jo sestavljajo ekotipi 'Ambrus', 'Črnuče', 'Kompolje' in 'Ribče', je bila v celoti značilna poudarjena generativna rast izven njene običajne sezone. Poleg tega sta bila ekotipa 'Kompolje' in 'Ambrus' zelo občutljiva za listne bolezni, ekotipa 'Črnuče' in 'Ribče' pa najbolj pokončne rasti med vsemi ekotipi. Vse te tri lastnosti so povzročile odmik navedenih ekotipov od drugih, kar ponazarjata smer in dolžina vektorjev.

Pri primerjavi ekotipov je zanimivo, da selekcijski pritisk, vezan na rodovitnost tal, ni bil tako velik, da bi povzročil grupacijo glede na vlažnost rastišča – vsaj med skrajnima skupinama na svežih in izrazito suhih tleh. Geografska oddaljenost izvornih lokacij ekotipov, ki v našem primeru ni bila velika, je ključnega pomena za na-



Slika 5: Pogostnostna porazdelitev prisotnosti generativnih poganjkov po posameznih ekotipih in standardnih sortah navadne pasje trave ($n = 20$). Popis je bil opravljen 26. septembra 2016. Oceni: 0 (brez), 1 (z generativnimi poganjki).
Figure 5: Frequency distribution of the presence of reproductive tillers by individual ecotypes and standard varieties of cocksfoot ($n = 20$). The estimation was conducted on September 26 2016. Estimates: 0 (without), 1 (with reproductive tillers).



Slika 6: Pogostnostna porazdelitev ocen vigorja po posameznih ekotipih in standardnih sortah navadne pasje trave ($n = 20$). Ocenjevanje je bilo opravljeno ob koncu 6. rastne sezone, 18. novembra 2019. Ocene: 1 (slab) do 5 (zelo dober rastni vigor).
Figure 6: Frequency distribution of the vigour estimates by individual ecotypes and standard varieties of cocksfoot ($n = 20$). The estimation was conducted at the end of the 6th growth season on the November 18 2019. Estimates: 1 (poor) to 5 (very good growth vigour).

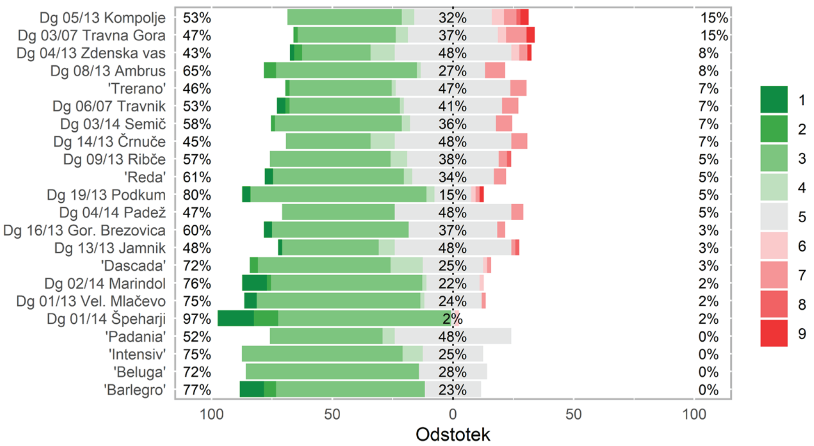
stanek genetsko in fenotipsko različnih ekotipov. Izjema se pri tem lahko pojavi, če so pridelovalne razmere zelo različne. Tak primer so švicarske populacije navadne pasje trave, ki so izvirale iz razmeroma bližnjih lokacij trajnega travinja (velikost območja 12 km^2), a je bila na njih pridelava krme različno intenzivna (Last in sod., 2014).

4 ZAKLJUČKI

Preučili smo raznolikost in agronomsko vrednost na novo nabranih slovenskih ekotipov navadne pasje trave. Pri tem smo kot standard uporabili kakovostne evropske sorte, namenjene za gojenje v zmerno celinskem podnebnem pasu, kamor spada tudi večji del Slovenije.

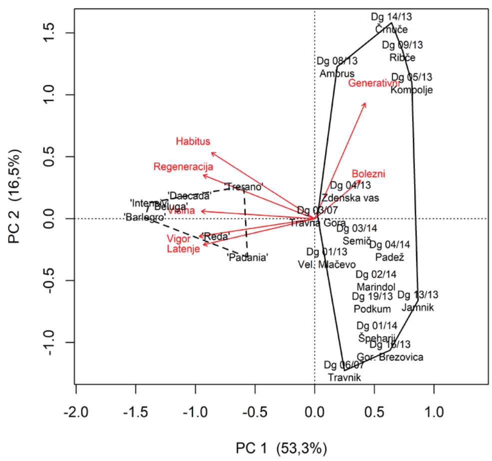
Med obravnavanimi entitetami so bile ugotovljene značilne razlike v vseh preučevanih agro-bioloških lastnostih. Značilne razlike so bile ugotovljene tudi pri primerjavi skupine ekotipov s skupino sort razen v okuženosti z listnimi boleznimi. Pri tem so bile z agronomskega vidika sorte boljše kot ekotipi. Znotrajpopulacijska raznolikost ekotipov v preučevanih lastnostih je bila večja kot tista pri sortah. Na njo je tudi odpadel večji del skupne variabilnosti ekotipov.

Žlahtnjenje navadne pasje trave se je odrazilo v manjši medsebojni raznolikosti sort v primerjavi z med-



Slika 7: Pogostnostna porazdelitev ocen okuženosti z listnimi boleznimi po posameznih ekotipih in standardnih sortah navadne pasje trave (n = 20). Ocenjevanje je bilo opravljeno 1. avgusta 2016. Ocene: 1 (1-5 %) do 9 (76-100 % pokritost listnih ploskev s simptomi).

Figure 7: Frequency distribution of the estimates of the infection with leaf fungal diseases by individual ecotypes and standard varieties of cocksfoot (n = 20). The estimation was conducted on the August 1 2016. Estimates: 1 (1-5 %) to 9 (76-100 % leaf blade cover by symptoms).



Slika 8: Rezultati analize glavnih komponent. Prikaz 15 slovenskih ekotipov in 7 standardnih sort navadne pasje trave skupaj z vektorsko težo obravnavanih lastnosti v dvorazsežnem prostoru glavnih komponent.

Figure 8: Results of principal component analysis. Arrangement of 15 Slovene ecotypes and 7 standard varieties together with the weight vectors for all investigated traits in 2D dimensional space of two main components

sebojno raznolikostjo ekotipov. Sorte so kot skupina tudi izrazito odstopale od skupine ekotipov. Čeprav sta kot rezultat analize glavnih komponent nastali dve podskupini ekotipov, za to nismo ugotovili nobenega z izvorom povezanega vzroka.

Velika znotraj populacijska raznolikost ekotipov navadne pasje trave bogati biotsko raznolikost travniških ekosistemov in izboljšuje njihovo funkcionalnost. To velja tudi za druge vrste. Genetsko različni ekotipi so nujni tudi za žlahtniteljski napredek pri vzgoji novih sort, saj so posamezni genotipi vir potencialno koristnih alelov, ki pri sortah več niso prisotni.

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Somatic embryogenesis of hypocotyl derived calli from an eggplant cultivar

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Somatic embryogenesis of hypocotyl derived calli from an eggplant cultivar

Abstract: Optimization of tissue culture and regeneration conditions of eggplant is necessary for achieving different goals such as gene transformation and the development of somaclonal variations. In this study, hypocotyl explants ware used to produce callus in a medium containing different concentrations of NAA and BAP. Moreover, the concentration of the elements Ca, Mn, Mg, Fe and K were measured and analysed between embryogenic and non-embryogenic calli. For shoot elongation, embryogenic calli were transferred to a new culture medium containing 3.5, 4 and 4.5 mg l^{-1} BAP plus 2 mg l^{-1} GA3. Finally, produced shoots were rooted in a culture medium containing 1, 1.5 and 2 mg l^{-1} NAA. Results showed that the best treatment for the embryogenic callus induction was MS medium containing 0.5 mg l^{-1} BAP plus 0.25 mg l^{-1} NAA. Two elements, Fe and K, had the highest amount in non-embryogenic calli compare to the embryogenic one. For plant regeneration, MS medium containing 4.5 mg l^{-1} BAP plus 2 mg l^{-1} GA3 and 2 mg l^{-1} NAA were the best treatments for shooting and rooting, respectively. In this study, the best treatments for plant regeneration produced 35 shoots from an explant with 92 % shooting. This regeneration protocol could be useful for gene transformation and micro-propagation studies.

Key words: eggplant; tissue culture; somatic embryogenic regeneration; BAP; NAA

Abbreviations:
NAA Naphthaleneacetic acid
BAP 6-Benzylaminopurine
GA3 Gibberellic acid
MS medium Murashige & Skoog Medium
IBA Indole-3-butyric acid
IAA Indole acetic acid

Somatska embriogeneza hipokotilnih kalusov izbrane sorte jajčevca

Izvleček: Optimizacija tkivnih kultur jajčevca in regeneracijskih razmer sta potrebni za doseganje različnih ciljev kot sta genska transformacija in razvoj somaklonalne variabilnosti. V tej raziskavi so bili uporabljeni hipokotilni izsečki za pridobitev kalusa v gojiščih, ki so vsebovala različne koncentracije NAA in BAP. Med embriogenimi in neembriogenimi kalusi so bile izmerjene koncentracije elementov kot so Ca, Mn, Mg, Fe in K. Za dolžinsko rast poganjkov so bili embriogeni kalusi premeščeni v drugo gojišče, ki je vsebovalo 3,5, 4 in 4,5 mg l^{-1} BAP in 2 mg l^{-1} GA3. Na koncu so bili poganjki ukoreninjeni v gojišču, ki je vsebovalo 1, 1,5 in 2 mg l^{-1} NAA. Rezultati so pokazali, da je bilo za indukcijo embriogenih kalusov najboljše MS gojišče, ki je vsebovalo 0,5 mg l^{-1} BAP in 0,25 mg l^{-1} NAA. Dva elementa, Fe in K , sta imela največjo vsebnost v ne-embriogenih kalusih v primerjavi z embriogenimi. Za regeneracijo rastlin, za nastanek in vkoreninjenje poganjkov, je bilo MS gojišče, ki je vsebovalo 4,5 mg l^{-1} BAP, 2 mg l^{-1} GA3 in 2 mg l^{-1} NAA najboljše. V raziskavi je najboljše regeneracijsko obravnavanje dalo 35 poganjkov iz enega izsečka z 92 % vkoreninjenjem. Ta regeneracijski protocol bi lahko bil koristen za gensko transformacijo in raziskave mikro propagacije.

Ključne besede: jajčevec; tkivna kultura; regeneracija s somatsko embriogenezo; BAP; NAA

Okrajšave:
NAA Naftalen ocetna kislina
BAP 6-Benzil aminopurin
GA3 Giberelinska kislina
MS gojišče Murashige & Skoog gojišče
IBA Indol-3-maslena kislina
IAA Indol ocetna kislina

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1 INTRODUCTION

Eggplant (*Solanum melongena* L., $2n = 2x = 24$) is one of the important plants of the Solanaceae family (Portis, et al., 2018), which grown for its often purple edible fruit. Different cultivars of eggplant have different sizes, shapes and colours ranging from oval or egg-shaped to long club-shaped; and from white, yellow, green through degrees of purple pigmentation to almost black (Aminifard, et al., 2010). Eggplant after potatoes and tomatoes represents the third most important solanaceous crop species, and it is believed that eggplant is native to India and China (Magioli & Mansour, 2005). Its bulk production is concentrated in China, India, Iran, Turkey, Egypt, Italy and Spain (Portis, et al., 2018).

Although the eggplant has a low calorie content, it is a rich source of magnesium, calcium, potassium and iron (Michalojc & Buczkowska, 2008). In addition, in traditional medicine, eggplant is used to treat diabetes, arthritis, asthma and bronchitis (Magioli & Mansour, 2005). Also, nasunin, as the major component of anthocyanin of eggplant, prevents lipid peroxidation (Igarashi, et al., 1993).

The tissue culture technique allows researchers to grow and manipulate plants under in-vitro sterile conditions and can be very useful to plant breeders (Bridgen et al., 2018). In fact, this technique can provide the diversities needed for selection the desired traits by inducing somaclonal variation. On the other hand, by optimizing the complete regeneration of callus, it is possible to transfer the desired genes to the desired plants (Chakravarthi, et al., 2010). Due to the reasons mentioned above, studies of the full plant regeneration from explants are of special importance in plants.

The fruit of the eggplant is found in the diet of many people in the world. For this reason, several studies have been carried out on regeneration of this plant through somatic embryogenesis using different explants such as leaf and cotyledon (Foo, et al., 2018), root (Franklin, et al., 2004) and hypocotyl (Statish, et al., 2015). However, it has been shown that plant regeneration can be affected by the cultivar. For example, Gandonou et al. (2005) showed that there is a significant difference between 9 genotypes of sugar beet in terms of callus induction capacity, embryogenic response and plant regeneration ability (Gandonou, et al., 2005), which indicates that the overall regeneration rate of the plant is genotype dependent. Hoque and Mansfield (2004), Schween and Schwenkel (2003) and Zale et al. (2004), working respectively on *Oryza sativa* L., *Primula* ssp. and wheat came to the same conclusions (Hoque & Mansfield, 2004; Schween & Schwenkel, 2003; Zale, et al., 2004). For this reason, in this study, regeneration of a native cultivar of Iran, Ghalami Varamin, which is commonly cultivated, was studied using somatic embryogenesis using hypocotyl explants.

2 MATERIALS AND METHODS

2.1 SEED SURFACE STERILIZATION AND IN-VITRO GERMINATION

This research was carried out on an Iranian eggplant variety called 'Ghalami Varamin'. Seeds of this variety were purchased from Pakan Bazr Esfahan Company (http://www.pakanbazr.com/en/). The seeds were surface-sterilized in 70 % ethanol for 30 seconds and then 2 % sodium hypochlorite for 10 minutes and rinsed 3 times with sterile distilled water. The MS medium was used for germination and seedlings growth of eggplant (Park & Facchini, 2000). The MS medium (Duchefa Company) was prepared as 4.43 g l^{-1}. After adjusting to pH 5.7 and adding agar (7.5 g l^{-1}) and sucrose (3 %), the culture medium was autoclaved. The MS medium was poured into autoclaved glass containers under sterile laminar hood. Seeds were cultured on the medium for germination, and placed in a growth chamber at 25 ± 2 °C with a 16/8 h light/dark period, light intensity of 32.38 µmol s^{-1} m^{2-1}.

2.2 CALLUS INDUCTION CULTURE MEDIUM

After 15 to 20 days, hypocotyl explants were placed in MS culture medium containing different concentrations of auxin (NAA) and cytokinin (BAP) to produce callus (Table 1). All treatments were carried out in 3 replicates. Then, the percentage of callus induction and embryogenic calluses, and fresh mass and dry mass of calluses were measured. All data were analysed by ANOVA as a factorial experiment based on a completely randomized design. BAP as first factor and NAA as second factor had seven and three levels, respectively. The mean comparisons were done based on the Duncan's Multiple Range Tests (Duncan, 1955).

2.3 NUTRIENTS MEASUREMENT

Embryogenic and non-embryogenic calli were washed in distilled water, oven dried at 70 °C for 48 h and grounded after being weighed. Different ions concentration was taken from the chloride acid (2 N) extract of the samples that were measured using atomic absorption spectrometer (Varian Spectra aa220; made in Italy). All data were analysed by ANOVA based on a completely

Table 1: Various hormonal treatments used to induce callus

Hormonal combination code	BAP(mg l^{-1})	NAA(mg l^{-1})
A	0	0
B	0	0.25
C	0	0.5
D	0.25	0
E	0.25	0.25
F	0.25	0.5
G	0.5	0
H	0.5	0.25
I	0.5	0.5
J	3.5	0
K	3.5	0.25
L	3.5	0.5
M	4	0
N	4	0.25
O	4	0.5
P	4.5	0
Q	4.5	0.25
R	4.5	0.5
S	5	0
T	5	0.25
U	5	0.5

randomized design with three replications. The mean comparisons were done based on the Duncan's Multiple Range Tests.

2.4 SHOOT PROLIFERATION MEDIUM

The embryogenic calli isolated from the whole callus masses and transferred to the MS medium culture with different concentrations of BAP (3.5, 4 and 4.5 mg l^{-1}) in order to shooting. Then, the percentage of shoot induction, shoot length, number of shoots and leaves were measured. In facts, the only treatments in this section were different concentrations of BAP that were analysed by ANOVA based on a completely randomized design with three replications. The mean comparisons were done based on the Duncan's Multiple Range Tests.

2.5 ROOT PROLIFERATION MEDIUM

For further prolongation and development of stem and leaf system, shoots were transferred to MS medium supplemented with 2 mg l^{-1} gibberellic acid (GA3). For root induction, shoot lengths of 3 to 4 cm were transferred to MS culture media containing different concentrations of NAA (1, 1.5 and 2 mg l^{-1}). The studied traits included root induction percentage and root length. The only treatments in this section were different concentrations of NAA that were analysed by ANOVA based on a completely randomized design with three replications. The mean comparisons were done based on the Duncan's Multiple Range Tests.

3 RESULTS AND DISCUSSIONS

3.1 CALLUS INDUCTION

In this study, callus induction from hypocotyl explant was successfully performed. Callus initiation and appearance of embryogenic calli were occurred after 7 and 16 days, respectively (Figure 1 and 5). Embryogenic calli were seen in white, frangible and sponge-like forms so that after a while, the formations of shoot primordia in these calli were observed (Figure 1). But, non-embryogenic types appeared as compact, non-frangible, and sometimes greenish callus masses (Figure 1), similar to what was reported by other researchers (Corral-Martinez & Segui-Simarro, 2012; Rivas-Sendra, et al., 2015). Eventually, these types of calli were necrosed and died on the medium.

The measured traits were the percentage of callus induction, percentage of embryogenic callus induction and fresh and dry mass of calluses. Variance analysis of data showed that there is a significant difference between different concentrations of BAP, NAA and their interactions at 1 % probability level (Table 2).

The mean comparison of interaction effects of NAA*BAP for induction of callus showed that the highest percentage of callus induction (85 %) observed in the MS media supplemented with the combination of 0.5 mg l^{-1} NAA + 4 mg l^{-1} BAP, and the lowest percentage of callus induction (zero) observed in combinations of 0 mg l^{-1} BAP + 0 mg l^{-1} NAA, 4.5 mg l^{-1} BAP + 0 mg l^{-1} NAA and 5 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Figure 2). Zayova et al., (2008) reported the highest induction of callus using hypocotyls (63.3%) in the treatment MS media supplemented with 0.5 mg l^{-1} BAP + 2.0 mg l^{-1} NAA (Zayova, et al., 2008). In another study, callus induction using cotyledons as explant, the highest callus induction (100%) occurred in some concentrations of Kinetin, BAP and combinations of both (Foo, et al., 2018).

In this study, the highest percentage of embryogenic calli (52 %) was related to 0.5 mg l^{-1} BAP + 0.25 mg l^{-1} NAA. In the treatment with BAP alone, induction of cal-

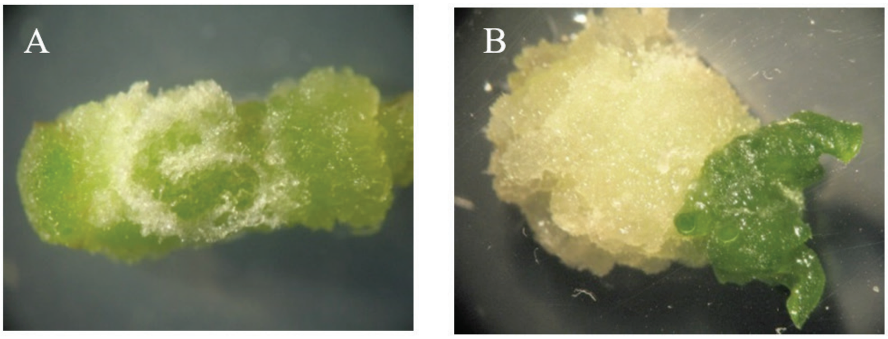

Figure 1: A: non-embryogenic callus. B: embryogenic callus

Table 2: Variance analysis of traits related to the callus induction section

		Mean Square			
Sources of variation	Degrees of freedom	Callusing percentage	Percentage of embryogenic calluses	Fresh mass (g)	Dry mass (g)
a (BAP)	6	0.35**	0.11**	0.027**	0.01**
b (NAA)	2	0.17**	0.067**	0.029**	0.017**
a*b	12	0.19**	0.06**	0.018**	0.009**
error	42	0.02	0.01	0.002	0.002

** Significant differences between the treatments at the level of 1 %

lus in Ghalami Varamin cultivar occurred in all BAP concentrations except for the 4.5 mg l^{-1} BAP, and 0.5 mg l^{-1} was the best concentration (Figure 2). However, embryogenic calli occurred in 0.25, 0.50, 3.5 and 4 mg l^{-1} treatments, and no embryogenic calli were recorded in the other two concentrations (4.5 and 5 mg l^{-1}). In the study of Foo et al., (2018), the highest shoot formation from the cotyledon explants was induced on Kinetin (2 mg l^{-1}) alone (Foo, et al., 2018). In our study, in addition to the best hormone composition selected, BAP alone induced embryogenic calli too while Foo et al. (2018), showed that kinetin alone is sufficient to induce shoots from the cotyledon explants (Foo, et al., 2018). It is likely, different concentrations of auxin and cytokinin hormones in the two tissues caused different results. Therefore, it seems that in the eggplant, depending on the type of explant, certain concentrations of auxin or cytokinin alone have the potential to induce embryogenic calli.

In our study, the NAA hormone (as a type of auxin) alone induced calli, but at a much lower rate than the BAP alone. In addition, the NAA hormone did not induce the embryogenic calli (Figure 2). Foo et al. (2018) revealed that all concentrations of another type of auxin, Kinetin, (0.5, 1, 1.5 and 2 mg l^{-1}) induced calli (Foo, et al., 2018). However, only two concentrations (1.5 and 2 mg l^{-1}) induced embryogenic calli (Foo et al., 2018). Therefore, depending on the type of auxin, explant and the concentration used, this hormone alone can induce calli, especially embryogenic calli.

In the case of fresh and dry weight of callus, the concentrations of 5 mg l^{-1} BAP + 0.25 mg l^{-1} NAA and 4.5 mg l^{-1} BAP + 0.25 mg l^{-1} NAA showed the highest results respectively (Figure 3).

3.2 COMPARISON OF IONS IN EMBRYOGENIC AND NON-EMBRYOGENIC CALLUS

Concentration of five elements, calcium, magnesium, iron, potassium and manganese were measured in embryogenic and non-embryogenic calli. The results of variance analysis showed that there is no significant difference between the two embryogenic and non-embryogenic calli for calcium, magnesium, and manganese. But

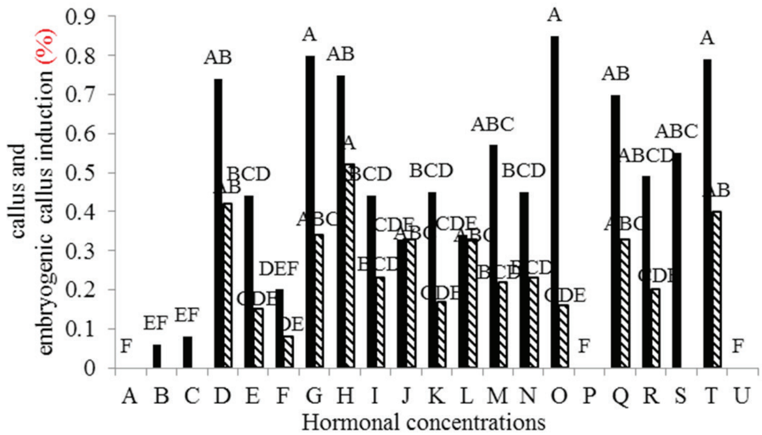

Figure 2: Mean comparison of the interaction effects of NAA*BAP for the percentage of callus induction and embryogenic callus induction. Black columns are percentage of callus induction and others are related to the percentage of embryogenic callus induction

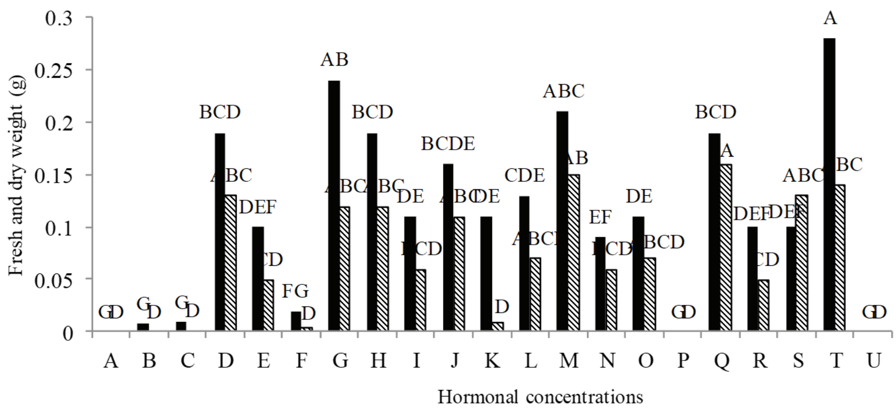

Figure 3: Mean comparison of the interaction effects of NAA*BAP for fresh and dry mass of calli. Black columns are fresh mass of calli trait and others are related to the dry mass of calli

iron and potassium were dissimilar between embryogenic and non-embryogenic calli (Table 3). Our results revealed that the amount of both iron and potassium were more in non-embryogenic calli than the embryogenic ones (Fig. 4). Unfortunately, there is no report so far on the difference between the embryogenic and non-embryogenic calli in terms of the amount of these elements.

3.3 PLANT REGENERATION

3.3.1 Shooting

Shooting was initiated by transferring embryogenic calli to MS medium containing 3.5, 4 and 4.5 mg l^{-1} BAP. For shooting regeneration, some traits including shooting percentage, number of shoots, Shoots length and number of leaves were measured. Variance analysis for these traits showed that there are significant differences between them (Table 4).

The mean comparisons showed that the highest percentage of shooting (94%), number of shoots (35 shoots), shoot length (25 cm) and leaf number (14 leaves) were observed with a MS medium containing 4.5 mg l^{-1} BAP + 2 mg l^{-1} GA3 (Table 5).

Different explants have been used for eggplant regeneration including hypocotyl (Mallaya & Ravishankar, 2013), leaf (Ray et al., 2011) and cotyledon (Rahman et al., 2006; Shivaraj & Rao, 2011), epicotyl and stem node, root (Ray et al., 2011). The highest percentages of shooting in various studies were different based on types of explant. Kaur et al. (2013) showed that cotyledon induced significantly the highest somatic embryogenesis on MS media fortified with 1.5 mg l^{-1} IBA + 1.0 mg l^{-1} BAP (89.62 %). In

Table 3: Variance analysis of iron and potassium amounts between embryogenic and non-embryogenic calli

		Mean Square	
Sources of variation	Degrees of freedom	K	Fe
treatment	1	81.99 **	0.133 **
error	1	1.47	0.002

** Significant differences at the level of 1 %

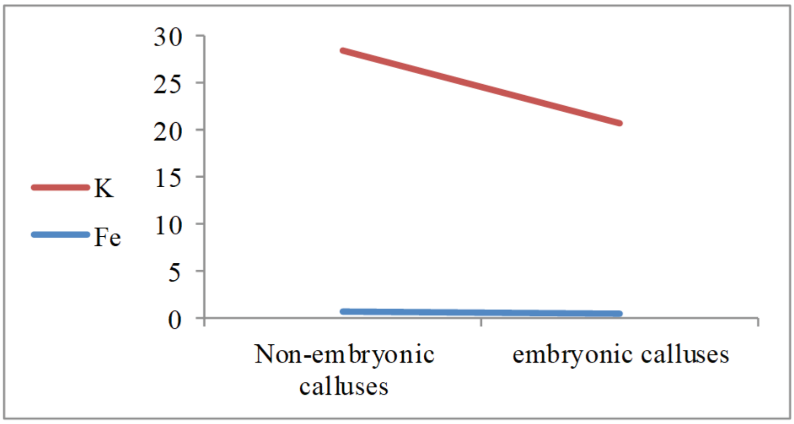

Figure 4: Mean comparison of iron and potassium amounts between embryogenic and non-embryogenic calli

Table 4: Analysis of variance of shooting traits

		Mean Square			
Sources of variation	Degrees of freedom	shooting percentage	number of shoots	Shoots length (cm)	number of leaves
treatment	2	0.172**	540.7**	352.44**	121.33**
error	6	0.001	10.33	3.77	1.22

** Significant differences at the level of 1 %

Table 5: Mean comparison of different concentrations of BAP on traits related to shoot elongation

number of leaves	Shoots length (cm)	number of shoots	shooting percentage	GA_3(mg l^{-1})	BAP(mg l^{-1})
2[b]	5[c]	9[b]	0.47[c]	2	3.5
3[b]	9[b]	15[b]	0.62[b]	2	4
14[a]	25[a]	35[a]	0.94[a]	2	4.5

Table 6: Analysis of variance of rooting traits

		Mean Square	
Sources of variation	Degrees of freedom	rooting percentage	root length (cm)
Treatment	2	0.06**	30.33**
Error	6	0.001	1.22

** Significant differences at the level of 1 %

Table 7: Mean comparison of different concentrations of NAA on traits related to root induction

NAA(mg l^{-1})	rooting percentage	root length (cm)
1	0.6^{b}	4.5^{b}
1.5	0.71^{b}	5^{b}
2	0.9^{a}	10^{a}

leaf explant, it was also the maximum on 1.5 mg l^{-1} IBA + 1.0 mg l^{-1} BAP (69.60 %). However, hypocotyl achieved the maximum of 38.41 % somatic embryogenesis on 0.5 mg l^{-1} IBA + 1.0 mg l^{-1} BAP (Kaur, et al., 2013). Mir et al., (2011) studied plant regeneration from different cultivars of eggplants with different explants. Their results showed that shoot induction percentage were different among the cultivars. The highest shoot induction percentage using hypocotyl (88.31 %), cotyledon (90 %) and root (60 %) were observed from PBSR-11 cultivar on MS medium containing 2.5 mg l^{-1} IAA + 0.5 mg l^{-1} BAP (Mir et al., 2011).

The number of shoots and shoot length in the most studies were low. For example, Ray et al. (2011) used stem, root and leaf explants for eggplant regeneration. They observed that the highest number of shoot regenerated through callus from stem containing 2.0 mg l^{-1} BAP and 0.5 mg l^{-1} NAA was 3.4 (Ray, et al., 2011). Shivaraj and Rao (2011) used cotyledonary leaf as explants for eggplant regeneration. Their results showed the highest number of shoots was 23.3 at 2 mg l^{-1} BAP + 0.5 mg l^{-1} Kinetin (Kn), and the highest shoot length was 11.2 cm at 2 mg l^{-1} BAP + 1.5 mg l^{-1} Kn from cultivar Pusa Purple long (Shivaraj & Rao, 2011).

Mallaya & Ravishankar (2013) showed that the maximum number of shoot buds elongated and shoot length using hypocotyl explant were 19 and 3.3 cm respectively on 0.5 mg l^{-1} TDZ + 0.1 mg l^{-1} GA3 (Mallaya & Ravishankar, 2013). By comparing the study of other researchers with this study, it can be concluded that the use of 4.5 mg l^{-1} BAP and 2 mg l^{-1} GA3 can provide a better result.

3.3.2 Rooting

Variance analysis of data showed that there was a significant difference between treatments for rooting percentage and root length traits (table 6).

In this study, rooting percentage and root length were 92 % and 10 cm respectively at 2 mg l^{-1} NAA (table 7; Figure 5). For root regeneration, the results of Shivaraj and Rao (2011) showed that the highest frequency and number of roots were 100% and 89.3 respectively on 3 mg l^{-1} IBA (Shivaraj & Rao, 2011). Mallaya, & Ravishankar (2013) reported that the highest number of root and root length were 4 and 5.6 cm respectively on 1 mg l^{-1} IBA (Mallaya & Ravishankar, 2013).

5 CONCLUSION

In this study, the best treatments for plant regenera-

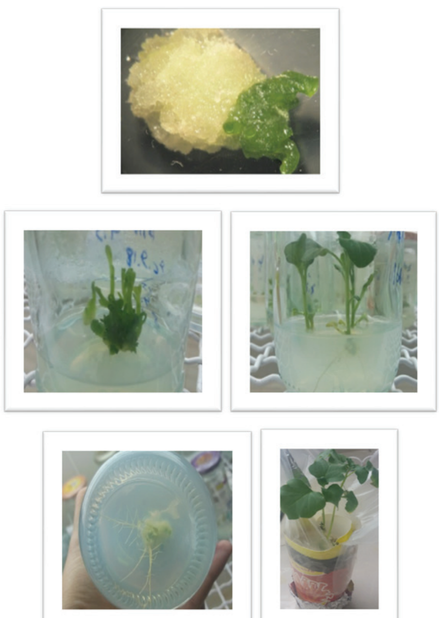

Figure 5: A: embryogenic callus. B: production of multiple shoots from embryogenic callus. C: production of roots in elongated shoots. D: production of roots. E: production of adult eggplant

tion resulted in 35 shoots and 90 % rooting from embryogenic calli. With our protocol, we could produce adult eggplant from embryogenic calli and then transferred to the pots (Figure 5). It seems that this protocol could be an efficient protocol for plant regeneration of eggplant specially Ghalami Varamin cultivar.

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Association of traits in Ethiopian fenugreek (*Trigonella foenum-graecum* L.) genotypes regarding to seed yield by using phenotypic data

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Association of traits in Ethiopian fenugreek (*Trigonella foenum-graecum* L.) genotypes regarding to seed yield by using phenotypic data

Abstract: Shortage of information on association of traits is one of the problems in fenugreek productivity. Field experiment was implemented at Jamma district of South Wollo Administrative Zone of Amhara National Regional State, in 2018/19 main rainy season to examine the nature and extent of correlation, direct and indirect effects among yield and yield related traits. Sixty-two nationally collected fenugreek genotypes along with standard and local checks were evaluated in simple lattice design. Seed yield plot^{-1} was significantly and positively correlated to biomass yield^{-1} (r = 0.5) and harvest index (r = 0.6***) at genotypic level. Seed yield was also significantly and positively correlated with harvesting index (r = 0.6***) and weakly and negatively (r = -0.01) correlated to biomass yield at phenotypic level. Path coefficient analysis revealed that biomass (0.951), harvesting index (0.283) and pod length (-0.163) had contributed the maximum positive and negative direct effect on seed yield respectively, at phenotypic level. At genotypic level biomass yield ha^{-1} (0.816) and harvesting index (0.930) had contributed strong positive direct effect and plant height (-0.004) had revealed weak negative direct effect.

Key words: correlation; path analysis; simple lattice

Povezava med fenološkimi značilnostmi pri različnih genotipih sabljastega triplata (*Trigonella foenum-graecum* L.)

Izvleček: Pomanjkanje informacij o povezanosti med fenološkimi lastnostmi je eden izmed problemov pri žlahtnenju sabljastega triplata. Za preučitev neposrednih in posrednih povezav med pridelkom in fenološkimi lastnostmi pri različnih genotipih je bil na območju Jamma, južno od upravnega območja Wollo, države Amhara, Etiopija, v glavnem deževnem delu sezone 2018/19 izveden poljski poskus. 62 genotipov sabljastega triplata, zbranih po vsej državi, je bilo ob standardnih in lokalnih preverbah ovrednoteno v nepopolnem bločnem poskusu. Pridelek semena na ploskev je bil statistično značilno pozitivno povezan s pridelkom biomase (r = 0,5) in žetvenim indeksom (r = 0,6***) na ravni genotipa. Pridelek semena je bil statistično značilno pozitivno povezan z žetvenim indeksom (r = 0,6***) in v šibki negativni korelaciji (r = -0,01) s pridelkom biomase na ravni fenotipov. Analiza neposrednih in posrednih vplivov nabora neodvisnih spremenljivk na odvisno spremenljivko je pokazala, da so biomasa (0,951), žetveni indeks (0,283) in dolžina stroka (-0,163) prispevali največji pozitivni in negativni neposredni učinek na pridelek zrnja na fenotipski ravni. Na genotipski ravni sta imela pridelek biomase na hektar (0,816) in žetveni indeks (0,930) močan, pozitivni neposredni učinek, višina rastlin je imela šibek neposredni negativni učinek (-0,004) na pridelek.

Ključne besede: korelacija; analiza posrednega in neposrednega vpliva; nepopolni bločni poskus

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1 INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant in the family Fabaceae, with leaves consisting of three small obovate to oblong leaflets. It is believed that fenugreek is native to Mediterranean region (Petropoulos, 2002). Correlation measures the mutual association between two variables but does not indicate the cause and effect relationship of traits contributing directly or indirectly towards economic yield (Shivanna et al., 2007). The value of correlation coefficient, which is a ratio of the covariance between the two variables and the geometric mean of their variances, ranges from -1 to +1, the extreme values indicating perfect negative and positive association, respectively (Gomez and Gomez, 1984). The correlations between characters indicate that the gene for the traits are either linked or are influenced by the same differences of environmental conditions (Falconer and Mackay, 1996).

Phenotypic correlation is the observable correlation between two variables; it includes both genotypic and environment effects and genotypic correlation on the other hand is the inherent association between two variables; it may be either due to a pleiotropic action of genes, linkage or, more likely both (Singh, 1993). Environment plays an important role in correlation. In some cases, environment affects two traits simultaneously in the same direction or sometimes in different directions. Genetic and environmental causes of correlation combine together and give phenotypic correlation. The dual nature of phenotypic correlation makes it clear that the magnitude of genetic correlation cannot be determined from phenotypic correlation (Usman et al., 2006). Correlation coefficient analysis helps to determine the nature and degree of relationship between any two measurable characters. Knowledge of the correlations that exist between important characters may facilitate the interpretations of the results that are already obtained, and provides the basis for planning more efficient breeding program. However, as the number of independent variables influencing a particular dependent variable, a certain amount of interdependence is expected (Aryo et al., 1973).

Mevlut et al. (2008) stated that correlation analyses are being widely used in many crop species by plant breeders to understand the nature of complex interrelationships among traits and to identify the sources of variation in yield. Yield is a quantitatively inherited trait; its expression is an outcome of complex interaction of several genes and environment. Therefore, proper understanding of association of different traits provides more reliable criterion for selection to achieve the goal of high yield (Mohammad et al., 2001). High yield through yield attributes, as primary interest in crop improvement, requires understanding the magnitude of correlations among various yield traits (Tadele Taddese et al., 2009).

Yield is the result of yield-correlated characters and some other undefined factors. Yield contributing traits can be ranked and specific characters producing a given correlation can be observed through path coefficient analysis (Ariyo et al., 1973). Therefore, the use of path coefficient analysis is important to come up with meaningful results of cause and effect. Therefore, this research is conducted to examine the nature and extent of correlation, direct and indirect effects among yield and yield related traits.

2 MATERIALS AND METHODS

2.1 DESCRIPTION OF THE EXPERIMENTAL SITE

The experiment was conducted at Jamma research site of Sirinka Agricultural Research Center (SARC) at Jamma district during the growing season of 2018. Jamma is located at 10°27'N and 39°16'E on an altitude of 2622 meters above sea level, South Wollo, Amhara National Regional State, Ethiopia. Based on the last ten years (2008-2017) meteorological data obtained from Ethiopian Meteorological Agency, Kombolcha station, Jamma receives an average annual rainfall of 1047 mm and minimum and maximum temperature of 9.2 °C and 26.2 °C, respectively. Jamma is 120 km and 320 km away from Dessie and Addis Ababa, respectively.

2.2. EXPERIMENTAL MATERIALS, DESIGN AND PROCEDURE

Sixty-two fenugreek accessions collected from Debre-Zeit Agricultural Research Center (DZARC) along with local and standard checks were evaluated at Jamma testing site of SARC. The experiment was laid out using simple lattice design (8 × 8) on plot size of 1.6 m^2, with an inter-row of 20 cm and intra-row spacing of 5 cm. The accessions were collected from different parts of the country.

Clean fenugreek seeds were sown at distances of 20 and 5 cm between rows and plants, respectively, as per the national recommendation. Each genotype grew on a gross plot size of 1.6 m^2 (0.8 m width × 2 m length). The distances between plots and blocks were 0.5 m and 1 m, respectively. Being fenugreek is leguminous crop, fertilizer was not applied at all. Weeding and thinning were carried out at the appropriate time. Data were obtainerd from the central two rows with net plot size of 0.8 m^2 (0.4 m × 2 m).

Table 1: Passport data of accessions

S.no	Accession Number	Region	Zone	S.no	Accession Number	Region	Zone
1	53003	Oromiya	N/Shewa	33	201627	NA	
2	53008	Amhara	S/ Gondar	34	201632	NA	
3	53009	Amhara	S/Gondar	35	202121	NA	
4	53014	Amhara	S/ Wollo	36	202122	NA	
5	53016	Oromiya	W/ Harerge	37	202124	NA	
6	53021	Amhara	E/Gojam	38	202125	NA	
7	53023	Oromiya	N/ Shewa	39	202126	NA	
8	53026	Amhara	E/Gojam	40	202127	NA	
9	53027	Amhara	E/Gojam	41	202129	NA	
10	53028	Amhara	E/Gojam	42	202132	NA	
11	53035	Amhara	E/Gojam	43	202133	NA	
12	53037	Amhara	E/Gojam	44	207361	Amhara	S/ Gondar
13	53039	Amhara	E/Gojam	45	207362	Amhara	N/ Gondar
14	53040	Amhara	E/Gojam	46	207363	Amhara	N/Gondar
15	53041	Amhara	E/Gojam	47	207364	Amhara	N/ Gondar
16	53042	Amhara	E/Gojam	48	207365	Amhara	N/ Gondar
17	53045	Amhara	E/Gojam	49	207390	Amhara	N/Gondar
18	53055	Amhara	E/Gojam	50	207391	Amhara	S/ Gondar
19	53056	Amhara	E/Gojam	51	207394	Amhara	S/ Gondar
20	53057	Amhara	E/Gojam	52	208680	Oromiya	E/ Harerge
21	53058	Amhara	E/Gojam	53	210864	NA	
22	53059	Amhara	E/ Gojam	54	212549	Amhara	N/ Shewa
23	53080	Amhara	E/ Gojam	55	212552	Amhara	N/ Shewa
24	53085	Oromiya	Bale	56	212777	Amhara	E/ Gojam
25	53086	Oromiya	N/Shewa	57	213115	Amhara	S/ Wollo
26	53094	SNNP	S/Omo	58	213116	Amhara	S/ Wollo
27	53097	Amhara	E/ Gojam	59	214942	Amhara	N/ Shewa
28	53098	Amhara	E/ Gojam	60	215056	Oromiya	Borena
29	53099	Amhara	E/ Gojam	61	216898	Oromiya	Arssi
30	53106	Amhara	N/ Shewa	62	216899	Oromiya	Arssi
31	53108	Amhara	N/Gondar	63	Jamma		
32	201577	NA		64	Local		

NA = not identified

2.3 DATA COLLECTION

The following data were recorded from the central two rows, leaving a guard row from both sides of the plot either plot or plant basis's.

Days to 50 % flowering: days to 50 % flowering was recorded as the number of days from planting to the time when 50 % of the plants in the plots produced flower.

Days to 90 % maturity: was recorded as number of days from planting to the time when 90 % of the plants in the plot reach physiological maturity.

Pod filling period**:** number of days from flowering or exertion of pods to the time when 50 % of the pod forms seeds.

Biomass yield (above ground): it was taken as the total above-ground biomass weight of the plants from the central two rows. Total above-ground biomass was harvested and sun-dried and weighed using spring balance

Seed yield**:** it was taken from the central two rows. Entire plants were harvested, threshed and winnowed. Clean seed were measured using electronic sensitive balance.

Thousand seeds mass: thousand seeds were counted and weighed using electronic sensitive balance for each replication.

Harvesting index: it was calculated as the ratio of seed yield to biomass yield in percent

Plant height: plant height was measured from the main stem, measured from the ground level to the tip of the plant using measurement tape at 90 % physiological maturity.

Pod length: pod length was measured from the tip to petiole of the pod at 90 % physiological maturity.

Number of branches per plant: the total number of branches arising from the main stem was counted at 90 % physiological maturity.

Number of pods per plant: the total number of pods per plant was counted at physiological maturity.

Number of seeds per pod: the total number of seeds per pod was counted at physiological maturity.

2.4 DATA ANALYSES

Correlations (genotypic and phenotypic) and path coefficient analysis were analyzed using SAS statistical software package, version 9.0 as per Gomez and Gomez (1984).

2.5 PHENOTYPIC AND GENOTYPIC CORRELATION COEFFICIENTS

Phenotypic correlation, the observable correlation between two variables, which includes both genotypic and environmental components between two variables, was estimated using the formula suggested by Miller *et al.* (1958).

The calculated value was compared with the tabulated 't' value at 5 % or 1 % level of significance for both phenotypic and genotypic correlations.

2.6 PATH COEFFICIENT ANALYSIS

Associations of yield with its components were estimated using correlation and path analysis. The use of path analysis requires a cause and effect situation among the variables. Path coefficient analysis is calculated using the formula suggested by Dewey and LU (1959) to assess direct and indirect effects of different traits on seed yield as:

$$r_{ij} = p_j + \sum r_{ik} \; p_{kj}$$

Where r_{ij} is mutual association between the independent traits (i) and the dependent trait (j) as measured by the correlation coefficient, p_{ij} is component of direct effect of the independent trait (i) on the dependent variable (j); and r_{ik} p_{kj} is the components of indirect effect of a given independent trait (i) on the dependent traits (j) via all other independent traits (k).

3 RESULT AND DISCUSSION

3.1 CORRELATION COEFFICIENT ANALYSIS

Yield is a complex character governed by several other yield attributing characters. Since, most of the yield attributing characters are quantitatively inherited and highly affected by environment, it is difficult to judge whether the observed variability is heritable or not. Correlation studies are helpful in determining the components of complex traits like yield. The correlation coefficient is the measures of degree of symmetrical association between two traits and helps us in understanding the nature and magnitude of association among yield and yield components. Association between any two traits or among various traits is of immense importance to make desired selection of combination of traits (Ahmad *et al.*, 2003). Therefore, phenotypic and genotypic correlation coefficients were estimated for all pairs of traits and results are presented in Table 2.

The magnitudes of genotypic correlation coefficients for most of the characters were higher than their corresponding phenotypic correlation coefficients, except few cases, which indicate the presence of inherent or genetic association among various characters. The degree of association between two characters was measured by the correlation coefficient.

Phenotypically seed yield had weak to strong positive correlation with days to flowering, pod filling period, plant height, number of branch per plan, number of pod per plant, harvesting index and thousand seed mass (r = 0.18, 0.119, 0.129, 0.115, 0.222, 0.6*** and 0.158 respectively). While weak and negatively correlated with days to maturity, biomass yield, number of seed per pod and pod length (r = -0.04, -0.02, -0.08 and -0.08 respectively). Harvesting index revealed very highly significance strong negative and positive phenotypic correlation with biomass yield and seed yield (r = -0.6*** and

0.6***) respectively. It also strongly negative correlated with biomass (r = -0.8***) and weakly correlated with the rest traits. Biomass yield had only weak positive phenotypic correlation with pod filling period and plant height, while it correlates negatively and weakly with the rest traits except harvesting index. Pod length revealed significance positive and negative phenotypic correlation with plant height and number of seed per plant and date of maturity (r = 0.31*, 0.43** and -0.26*) respectively. Plant height had positive significance positive phenotypic correlation with number of branches (r = 0.3*).

Therefore, phenotypically traits like number of pods plant^{-1}, plant height, pod length and harvesting index could be utilized in breeding program to improve fenugreek genotypes for higher yield. Selection simultaneously based on these characters would be effective and a worthwhile for increasing seed yield and can be taken as primary selection criteria in fenugreek.

Genotypically seed yield revealed positive and significance correlation with biomass and harvesting index (r = 0.5** and 0.6***). It is also weak negatively correlated with days to maturity, pod filling period and number of seed per plant, while weak and positive correlation with the rest of the traits. Pod length had positive significance genotypic correlation with days to flowering and negative significance with days to maturity and plant height (r = 0.4**, -0.29* and -0.25*). Number of seed per plant had strong significance negative correlation with thousand seed mass (r = -0.4**), while number of branches had strong positive correlated with plant height and negatively significant correlated with pod filling period (r = 0.5** and-0.28*). Biomass yield revealed positively correlated with all traits except days to maturity. Generally genotypic correlation was higher magnitude than their phenotypic correlation and most traits weakly correlated to each other. Therefore, genotypically improving of biomass yield and harvesting index will leads to develop high yielding fenugreek genotypes. Generally, in the present study, seed yield revealed strong positive correlations with harvesting index both at genotypic and phenotypic levels, implying pleiotropic effect of harvesting index with seed yield. Hence, selection genotypes having high harvesting index would improve seed yield of fenugreek. Biomass yield also affected seed yield positively and significantly at genotypic level.

In agreement with Mahendra (2015) reported, negative and weakly phenotypic correlation was achieved between days to flowering to number of branches per plant, number of seeds per plant, pod length and thousand seed mass, while opposite in level of significance and relation of days to flowering with plant height and harvesting index. Krishan et al. (2013) reported seed yield had positive correlation with plant height, number of seed plant^{-1} and number of branch per plant at both levels. Betelhiem Belete (2018) also reported similarly in days to flowering to days to maturity, thousand seed mass to days to flowering, days to flowering to seed yield, seed yield to thousand seed mass and days to flowering to number of seed plant^{-1} both at phenotypic and genotypic level. Anbuha et al. (2013) also reported similar result that positive correlation with days to flowering to number of pods plant^{-1} at genotypic level, significant negative and positive correlation with days to flowering to pod length and plant height to number of pods plant^{-1} at genotypic and phenotypic level respectively and number of seed plant^{-1} had positive correlation to plant height both at genotypic and phenotypic level.

3.2. PATH COEFFICIENT ANALYSIS

Path coefficient analysis was computed to estimate the contribution of individual characters to seed yield. It is performed to understand the causes and effects of chain relationships of different yield contributing characters with yield. The path coefficient analysis was conducted using seed yield as dependent variable and all other traits studied as independent variables. The results of phenotypic and genotypic path coefficient analysis are presented in Table 3 and 4 below.

3.3 PHENOTYPIC DIRECT AND INDIRECT EFFECTS OF OTHER TRAITS ON SEED YIELD

Correlation coefficients were further partitioned into direct and indirect effects. Biomass yield (0.95) had exerted the highest positive direct effects on seed yield. Plant height (0.15), number of branches per plant (0.05), thousand seed mass (0.07) and harvesting index (0.28) exerted positive direct effects on seed yield (Table 3). High values of direct effects suggested that the true relationship and direct selection for these traits may also increase and give better response for improvement of seed yield and can be major selection criteria in fenugreek breeding programs. Number of pods per plant (-0.14), number of seeds per pod (-0.01) and pod length (-0.16) on the other hand, exerted negative phenotypic direct effect and unfavourable effect on seed yield (Table 3). The negative phenotypic direct effects on seed yield indicated that selection for these traits would not be rewarding for yield improvement. The direct effect of biomass yield (0.95) and number of pods per plant (-0.014) but their association to seed yield was (r= -0.14 and 0.12) opposite in direction. Indicating that fenugreek genotypes having maximum biomass yield and number of pods would

Table 2: Genotypic correlation (below diagonal) and phenotypic correlation (above diagonal) among 12 traits of 64 fenugreek genotypes studied

	DF	DM	PFP	PH	NB	BM	NP	NS	PL	SY	HI	TSM
DF	1	0.107	-0.013	0.074	-0.152	-0.182	0.075	-0.079	-0.026	0.184	0.238	-0.017
DM	0.191	1	0.097	-0.061	-0.189	-0.112	-0.012	-0.205	-0.262*	-0.044	0.007	0.111
PFP	-0.023	0.118	1	0.162	-0.173	-0.045	-0.001	0.088	-0.004	0.119	-0.006	0.098
PH	0.245	0.138	-0.049	1	0.237	0.015	0.31*	0.017	0.309*	0.129	0.004	0.064
NB	0.031	-0.231	-0.049	-0.079	1	0.133	-0.010	0.112	0.198	0.115	-0.046	-0.041
BM	0.055	-0.138	0.082	0.029	0.059	1	-0.005	-0.133	-0.008	-0.014	-0.8***	-0.001
NP	0.188	0.199	-0.28*	0.5**	-0.005	0.051	1	0.032	0.192	0.222	0.165	0.206
NS	0.083	-0.114	-0.072	0.046	0.048	0.135	-0.118	1	0.432**	-0.084	0.088	-0.071
PL	-0.4**	-0.291*	-0.065	-0.25*	0.039	0.215	-0.104	0.032	1	-0.078	0.034	-0.045
SY	0.234	-0.056	-0.094	0.139	0.168	0.5**	0.206	-0.029	0.091	1	0.6***	0.158
HI	0.138	0.112	-0.061	0.124	0.111	-0.4**	0.145	-0.181	-0.153	0.6***	1	0.060
TSW	0.133	0.155	0.142	0.100	0.139	0.141	0.107	-0.4**	-0.048	0.225	0.117	1

Where DF = date of flowering, DM = date of maturity, PEP = pod filling period, PH = plant height, NB = number of branch per plant, BM = biomass, NP = number of pod/plant, PL = pod length, SY = seed yield, HI = harvesting index, TSM = thousand seed mass

Table 3: Estimates of direct (bold diagonal) and indirect effect (off diagonal) at phenotypic level 8 characters on seed yield in fenugreek genotypes

	PH	NB	BM	NP	NS	PL	HI	TSM
PH	**0.1487**	0.0115	0.0144	-0.0044	0.0000	-0.0504	0.0051	0.0045
NB	0.0352	**0.0486**	0.1263	0.0001	-0.0003	-0.0324	-0.0596	-0.0029
BM	0.0022	0.0065	**0.9511**	0.0001	0.0003	0.0013	-0.9757	-0.0001
NP	0.0460	-0.0005	-0.0048	**-0.0141**	-0.0001	-0.0313	0.2121	0.0142
NS	0.0025	0.0054	-0.1264	-0.0004	**-0.0023**	-0.0706	0.1128	-0.0049
PL	0.0459	0.0097	-0.0073	-0.0027	-0.0010	**-0.1632**	0.0439	-0.0031
HI	0.0006	-0.0023	-0.7231	-0.0023	-0.0002	-0.0056	**0.2833**	0.0042
TSM	0.0096	-0.0020	-0.0007	-0.0029	0.0002	0.0073	0.0776	**0.0692**

PH = plant height, NB = number of branches per plant, BM = biomass, NP = number of pod/plant, PL = pod length, SY = seed yield, HI = harvesting index, TSM = thousand seed mass.

negatively affect seed yield, therefore; keeping other traits constant, selection of fenugreek genotypes having average amount biomass yield and number of pods would improve seed yield in fenugreek breeding.

Number of seeds per pod exerted the highest positive phenotypic indirect effect on seed yield through harvesting index. Number of seed per plant through harvesting index and pod length through plant height exerted second and third highest positive indirect effect on seed yield. While the first and second highest negative phenotypic indirect effect on seed yield exerted by biomass yield (-0.96) through harvesting index and harvesting index (-0.723) through biomass yield. Genetic improvement in seed yield can be accelerated if this phonological attribute is used as selection criteria. For this purpose, it is necessary not only to identify indirect linkage to seed yield potential, but also to improve the understanding of the genetic basis controlling this trait for easy handling (Garcia et al., 2011). Harvesting index had indirect and positive effect on seed yield through plant height and thousand seed mass and harvesting index had indirect negative effects was exhibited via number of branches per plant, biomass yield, number of pods, number of seeds per plant and pod length. The high positive direct magnitude effect of biomass yield on seed yield was counter balanced by high negative indirect effect on seed yield through harvesting index. The results of phenotypic path coefficient analysis revealed that pod length exerted positive indirect effects on seed yield through biomass yield and harvesting index and negative indirect effect in plant height, number of branches per plant, number of pods per plant, number of seeds per pod and thousand seed mass (Table 3). negative indirect effect of biomass yield on seed yield was observed through thousand seed mass and harvesting index that cannot be generalized as traits for indirect selection for higher grain yield improvement. Therefore, plant height, biomass yield and harvesting index could be used as indirect selection criteria for developing high yielding fenugreek variety.

In agreement with the current findings, Ali et al. (2012) also reported positive direct effects of harvest index and biological yield on seed yield. Analogous magnitude of path result was reported by Mahendra et al. (2015) that harvesting index, number of branches per plant and thousand seed mass revealed positive direct effect and number of seeds per plant and number of pod and revealed negative direct effect on seed yield, while opposite result in plant height and pod length.

3.4 GENOTYPIC DIRECT AND INDIRECT EFFECTS OF OTHER TRAITS ON SEED YIELD

The genotypic association between dependant and independent traits was explained by the genotypic path coefficient analysis method. Number of branches per plant (0.01), number of seeds per plant (0.04), biomass yield (0.82), number of pods plant^{-1} (0.04), thousand seeds mass (0.01) and harvesting index (0.93) exerted positive genotypic direct effects on seed yield, while plant height had negative direct effects on seed yield (Table 4).

The genotypic correlations of biomass yield plot^{-1}, pod length and harvesting index on seed yield were almost equal to their corresponding genotypic direct effect indicating that genotypic correlation coefficient explained true relationship between them. The highest indirect positive genotypic effect was recorded on pod length (r_g = 0.18) through biomass yield, while the highest negative indirect effect on seed yield was biomass yield (r_g = -0.39) through harvesting index. The minimum negative direct effect of plant height on seed yield was counterbalanced by significant positive correlation

Table 4: Estimates of direct (bold diagonal) and indirect effect (off diagonal) at genotypic level for 8 characters on seed yield in fenugreek genotypes

	PH	NB	BM	NP	NS	PL	HI	TSM
PH	**-0.0044**	-0.0008	0.0234	0.0178	0.0016	-0.0150	0.1151	0.0012
NB	0.0003	**0.0107**	0.0484	-0.0002	0.0017	0.0023	0.1029	0.0016
BM	-0.0001	0.0006	**0.8158**	0.0020	0.0048	0.0127	-0.3906	0.0017
NP	-0.0019	0.0000	0.0414	**0.0404**	-0.0042	-0.0062	0.1349	0.0013
NS	-0.0002	0.0005	0.1103	-0.0047	**0.0355**	0.0019	-0.1685	-0.0042
PL	0.0011	0.0004	0.1754	-0.0042	0.0011	**0.0592**	-0.1419	-0.0006
HI	-0.0005	0.0012	-0.3424	0.0059	-0.0064	-0.0090	**0.9304**	0.0014
TSM	-0.0004	0.0015	0.1150	0.0043	-0.0128	-0.0028	0.1089	**0.0118**

PH = plant height, NB = number of branches per plant, BM = biomass, NP = number of pod/plant, PL = pod length, SY = seed yield, HI = harvesting index, TSM=thousand seed mass.

between plant height and number of pods per plant. This implies maximum number of branches per plant, biomass yield, number of pods per plant, harvesting index, thousand seed mass and number of seeds per pod will improve seed yield of fenugreek genotypes.

The indirect effect of plant height through biomass yield (-0.0001), number of seeds per pod (- 0.0002), number of pods per plant (-0.02), harvesting index (-0.0005) and thousand seed mass (-0.0004) counter balanced the direct effect of plant height on seed yield per plot and resulted in the correlation coefficient of 0.12. It was noted that, even if the indirect influence of plant height on seed yield per plot through the above mentioned characters was too small (negative), their cumulative effect (the direct effect of plant height with seed yield) is high, therefore all those traits, were considered in selection. Anubha et al. (2013) reported similar result that positive genotypic direct effect on seed yield through number of pods plant^{-1}, number of seeds per plant and thousand seed mass, while opposite result revealed on genotypic direct effect of plant height and pod length on seed yield. Pushpa (2010) also reported that number of branches had positive indirect effect on seed yield through number of seeds per plant, biomass yield and harvesting index and negative indirect effect of number of branches via number of pods per plant. In contrast to this finding Betelheim Belete (2018) reported that genotypically plant height exerted positive direct effect on seed yield plot^{-1}, while number of pods per plant and number seeds plant^{-1} had negative direct effect on seed yield.

4 CONCLUSION

Ethiopian fenugreek genotype traits showed very highly significant difference in between. Biomass and harvesting index have high magnitude of direct effect on productivity of fenugreek yield. Number of seeds per pod and number of pods per plant through harvesting index also have high indirect effects on productivity of fenugreek. Generally, indirect selection of genotypes based on high mean values of these positive significantly correlated and high positive direct effect traits will be used to improve the productivity of fenugreek. In addition, by selecting genotypes having medium mean negative values of association will be used to increment of the productivity of fenugreek.

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Evaluation of yield, chemical composition and yield of essential oil of four cultivars of sweet basil (*Ocimum basilicum* L.) affected by different levels of nitrogen

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Evaluation of yield, chemical composition and yield of essential oil of four cultivars of sweet basil (*Ocimum basilicum* L.) affected by different levels of nitrogen

Izvleček: A field experiment was conducted to evaluate yield and some qualitative characteristics of four cultivars of sweet basil treated with different levels of nitrogen fertilizer. The experiments were located on the Agricultural Research Station of Khorramabad, Iran, during the 2016-2017 growing season. Treatments were arranged in factorial split-plot-in time in randomized complete block design with three replications. Experimental treatments were four cultivars of sweet basil (*Ocimum basilicum* 'Italian Large Leaf', *O. basilicum* 'Mobarakeh', *O. basilicum* 'Cinnamon' and *O. basilicum* 'Thai'), three levels of nitrogen fertilizer (0, 100 and 200 kg ha^{-1} urea) and three harvests. The highest total dry mass (3482.4 kg ha^{-1}) was related to 'Italian Large Leaf' with the application of 200 kg ha^{-1} urea at the second harvest. The maximum (26.79 kg ha^{-1}) essential oil yield belonged to 100 kg ha^{-1} urea. The highest concentrations of main constituents of essential oil, except methyl chavicol, 1,8-cineole and methyl cinnamate were obtained by control (without nitrogen fertilizer). Italian Large Leaf cultivar and application of 100 kg ha^{-1} urea are recommended to access an acceptable agricultural yield and essential oil yield in sweet basil under the environmental condition similar to Khorramabad.

Key words: basil; dry mass; essential oil; main constituents of essential oil; urea

Ovrednotenje pridelka, kemijske sestave in pridelka eteričnih olj štirih sort navadne bazilike (*Ocimum basilicum* L.) pri različnem gnojenju z dušikom

Abstract: Za ovrednotenje pridelka in nekaterih kakovostnih lastnosti štirih sort navadne bazilike je bil izveden poljski poskus z različnimi odmerki dušikovih gnojil. Poskus je potekal na Agricultural Research Station of Khorramabad, Iran, v rastni sezoni 2016-2017. Obravnavanja so bila izvedena v naključnem faktorskem bločnem poskusu z deljenkami s tremi ponovitvami. Obravnavane so bile štiri sorte navadne bazilike (*Ocimum basilicum* 'Italian Large Leaf', *O. basilicum* 'Mobarakeh', *O. basilicum* 'Cinnamon' and *O. basilicum* 'Thai'), tri ravni gnojenja z dušikovimi gnojili (0, 100 in 200 kg ha^{-1} uree) in tri žetve. Največjo celukupno suho maso (3482,4 kg ha^{-1}) je imela sorta Italian Large Leaf pri uporabi 200 kg ha^{-1} uree v drugi žetvi. Največji pridelek eteričnih olj (26,79 kg ha^{-1}) je bil dosežen pri 100 kg ha^{-1} uree. Največja vsebnost glavnih komponent eteričnih olj, razen metil kavikola, 1,8-cineola in metil cinamata, je bila pri kontroli (brez gnojenja z dušikovimi gnojili). Sorta Italian Large Leaf in uporaba 100 kg ha^{-1} uree sta priporočeni za doseganje sprejemljivega agronomskega pridelka in pridelka eteričnih olj navadne bazilike v okoljskih razmerah podobnih tistim v Khorramabadu.

Ključne besede: navadna bazilika; suha masa; eterično olje; glavne sestavine eteričnega olja; urea

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1 INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) is one of the oldest spices belonging to the Labiatae family, an herbaceous annual plant, native to Asia, Africa, America and the Subtropics (Roman, 2012; Borloveanu, 2014). The basil is rich in secondary metabolites and essential oil of therapeutic importance. Therefore, it has been used in traditional medicine as a tonic, diuretic, analgesic, anti-inflammatory, anticancer and also to prevent cardiovascular disease complications (Krishnaiah et al., 2009; Srivastava et al., 2014; Li et al., 2017). Basil is an important economic crop and widely used in the culinary arts, food processing and pharmaceutical industries (Al-Maskri et al., 2011; Beatović et al., 2015). There are many cultivars of sweet basil which vary in their leaf colors (green or purple), flower color (white, red, purple) and aroma. Sweet basil has been classified into seven different morphotypes, which include 1) tall, slender types; 2) large-leafed types ('Italian' basils); 3) dwarf types ('Bush' basils); 4) compact types ('Thai' basils); 5) purple types (with clove-like aroma); 6) 'Purpurascens' types (sweet purple colored basils); and 'Citriodorum' types (flavored basils) (Simon et al., 1999). The chemical composition of sweet basil essential oil depends on genetic, season, environmental factors and the plant growth stage (Bilal et al., 2012). Padalia et al. (2014) has reported linalool, methyl chavicol, methyl eugenol, eugenol and geraniol as dominant components in the basil essential oil.

Nitrogen is one of the most important nutrients for plant production that plays a major role in photosynthetic activity and crop yield capacity and its availability influence plants growth and biochemical parameters (Werner & Newton, 2005; Caliskan et al., 2008). The promoting effect of nitrogen on growth parameters can be explained on the basis of the fact that nitrogen is involved in all parameters of growth through structure and regulation, among them supply increases the number and size of meristematic cells which leads to the formation of new shoots and leaf expansion (Lawlor, 2002).

Growth and yield of basil, like in other cultivated plants, depend upon the availability of all nutrients in the nutritional environment; besides, the yield quality is closely connected with macro- and micro-elements taken up. It is reported that nitrogen application significantly increase the herb yield of basil grown in different climatic conditions (Zheljazkov et al., 2008). According to Zheljazkov et al. (2008), the relationship between nitrogen levels and basil plants growth was characterized by quadratic polynomial model, the highest values being reached at approximately 60 kg ha^{-1} N. However, Sifola & Barbieri (2006) reported that the highest fresh mass of basil occurred in 300 kg ha^{-1} of nitrogen fertilization. The conditions such as climatic and nutrient factors suitable for plant growth are of the most important factors affecting the growth of medicinal plants and qualitative and quantitative characteristics of essential oil (Street, 2012). Reporting research findings showed that nitrogen markedly changed the amount of linalool, eugenol, methyl chavicol, Z-citral (neral), geranial, (E)-caryophyllene, trans-α-bergamotene, α-humulene and eucalyptol of essential oil of sweet basil (Zheljazkov et al., 2008; Nurzyńska-Wierdak et al., 2013; Kordi, 2017). In a field research with mint (*Mentha arvensis* L.), the effect of three levels of nitrogen fertilizer (0, 100 and 200 kg ha^{-1} nitrogen) on essential oil yield was investigated. The results showed that the highest amount of essential oil in mint was obtained from application of 100 kg ha^{-1} nitrogen (Ram et al., 1998).

Excessive nitrogen fertilizer may negatively affect the uptake of other nutrients, and also its high water-solubility of nitrogen leads to increased runoff into surface water as well as leaching into groundwater, thereby causing groundwater pollution. So, determining an appropriate dose of N fertilizer in line with feeding crops results in not only higher yield quantitatively and qualitatively, but also less damage to agricultural system and environment. Moreover, the yield and qualitative characteristics of the cultivars, investigated fully throughout this experiment, have not been studied under a certain climate in the previous researches, so far. The aim of the present research was to determine the effect of the rate of nitrogen fertilizers on agricultural yield, essential oil content and composition in four basil cultivars: 'Italian Large Leaf', 'Mobarakeh', 'Cinnamon' and 'Thai'.

2 MATERIALS AND METHODS

2.1 LOCATION AND PLANT MATERIALS

This field experiment was conducted in the Agricultural Research Station of Khorramabad, Iran (33°27′N, 48°17′E, and altitude 1,162 m), during 2016 - 2017 growing season. Physical soil analysis and chemical characteristics of soil at the depth 0-30 cm are shown in Table 1.

Before cultivation, 100 kg triple superphosphate ha^{-1} and 30 kg potassium sulfate ha^{-1} were added to plots according to soil test. Nitrogen (as urea; 46 % N) in two phases was distributed: half of the amount of nitrogen was added to plots with the last tilling before planting and the rest of the nitrogen after the first harvest. Seeds of sweet basil were planted by hand in plots, whose area was 5 m^2, consisting of five 2-meter rows spaced 50 cm apart and 2.5 cm intra-row plant spacing on May 25th 2016. All plots were irrigated immediately after sowing. Sub-

Table 1: Physical and chemical analysis of soil before the experiment

Soil texture	Clay (%)	Silt (%)	Sand (%)	pH	EC (dS m^{-1})	OC (%)	Total N (%)	Available P (ppm)	Available K (ppm)
Silty clay loam	39	48	13	7.7	0.5	1.04	0.22	7	335

sequent irrigations were carried out every 4 days. Hand weeding of the experimental area was performed when required.

2.2 EXPERIMENTAL DESIGN AND TREATMENTS

This experimental design was a factorial split-plot-in time experiment based on Randomized Complete Block Design (RBCD) with three replications. Experimental treatments were four cultivars of sweet basil (*O. basilicum* 'Italian Large Leaf', *O. basilicum* 'Cinnamon', *O. basilicum* 'Thai' and *O. basilicum* 'Mobarakeh' (native basil cultivar has been cultivated in many regions of Iran), three levels of nitrogen fertilizer included; N1: control (without urea fertilizer); N2: 100 kg ha^{-1} urea; N3: 200 kg ha^{-1} urea, and three harvests. Mobarakeh cultivar was provided from Pakan-Bazr institute, Isfahan and three foreign cultivars of basil were obtained from Eden Brothers Company in the United States.

2.3 TRAITS MEASUREMENT

The traits measured in this study included leaf dry mass, total dry mass, essential oil percentage, chemical composition and yield of essential oil sweet basil.

2.4 MEASURING LEAF DRY MASS AND TOTAL DRY MASS

The sweet basil was harvested three times each season in early flowering stage on the July 12th, August 15th, and September 25th. Samples along 1 m of length were taken from the center of two rows, located in the middle of each plot. Plants above ground was cut and transferred into a lab to measure leaf and total mass. To measure dry mass, the samples were dried in an oven at 75 °C for 72 h and then weighed. For essential oil extraction the aerial parts of sweet basil plants were dried naturally in the shade.

2.5 MEASURING ESSENTIAL OIL CONTENT

Fifty grams of dried aerial parts (both stems and leaves) were sampled for analysis. Woody parts were separated and the remainder hydro-distilled for 4 h, using a Clevenger-type apparatus (Anonymous, 1996). The distilled essential oils were dried over anhydrous sodium sulfate, filtered and stored in sealed vials at 4 °C, prior to further analyses. To measure main constituents of essential oil, all treatments belonged to a given repetition were chosen in second harvest.

2.6 GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

The oil samples were analyzed in a gas chromatography Agilent model 7890 using HP-5MS column (30 m × 0.25 mm, 0.25 mm in thickness). The oven temperature was programmed from 50 °C (held for 2 min) and increased to 240 °C at a rate of 3 °C min^{-1} then 240 °C to 300 °C at a rate of 15 °C min^{-1}. Helium was used as carrier gas with a constant flow rate of 1 ml min^{-1}. The mass spectra were recorded on electron ionization (EI) mode, with ionization energy of 70 eV. Temperature at the injection site was 290 °C. The identification of constituents was carried out based on the retention indices (calculated using from C8 to C20 alkanes) and by comparing the mass spectra with a computer databank (Wiley 7 and Nist 62) and with reference to published data (Adams, 2007; Carneiro et al., 2017).

2.7 DATA ANALYSIS

SAS (version 9.1) and MSTAT-C programs were used to conduct an analysis of variance (ANOVA) and means comparison, respectively. Treatment mean differences were separated by Duncan test at the 5 % level of probability. The graphs were drawn by Excel and error bars were assigned on the basis of standard deviation (SD).

3 RESULTS AND DISCUSSION

3.1 LEAF DRY MASS AND TOTAL DRY MASS

The analysis of variance showed that cultivar had a significant effect on leaf dry mass and total dry mass.

Table 2: The variance analysis of agricultural yield and some qualitative properties of sweet basil cultivars

Sources of variation	df	Leaf dry mass	Total dry mass	Percentage of essential oil	Essential oil yield
Replication (R)	2	4372.81	61975.13	0.008	13.99
Cultivar (A)	3	100263.97**	865900.19**	2.63**	2701.25**
Fertilizer (B)	2	1993129.8**	5107691.31**	0.35**	246.26**
A× B	6	17972.91 ns	83695.26**	0.007 ns	7.36 ns
Error 1 (A×B×R)	22	15800.19	20256.08	0.006	8.24
Harvest (C)	2	3728940.4**	15436181.5**	0.37**	3046.94**
Error 2 (C×R)	4	2722.3	17939.5	0.05	21.95
A× C	6	8325.49 ns	58284.72**	0.05**	207.93**
B× C	4	164325.9**	187653.87**	0.003 ns	8.72 ns
A× B× C	12	2546.9 ns	35986.78**	0.004 ns	7.82 ns
Error 3	48	5140. 8	6871.59	0.01	8.26
C.V (%)	-	6.07	3.37	10.78	11.97

*,** and ns show significant difference at probability of 5 %, 1 % and no significant difference, respectively

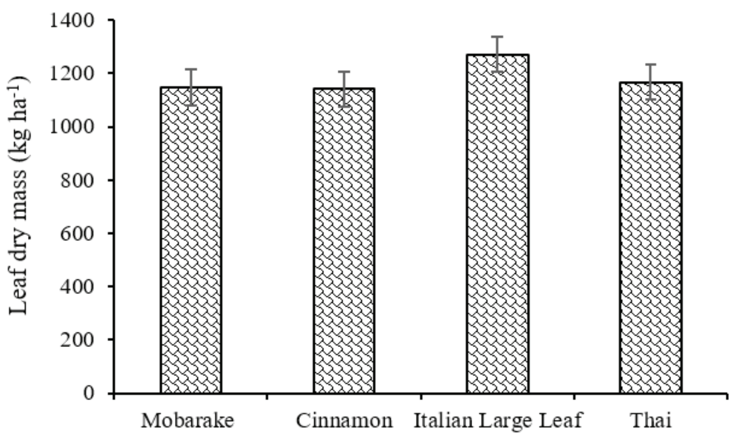

Figure 1: Leaf dry mass in different cultivars of basil

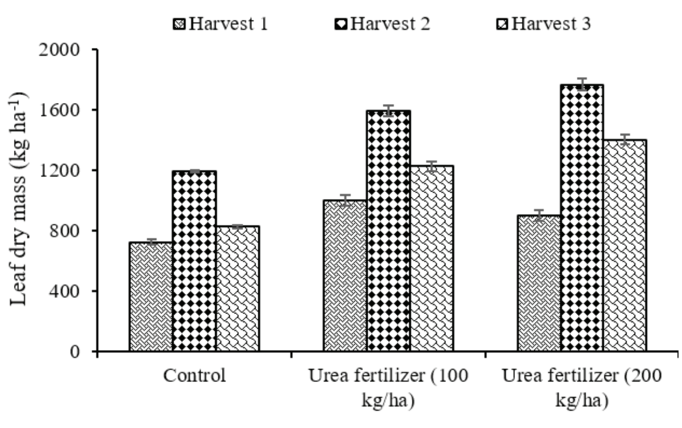

Figure 2: Leaf dry mass response of basil to different harvests under various levels of nitrogen fertilizer

Also, simple effects of fertilization and harvest were significant on leaf dry mass and total dry mass in this study. Cultivar × harvest interaction effect was significant for the total dry mass. Also, the leaf dry mass and total dry mass were influenced by fertilizer × harvest. The interaction effects of cultivar × fertilizer and cultivar × fertilizer × harvest were only significant for total dry mass (Table 2).

Application of nitrogen fertilizer significantly improved yield in all four cultivars. Nitrogen fertilizers provide during the growing season provides a favorable environment for the production of basil, by providing the nitrogen needed for the basil plant growth and biomass production.

The highest leaf dry mass (1271.3 kg ha^{-1}) was related to Italian Large Leaf cultivar, but there was no significant difference among other studied sweet basil cultivars (Fig. 1). In all harvests, plants fed with urea had greater leaf dry mass compared to untreated plants. Plants in the second harvest had higher leaf dry mass compared to the first and the third harvests. The maximum leaf dry mass (1766.5 kg ha^{-1}) was recorded with the application of 200 kg ha^{-1} urea at the second harvest. Untreated plants had the lowest leaf dry mass in all harvests (Fig. 2). Kandil et al. (2009) reported that nitrogen fertilization significantly increases basil leaf mass, which can be due to an increase in leaf thickness, and not only its size. Also, Kordi (2017) reported that among different sources of nitrogen fertilizers, the highest fresh and dry mass of basil leaves were recorded for plants fed with urea fertilizer in the second harvest.

The comparison of the mean values of the total dry mass (Fig. 3) showed that total dry mass of the all studied basil cultivars increased with application of nitrogen fertilizer in all harvests. Similar to leaf dry mass (Fig. 2), plants in the second harvest had the highest total dry mass compared to the first and the third harvests. The highest total dry mass (3482.4 kg ha^{-1}) was related to Italian Large Leaf cultivar with the application of 200 kg ha^{-1} urea at the second harvest, although in terms of this trait had no significant difference with Italian Large Leaf cultivar with the application of 100 kg ha^{-1} urea at the second harvest (Fig. 3). The increment in total dry mass of basil by application of nitrogen fertilizer (Fig. 3) can be attributed to increment in chlorophyll content (unpublished data) and better growth of plants and subsequently the better canopy development which ultimately leads to the better use of solar irradiance, higher photosynthesis and finally higher dry mass in basil plants. Nitrogen has an important role in the improvement of vegetative growth, resulting in increased yield. Sifola & Barbieri (2006) reported that applying nitrogen doses (ranging between 0 to 300 kg ha^{-1}) resulted in a dry mass rise of the above-ground part. In other research, the highest basil herb yield (23.2 t ha^{-1}) was obtained by application of 120 kg ha^{-1} N (Yassen et al., 2003). Also, Biesiada & Kus (2010) reported that nitrogen amount affects basil herb yield so that the highest yields were recorded at the dosage of 150-250 kg ha^{-1} N.

Although all plants were harvested at identical growth stage, a reduction in total dry mass of the first harvest (Fig. 3) is attributed to the effects of higher temperature of June and consequently stimulating premature flowering in spite of lower vegetative growth. In this respect, lateral branch and height of plants relative to other harvests were reduced.

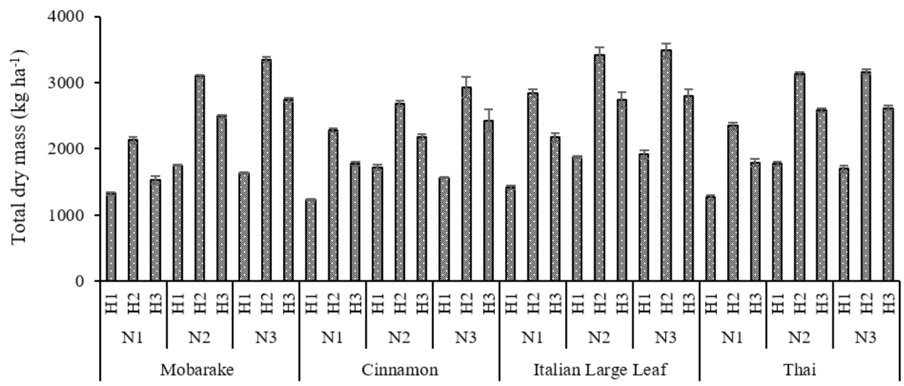

Figure 3: Total dry mass response of basil cultivars to different harvests under various levels of nitrogen fertilizer
N1: (control), N2: 100 kg ha-1 urea, N3: 200 kg ha-1 urea;
H1, H2 and H3: first harvest, second harvest and third harvest, respectively

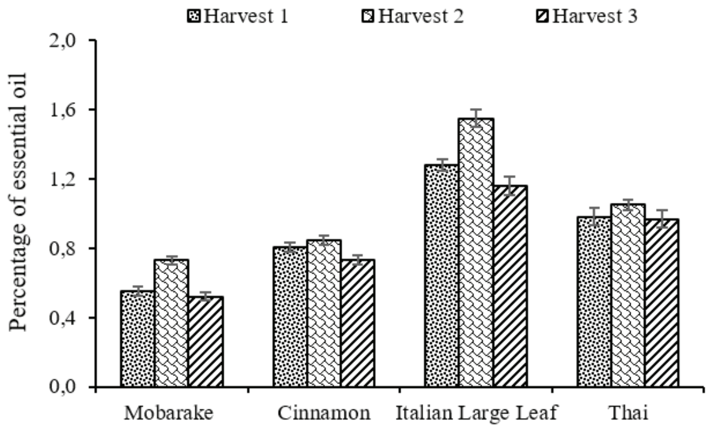

Figure 4: Percentage of essential oil response of basil cultivars to different harvests

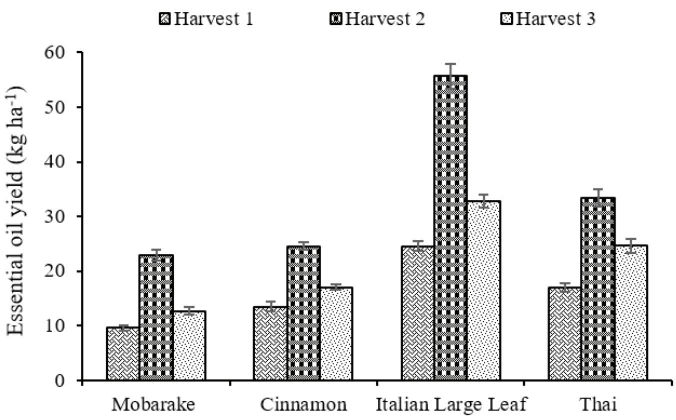

Figure 5: Essential oil yield response of basil cultivars to different harvests

3.2 PERCENTAGE OF ESSENTIAL OIL AND ESSENTIAL OIL YIELD

The results of variance analysis showed a significant influence of cultivar, fertilization and harvest on the percentage of essential oil and essential oil yield (kg ha^{-1}) (Table 2). Also, cultivar × harvest interaction effects were significant for both traits (Table 2). The comparison of the mean values showed that the highest percentage of essential oil (1.55 %) and essential oil yield (55.64 kg ha^{-1}) were related to Italian Large Leaf cultivar at the second harvest (Figs 4 and 5). Although all harvests were performed at the same developmental stage, plants of second harvest generated more secondary metabolites (Fig. 4) due to exposition to abundant light and carrying out more photosynthesis activities. Among three harvests of sweet basil, Jahan et al. (2012) demonstrated that the highest and lowest percentage of essential oil and essential oil yield were found in the second and the first harvests, respectively.

The results of mean comparisons showed that the highest (1.01 %) and the lowest (0.82 %) essential oil content was related to control and 200 kg ha^{-1} urea, respectively, but the maximum (26.79 kg ha^{-1}) and minimum (21.58 kg ha^{-1}) essential oil yield belonged to 100 kg ha^{-1} urea and control (Figs 6 and 7). According to the results of this research, it seems that there is an inverse relationship between essential oil percentage of sweet basil and using nitrogen chemical fertilizer (Fig. 6). The superiority of controls over other treatments with high agricultural yield is attributed to an increase in secondary metabolites under environmental stress and nutritional deficiency conditions. Nitrogen fertilizer application adequately paved the way for plants to grow adequately through supplying nutritional resources. Tahami Zarandi et al. (2010) revealed that higher essential oil percentage of sweet basil was produced by not using fertilizer than with chemical fertilizer. Control (without nitrogen fertilizer) treatment gave higher essential oil percentage compared to the ni-

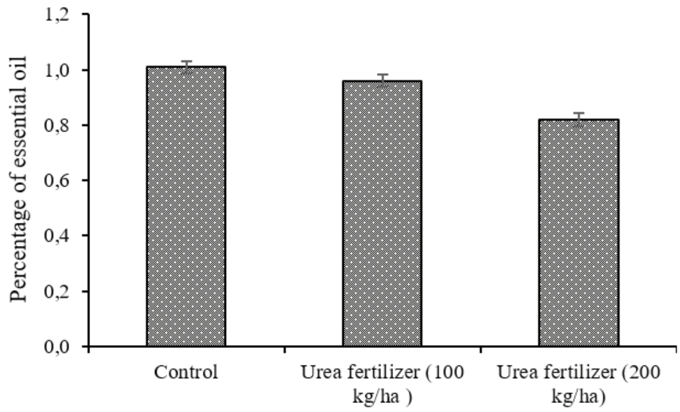

Figure 6: Percentage of essential oil response of basil to different levels of nitrogen fertilizer

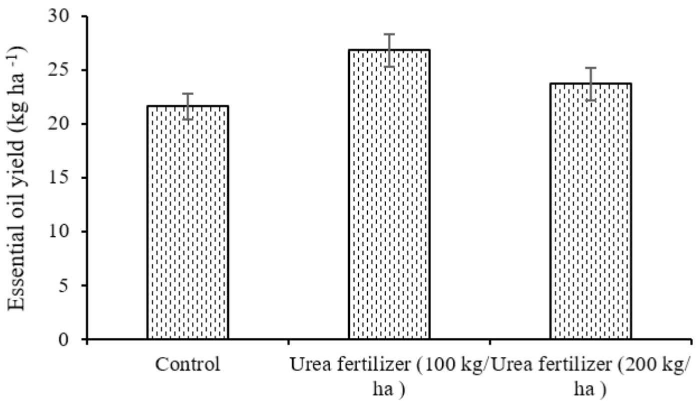

Figure 7: Essential oil yield response of basil to different levels of nitrogen fertilizer

trogen chemical fertilizers (Fig. 6). This reflected the facts that essential oil yield of sweet basil was highly affected by agricultural yield and less by essential oil percentage (Fig. 7). This experiment confirmed the hypothesis that application of nitrogen chemical fertilizer could increase essential oil yield of sweet basil, mainly due to increase in agricultural yield. Biesiada & Kus (2010) documented that highest yield of sweet basil's aerial organs was obtained by applying 150 kg ha^{-1} N. Added nitrogen supply increases photosynthesis rate and enables the plant to grow rapidly and produced considerable biomass and basic metabolism, which may increase production and accumulation of secondary metabolites, such as essential oil (Sifola & Barbieri, 2006).

3.3 THE MAIN COMPONENTS OF ESSENTIAL OIL

In addition to quantity, the quality (in terms of type and amount of constituents) of essential oil is also received great attentions while cultivation medicinal plants. The analysis of essential oil carried out on aerial parts of basil cultivars under different treatments revealed the presence of 29-35 constituents, shown in Table 3. In current research, the results revealed that the types of essential oil components could remarkably vary depending on the cultivar and the rate of applied nitrogen. Eight constituents including methyl chavicol (38.2-48.9 %), Z-citral (neral) (13.1-17.0 %), geranial (17.3-23.0 %), (E)-caryophyllene (4.50-4.90 %), trans-α-bergamotene (1.27-1.80 %), α-humulene (1.67-1.90 %), germacrene-D (0.82-1.50 %), and γ-cadinene (3.00-3.20 %) were the main constituents of essential oil in the cultivar Mobarakeh (Table 3). 1,8-cineole (4.70-5.20 %), linalool (34.5-36.1%), methyl chavicol (5.80-7.20 %), eugenol (1.50-1.81 %), methyl cinnamate (38.8-41.0 %) and germacrene-D (1.80-2.30 %) were the dominant compounds in the essential oil of cultivar Cinnamon (Table 3). In the

Table 3: Essential oil constituents in sweet basil cultivars under different levels of nitrogen fertilizer (based on percentage)

	'Mobarakeh'			'Cinnamon'			'Italian Large Leaf'			'Thai'		
Compound	N1	N2	N3	N1	N2	N3	N1	N2	N3	N1	N2	N3
α-Pinene	0.20	0.21	0.21	0.28	0.25	0.32	0.26	0.30	0.31	0.16	0.20	0.22
Camphene	0.69	0.60	0.63	tr	tr	tr	0.11	tr	tr	tr	tr	tr
β -Pinene	0.36	0.37	0.30	0.45	0.50	0.40	0.67	0.60	0.61	0.29	0.33	0.30
Myrcene	0.15	0.15	0.10	0.29	0.26	0.30	0.49	0.53	0.5	0.40	0.45	0.32
Limonene	0.12	0.13	0.14	0.10	0.10	0.12	0.30	0.35	0.30	0.21	0.20	0.18
1,8-Cineole	0.31	0.40	0.35	**4.70**	**5.20**	**4.70**	**8.14**	**8.30**	**8.40**	**1.45**	**1.60**	**1.50**
(E)-β- Ocimene	0.13	0.20	0.26	0.60	0.65	0.60	0.32	0.30	0.35	**1.14**	**1.20**	**1.20**
Terpinolene	-	-	-	0.17	0.15	0.10	0.25	0.20	0.23	0.16	0.20	0.20
Fenchone	0.10	0.15	0.20	0.22	0.30	0.30	0.30	0.30	0.35	0.24	0.20	0.30
Linalool	0.20	0.30	0.25	**34.5**	**36.1**	**34.6**	**42.4**	**44.8**	**41.9**	**40.0**	**40.1**	**39.0**
Camphor	tr	tr	tr	0.70	0.40	0.30	0.53	tr	0.30	0.75	0.50	0.50
Borneol	0.32	0.20	0.25	0.15	tr	tr	0.42	0.30	0.30	0.40	0.40	0.20
Terpinen-4-ol	-	-	-	0.73	0.80	0.95	-	-	-	0.21	0.40	0.40
α-Terpineol	0.26	0.30	0.20	0.33	0.35	0.30	0.71	0.60	0.55	**1.10**	**0.90**	**0.80**
Methyl chavicol	**38.2**	**45.2**	**48.9**	**5.80**	**6.00**	**7.20**	**30.7**	**33.2**	**37.0**	**9.30**	**10.1**	**11.3**
Z-Geraniol (Nerol)	0.21	0.40	0.68	-	-	-	-	-	-	-	-	-
Z-Citral (Neral)	**17.0**	**14.2**	**13.1**	-	-	-	-	-	-	-	-	-
Chavicol	0.30	0.20	0.20	0.40	0.40	0.50	0.80	0.75	0.90	-	-	-
Geraniol	0.85	0.50	0.23	-	-	-	0.10	tr	tr	-	-	-
Geranial	**23.0**	**21.4**	**17.3**	-	-	-	-	-	-	-	-	-
Bornyl acetate	0.30	0.30	0.20	0.26	0.20	0.20	0.60	0.70	0.50	0.42	0.50	0.40
α-Cubebene	0.20	0.18	0.15	0.15	0.20	0.15	0.40	0.30	0.30	-	-	-
Eugenol	0.19	0.15	0.10	**1.81**	**1.60**	**1.50**	**1.20**	**1.10**	**0.90**	**3.21**	**3.00**	**2.80**
α-Copaene	0.16	0.21	0.17	0.14	0.10	0.10	tr	tr	tr	-	-	-
β- Cubebene	0.50	0.45	0.40	-	-	-	0.13	tr	tr	0.75	0.60	0.60
β- **Elemene**	0.14	tr	tr	0.68	0.50	0.60	0.15	tr	tr	**1.03**	**0.90**	**0.90**
Methyl cinnamate	tr	tr	0.10	**38.8**	**40.3**	**41.0**	-	-	-	**30.1**	**31.2**	**32.0**
Methyl eugenol	0.60	0.50	0.46	0.16	tr	tr	0.52	0.30	0.40	**1.60**	**1.30**	**1.30**
(E)-Caryophyllene	**4.90**	**4.50**	**4.78**	0.40	0.30	0.30	**2.80**	**2.50**	**2.20**	0.30	0.20	tr
Trans-α-Bergamotene	**1.80**	**1.60**	**1.27**	0.53	0.30	0.30	**1.02**	**0.90**	**0.75**	**1.18**	**0.90**	**0.75**
α-Guaiene	-	-	-	0.23	0.25	0.15	0.26	0.20	0.10	0.37	0.30	0.30
α-Humulene	**1.90**	**1.80**	**1.67**	0.53	0.30	0.10	0.42	0.30	0.25	0.23	0.10	tr
(E)-β-Farnesene	0.50	0.40	0.42	0.33	0.30	0.20	-	-	-	-	-	-
Germacrene-D	**1.50**	**1.10**	**0.82**	**2.30**	**2.00**	**1.80**	**1.71**	**1.60**	**1.30**	0.59	0.40	0.40
γ-Cadinene	**3.20**	**3.00**	**3.01**	0.53	0.50	0.45	0.66	0.60	0.50	0.40	0.40	0.30
β-Bisabolene	0.25	0.20	0.23	-	-	-	-	-	-	-	-	-
Nerolidol	0.37	0.30	0.30	0.15	0.15	0.10	-	-	-	0.13	0.10	0.15
Caryophyllene oxide	0.10	0.10	0.14	0.13	tr	tr	-	-	-	-	-	-
α-Cadinol	-	-	-	0.57	0.40	0.30	-	-	-	0.23	0.20	0.20

N1: (control), N2: 100 kg ha^{-1} urea , N3: 200 kg ha^{-1} urea and tr: trace amounts < 0.05 %

essential oil extracted from the basil herb in the cultivar Italian Large Leaf seven constituents, i.e. 1,8-cineole (8.14 - 8.40 %), linalool (41.9-44.8 %), methyl chavicol (30.7-37.0 %), eugenol (0.90-1.20 %), (E)-caryophyllene (2.20-2.80 %), trans-α-bergamotene (0.75-1.02 %) and germacrene-D (1.30-1.71 %) were the main constituents of essential oil (Table 3). Ten constituents including 1,8-cineole (1.45-1.60 %), (E)-β-ocimene (1.14-1.20 %), linalool (39.0-40.1 %), α-terpineol (0.80-1.10 %), methyl chavicol (9.30-11.3 %), eugenol (2.80-3.21 %), β-elemene (0.90-1.03 %), methyl cinnamate (30.1-32.0 %), methyl eugenol (1.30-1.60 %) and trans-α-bergamotene (0.75-1.18 %) were the main constituents of essential oil in the cultivar Thai (Table 3).

In order to monitor the change occurred in type and amount of essential oil's constituents, their contents in different treatments were carefully assayed. The highest amount of 1,8-cineole (8.40 %) and linalool (44.8 %) were achieved by Italian Large Leaf cultivar and the application of 200 and 100 kg ha^{-1} urea, respectively (Table 3). Nurzyńska-Wierdak et al. (2013) reported that linalool concentration increased after the application of the medium rate of nitrogen fertilizer and subsequently decreased after the highest rate was applied.

The highest amount of methyl chavicol (48.9 %) was achieved by Mobarakeh cultivar with the application of 200 kg ha^{-1} urea; while the lowest (5.80 %) was obtained by Cinnamon cultivar without nitrogen fertilizer (Table 3). The availability of nitrogen in chemical fertilizer seemingly increased methyl chavicol concentration compared to other treatments. Zheljazkov et al. (2008) stated that nitrogen markedly changed the amount of linalool, eugenol, bornil-acetate and eucalyptol of essential oil of sweet basil. They continued that application of more nitrogen increased methyl chavicol while it decreased linalool of essential oil.

The highest content of Z-citral (neral) (17.0 %), geranial (23.0 %), (E)-caryophyllene (4.90 %), trans-α-bergamotene (1.80 %), α-humulene (1.90 %) and γ-cadinene (3.20 %) were observed by Mobarakeh cultivar without nitrogen fertilizer (Table 3). Some of detected constituents such as Z-citral (neral) and geranial were not observed in Cinnamon, Italian Large Leaf and Thai cultivars (Table 3). It appears that some factors like nutrient deficiency (especially nitrogen deficiency in the control treatment) could be considered as factors stimulating production of these constituents. Main constituents of essential oil are affected by diverse factors: water stress, salt stress and nutrition deficiencies resulting in alteration of essential oil constituents (Ekren et al., 2012; Barbieri et al., 2012). Based on Nurzyńska-Wierdak et al. (2013), different levels of nitrogen was shown to have a significant effect on the main constituents in the essential oil of basil in such that the highest amount trans-α-bergamotene in sweet basil was found by lowest level of nitrogen. Kordi (2017) reported that among different sources of nitrogen, the highest amounts of Z-citral (neral), geranial, (E)-caryophyllene, trans-α-bergamotene and α-humulene were obtained by control (without nitrogen fertilizer).

One of the predominant components of the essential oil in Cinnamon and Thai cultivars was methyl cinnamate. The maximum amount of methyl cinnamate was achieved by Cinnamon cultivar and applying 200 kg ha^{-1} urea, while the highest amount of germacrene-D was obtained by Cinnamon cultivar without nitrogen fertilizer (Table 3). In this regard, Kordi (2017) stated that the amount of germacrene-D decreased with increasing N supply. The highest content of eugenol (3.21 %) was observed by Thai cultivar without nitrogen fertilizer. In all cultivars, the trend of eugenol changes was similar to that of germacrene-D in fertilizer treatments and amount of this compound decreased with increasing nitrogen consumption (Table 3).

4 CONCLUSIONS

The results of the present study revealed that among different basil cultivars, Italian Large Leaf cultivar had the highest mean leaf dry mass, total dry mass, essential oil percentage and essential oil yield as compared to other cultivars. The lowest essential oil percentage and essential oil yield belonged to Mobarakeh cultivar. Application of urea fertilizer significantly improved vegetative growth, resulting in increased yield. Except essential oil percentage, all parameters evaluated in this study in the first harvest were lower than those at the other harvests. The highest yield of essential oil was attained by 100 kg ha^{-1} urea fertilizer; although no-fertilizer treatment gave higher essential oil percentage than did nitrogen chemical fertilizer treatments, but 100 kg ha^{-1} urea fertilizer treatment gave higher essential oil yield. This can be justified as essential oil yield was mainly affected by agricultural yield rather than essential oil percentage. The highest concentrations of main constituents of essential oil, except methyl chavicol, 1,8-cineole and methyl cinnamate were obtained by control (without nitrogen fertilizer); and this indicated an increase in content of the most main constituents of essential oil under treatment without nitrogen fertilizer, as compared to its nitrogen chemical fertilizers. Due to the fact that in terms of total dry yield there was no significant difference between application of 100 and 200 kg ha^{-1} urea fertilizer and the highest yield of essential oil belonged to 100 kg ha^{-1}urea fertilizer and also reduce the consumption of chemical fertilizers generating pollution, Italian Large Leaf cultivar and application of 100 kg ha^{-1} urea are considered to access an acceptable agricultural yield and essential oil yield in sweet basil under environmental condition similar to Khorramabad.

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Optimizing sowing time for boosting productivity and nutritional quality of amaranth (*Amaranthus cruentus* L.) genotypes under Mediterranean climate

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Optimizing sowing time for boosting productivity and nutritional quality of amaranth (*Amaranthus cruentus* L.) genotypes under Mediterranean climate

Abstract: Currently, there is increasing interest in the cultivation of alternative grain crops, such as amaranth (*Amaranthus cruentus* L.) in Italy. However, few information exist for the most optimal sowing date (SD) of amaranth especially under rainfed conditions. A field-trial was conducted in Tuscany during 2018 to evaluate SD effects on amaranth genotypes ('Kharkov' and two new breeding lines coded 'A-61' and 'A-67'). The three SD were March 27 (first), April 20 (second), June 4 (third). Only the first and second SD coincided with increasing photoperiod. Cumulative Growing Degree Days (GDD) for ripening were 2282, 1990 and 1480 for the first, second and third SD, respectively. Compared to the first two SD, ground-cover was 20 % less from panicle formation in the third SD, therefore potentially less competitive towards weeds. A-67 was more "palatable" to the incidence of sugar beet flea beetle (*Chaenoctema tibialis* Illinger) from the earliest stages of growth. In contrast, 'Kharkov', was significantly less attacked by the insect over all three SD.

'Kharkov', demonstrated greater flexibility with stable yields of 1.3 t ha^{-1} for the first and second SD. In contrast, significant yield reductions (1.5 to 1.2 t ha^{-1}, from the first to second SD, respectively) were evident for both lines of amaranth in response to SD delay. Adverse effects of the third SD, included significant decline in yield, protein content and 1000 seed mass. In conclusion, amaranth, March-April SD may be recommended for obtaining optimal grain yield of amaranth along with making it successful as a viable alternative grain crop under agro-ecological conditions of Central Italy.

Key words: grain amaranth; breeding lines, *Amaranthus cruentus*; date of sowing; Central Italy

Optimizacija časa setve za povečanje produktivnosti in prehranske kakovosti genotipov zrnatega ščira (*Amaranthus cruentus* L.) v mediteranskih klimatskih razmerah

Izvleček: V zadnjem času narašča v Italiji interes za gojenje alternativnih zrnatih poljščin kot je zrnati ščir (*Amaranthus cruentus* L.), a je za najprimernejši čas setve (SD) v razmerah brez namakanja na razpolago le malo podatkov. V ta namen je bil v Toskani, v rastni sezoni 2018, izveden poljski poskus za ovrednotenje časa setve treh genotipov zrnatega ščira (sorta Kharkov in dve novi žlahtniteljski liniji, označeni kot 'A-61' in 'A-67'). Izbrani so bili trije termini setve in sicer: 27. marec (prvi), 20. april 20 (drugi), 4. junij (tretji). Samo prvi in drugi termin setve sta soupadala z naraščajočo fotoperiodo. Komulativno število rastnih dni (GDD) do zorenja je bilo 2282, 1990 in 1480 za prvi, drugi in tretji čas setve. Primerjalno s prvima terminoma setve je bil pri tretjem sklop posevka za 20 % manjši v času latenja, kar je zmanjšalo kompetitivnost proti plevelom. Linija 'A-67' je bila bolj palatibilna in bolj dovzetna za napad hrošča bolhača sladkorne pese (*Chaenoctema tibialis* Illinger) v začetnih fazah rasti. Nasprotno je bila sorta Kharkov značilno manj napadena s tem hroščem pri vseh treh datumih setve. Sorta Kharkov je pokazala večjo prožnost s stabilnim pridelkom, 1,3 t ha^{-1}, za prvi in drugi termin setve. Nasprotno je bil pri obeh linijah zrnatega ščira značilen upad pridelka (1,5 to 1,2 t ha^{-1}) kot odziv na zakasnitev setve iz prvega na drugi termin. Negativni učinek tretjega termina setve je obsegal značilen upad pridelka, zmanjšanje vsebnosti beljakovin in zmanjšanje mase 1000 semen. Zaključimo lahko, da lahko obdobje marec-april priproročamo za setev zrnatega ščira za doseganje optimalnega pridelka zrnja in s tem uspešno uvedemo alternativno poljščino za pridelovanje zrnja v agroekoloških razmerah osrednje Italije.

Ključne besede: zrnati ščir; žlahtniteljske linije, *Amaranthus cruentus*; datum setve; osrednja Italija

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1 INTRODUCTION

There is an increasing focus on alternative crops, predominantly in areas where the cultivation of most common cereals (wheat, maize, barley, etc.) no longer provides a sufficient income for farmers due to low international selling prices. In addition, there is the necessity for boosting crops production leading to enhanced food safety levels under changing climate (Yarnia, 2010). In particular, increasing occurrences of drought in temperate areas necessitate irrigation for the most critical phases of spring-summer sown crops, which is often costly and difficult to implement. Hence, there is the need to look for alternative crops having higher grain potential under drought and water deficit conditions. Amaranth is cultivated in a wide variety of climates and cultivation systems in large areas, as well as at subsistence agriculture levels, either as a vegetable or for dried seeds (Borneo and Aguirre, 2008).

Different species of amaranth such as *Amaranthus cruentus* L., *A. hypochondriacus* L., *A. caudatus* L. and *A. hybridus* L., are receiving considerable research attention as grain and fodder crop owing to their diverse genetic makeup and superior agro-botanical characteristics (Cervantes, 1996). Some of the prime characteristics of these species include the high protein (15-18 %), lysine (5.2 g per 100 g^{-1} dry matter) and calcium (0.37 g per 100 g^{-1} dry matter) (Petr et al., 2003). Moreover, these species have become distinguishable owing to absence of gluten and highly cherished by celiac patients (Ballabio et al., 2011).

Recently, a number of studies have been executed to determine the adaptability of amaranth in Italy primarily centered on the areas of Central and Southern Italy. First experiments have been performed to test the adaptation of amaranth plants, and qualitatively evaluate the grain (Massantini et al, 1987; Ercoli et al., 1987; Alba et al., 1997; Lovelli et al., 2005; Rivelli et al., 2008; Casini and La Rocca, 2014; Pulvento et al., 2015; El Gendy et al., 2018). The results of these tests highlighted an improved adaptability of *A. cruentus*, with a spring sowing, for seed production purposes. In the context of the provenances of the accessions tested in Italy, those derived from Mexico seem most adaptable. Not all aspects of the agronomic techniques have been addressed. Even if precise indications relating to the sowing density were obtained for *A. cruentus* (Casini and La Rocca, 2014), there is uncertainty with regard to the best sowing period, which is particularly important for a spring-summer crop.

Although amaranth is tolerant to drought (Roitner-Schobesberger et Kaul, 2013; Kauffman et Weber, 1990), the identification of the best SD in the Mediterranean environment, can aid plants in escaping, at least in part, periods with higher temperatures and scarcity of rains, especially coinciding with the formation of the panicle. In this phenological phase, possible water shortages and high temperatures negatively influence seed yield in *A. cruentus* (Mlakar et al., 2012). Within this species and with regard to drought tolerance, there is a certain degree of variability and the different accessions could be screened to choose the most suitable using the Van der Mescht and De Ronde (1993) method based on the accumulation of proline.

The choice of the most suitable sowing date has a direct effect on crop production. The emission of the inflorescence and flowering occur in optimum conditions, favoring maximum production (O'Brien et Price, 2008; Zubillaga et al., 2019). In a temperate Mediterranean environment, the spring sowing of amaranth cannot be performed too early, because the minimum temperature required for germination is approximately 12°C (Casini and La Rocca, 2015). Generally, the sowing period for this crop is similar to that of maize.

Each genotype reacts uniquely to the different sowing periods that directly influence the flowering and, above all, the ability to reach physiological maturation. In this context, the trends in photoperiod and temperatures play an important role in selecting the correct SD (Boote et al., 1994). Moreover, in temperate plains, amaranth has a tendency to wither with difficulty, hence the need to use medium-early cycle varieties to maximize yield and permit the complete maturation of the seeds.

The aim of the present research was to evaluate the effects of sowing date on grain yield, seed protein and mass in two new breeding lines of *Amaranthus* cruentus (A-61 and A-67), compared to a commercial variety ('Kharkov').

2 MATERIALS AND METHODS

Field experiment was carried out during 2018 in Tuscany, Central Italy at the "Centro per il Collaudo ed il Trasferimento dell'Innovazione di Cesa (Arezzo)" (43° 18' north; 11° 47' east, 246 m asl), on a neutral, loamy-sandy soil. Sampling of soil using a "W" shaped path were performed. The principle physical and chemical characteristics of the soil (depth of 20 cm) resulted as follows: sand 36.4 %; loam 37.7 %; clay 25.9 %; total N 0.114 %; P (Olsen) 11 ppm; exchangeable Ca, Mg and K: 4250, 620 and 136 ppm, respectively. The experiment was carried out according to a RCB split-plot design with four replicates. The size of the overall plot was 18.0 x 7.2 m, which constituted the main factor, comprising two new breeding lines coded A-61 and A-67, as well as 'Kharkov', an Ukrainian commercial variety. The subplots (2.4 x 6.0 m, four rows wide with 0.6 m row spacing) constituted four different sowing dates as follows: March 27; April 20, June 4 and June 20 (hereon referred to as the first, second, third and fourth SD).

Fertilizer treatment before seeding was as follows (Zubillaga et al., 2019): 76 kg ha^{-1} of N as ammonium nitrate, and 100 kg ha^{-1} of P_2O_5 as superphosphate. A seed quantity of 9 kg ha^{-1} was used. In order to attain the planting density of 30 plants m^{-2}, seedlings were thinned at the two-true leaf stage. Plots were hand-weeded twice (33 and 50 Days After Emergence [DAE]) during the growth cycle. The incidence of sugar beet flea beetle (*Chaetocnema tibialis* (Illiger, 1807), was estimated at emergence, and at the two-, four- and six-true leaf stage. Immediately after the last estimation, the seedlings were treated with the insecticide, deltamethrine (50 ml dissolved in 100 l water).

The following field measurements were recorded: emergence, 2-, 4-, 6- and 10- true-leaf stages; early panicle appearance; full panicle appearance; early flowering; milky maturation, waxy maturation and maturation at 75 %. For the maturation stage, seed consistency was taken in consideration together with complete filling (non-translucent endosperm).

The harvest was performed manually starting from September 7. The duration of maturation was accession dependent, and the different plots were harvested accordingly.

After drying the seeds to a standard humidity of 12 %, (airflow at 35 °C for 48 h), the yield calculations were performed. A sample from a seed batch was used to determine the mass of 1000 seeds. Total protein was determined from the N content (N x 6.25) using an Elemental Analyser EA FLASH 1112 of Thermo Fisher Scientific.

Day length records were provided by "Centro Interdipartimentale di Bioclimatologia-CIBIC" (University of Florence). Cumulative Growing Degree Days (GDD) were recorded from the first sowing (March, 27) to the last harvest period with a T_z (base temperature) equal to 8 °C (Mujica et al, 1997) as follows:

T_m is the daily mean temperature:

$$GDD = \sum_{days} (Tm - Tz)$$

Differences between response variables were assessed with COSTAT 6.45 software. Statistical differences were tested at $p \leq 0.05$, $p \leq 0.01$ or $p \leq 0.001$. The Tukey's HSD test was used to evidence significant differences between means and homogenous groups.

3 RESULTS AND DISCUSSION

The intense and persistent rains over March-April (110 mm) (Figure 1) led to a slight delay in the first sowing period compared to that predicted to be most suitable. Subsequently, this then led to a consequential delay of the remaining sowing periods envisaged. Even over the May-June period, the rains were of unusual frequency and intensity (75 mm), resulting in an extensive delay of the fourth SD, that was nonetheless carried out on June 20. This last SD was unsuccessful, due to high emergence failures. For this reason, only the first three sowing dates were considered in the statistical analysis of the results.

The increasing trend in photoperiod up to 65 Days After Sowing (DAS) the first SD, and up to 40 DAS from the second SD (Figure 2). In contrast, the entire growth cycle of the crop sown on the third SD was subjected

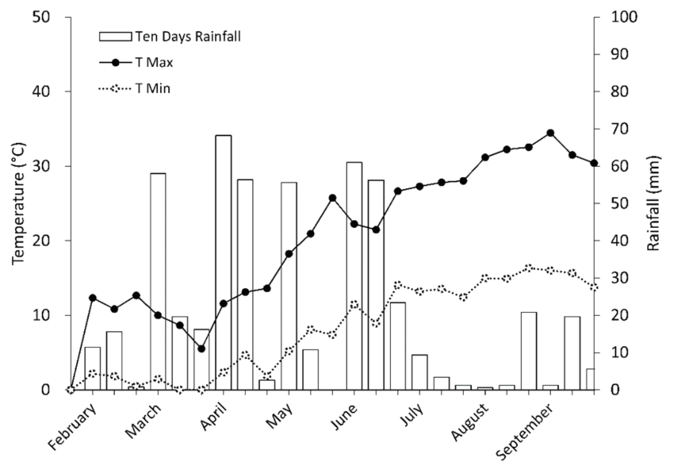

Figure 1: Temperature and rainfall recorded during the field experiment

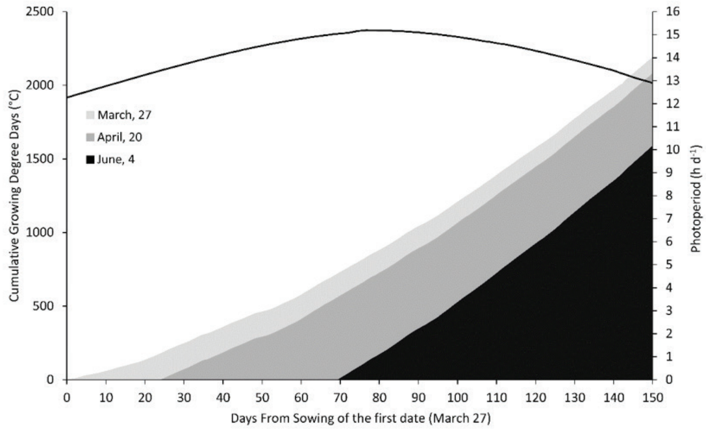

Figure 2: Cumulative Growing Degree Days (GDD) and day-length recorded during the field experiment according to the three sowing dates

Table 1: Analysis of variance of the growth stages

Source of Variation	DF	Emergence	Two true leaves	Four true leaves	Six true leaves	Ten true leaves	Early panicle	Panicle	Flowering
Blocks	3	1.63	29.44	141.64	222.97	73.22	45.00	40.75	27.33
Date of sowing (D)	2	515.06***	1500.67***	1264.39**	1120.39**	2752.17***	1416.72***	2067.17***	1963.72***
Error D	6	14.94	77.56	312.44	196.94	94.94	67.50	38.83	57.83
Lines (L)	2	12.72*	2.00	2.39	17.39	20.67	9.72	32.67*	23.83*
Error L	6	5.94	4.89	8.28	10.61	18.44	23.83	18.00	13.50
D x L	4	16.94	7.33	11.44	5.28	21.67	12.94	6.67	20.78
Error D x L	12	15.72	9.11	13.89	42.72	25.89	40.17	36.67	28.33

Source of Variation	DF	Milky Maturity	Waxy Maturity	Maturity	Seed humidity	Seed yield	1000 seeds mass	Seed protein
Blocks	3	16.56	66.97	1.42	14.45	1.57	0.02	0.55
Date of sowing (D)	2	4503.72***	4397.00***	7842.72***	88.21**	11.37**	0.03	29.32**
Error D	6	77.61	81.78	2.83	15.48	1.94	0.03	6.57
Lines (L)	2	3.72	0.5	0.06	0.91	0.01	0.01	8.42*
Error L	6	35.61	7.94	2.83	2.55	0.43	0.02	2.67
D x L	4	17.78	1.00	0.11	4.78	1.55	0.03*	4.10
Error D x L	12	36.22	16.56	5.67	5.96	0.72	0.02	10.63

*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

to decreasing photoperiod conditions. The three SD received 2282, 1990 and 1480 GDD, respectively.

The analysis of the variance, indicated significant differences largely attributable to the average effect of SD (Table 1 & 2). The variety effect was only significant with regard to the time of emergence, the emission of the panicle, flowering, and ground-cover with two-true leaves. For the 'Sowing date and Genotype' interaction, the only significant features at $p \leq 0.05$ were ground-cover of six-true leaves, and corresponding full maturation, as well as the weight of 1000 seeds.

Overall, the number of days required for the appearance of the panicle and flowering date decreased significantly from the first to the second SD, whilst the maturation period showed a progressive and significant decrease from the first to the third SD, respectively. In detail, the first appearance of the panicle occurred after approximately 66 DAE for the first SD, whilst the appearance of the panicle was anticipated by 15 d for the second

Table 2: Analysis of variance of the ground cover recorded at different growth stages

Source of Variation	DF	Two true leaves	Four true leaves	Six true leaves	Ten true leaves	Early panicle
Blocks	3	37.00	0.75	47.22	505.56	333.33
Date of sowing (D)	2	48.39	162.67**	151.39	59.72	1666.67
Error D	6	157.83	41.33	81.94	406.94	1400.01
Lines (L)	2	13.56*	19.50	26.39	243.06	66.67
Error L	6	5.33	21.83	56.94	223.61	200.00
D x L	4	10.44	3.33	61.11*	406.94	216.67
Error D x L	12	13.33	17.33	38.89	576.39	2516.67
Source of Variation	**DF**	**Panicle**	**Flowering**	**Milky Maturity**	**Waxy Maturity**	**Maturity**
Blocks	3	2155.56	1941.67	1488.89	646.31	385.33
Date of sowing (D)	2	2866.67	4205.56**	4310.22**	3493.39*	1648.39
Error D	6	1444.44	816.67	777.78	1464.61	1816.50
Lines (L)	2	466.67	105.56	288.89	115.06	44.22
Error L	6	377.78	583.33	511.11	508.94	463.33
D x L	4	166.67	344.44	361.11	394.44	476.78*
Error D x L	12	1722.22	1033.33	972.22	706.89	438.33

*: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$.

SD. A similar trend was observed for flowering. In comparison to the 85 DAE necessary for the flowering of the first SD, flowering occurred at 69 DAE for second SD, on April 20. A significant shortening in time for maturation, in comparison for the first SD, was observed for the second SD (-16 d) but above all for the third SD (-36 d).

This trend can be attributed to the fact that amaranth is a quantitative short-day species (Gimplinger et al., 2007). This means that it can flower in long photoperiod conditions, but starts the anthesis phase early when plants are exposed to shorter photoperiods. An anticipation or shortening in both flowering and maturation dates, similar to what is observed in this experiment, was in line as reported by Henderson et al. (Henderson et al., 1998). The same authors suggests that this tendency may also be attributed to the higher temperatures recorded during the delayed sowing dates, and an accelerated increase in temperature units. It was observed for species such as amaranth, in which the rate of development is temperature dependent (Gardener et al, 1991).

As regards the period between the emergence and the flowering, it must be pointed out that the first two SD benefited from an increasing photoperiod of 2.7 and 1.7 h, respectively. Simultaneously, all three amaranth lines, were on average, able to take advantage of GDD equal to 974, 671 and 1021 °C for the first, second and third SD respectively. The photoperiod from flowering to maturation decreased for all SD but this was particularly evident from the second (1305 °C) to the third (451-474 °C) SD.

All three SD permitted amaranth to attain to full maturation, even if with different yields, as will be discussed. The results of Zubillaga et al. (2019) were corroborated by the present work, reporting a minimum 1600-1700 GDD necessary for the completion of the crop cycle. In the present experiment, the third SD benefited from a minor GDD (1480 °C), thereby imposing a shortening of the phenological phases (Table 2). The present study verified *A. cruentus* as a species capable of attaining economically viable yields in a Mediterranean environment when compared to *A. hypochondriacus* (Casini and La Rocca, 2014). Excellent production yields were obtained under conditions of both 12 h of light (Bavec et Mlakar, 2002) and in the presence of less than 12 h, equivalent to just under three months of cultivation, as was reported by Wu et al. (2000) and Whithead et al.(2002) of which a minimum of 342 GDD was necessary to permit an efficient accumulation of biomass (biomass build-up) for harvesting (Nyathi et al., 2018).

The data shown in Figure 3 illustrates the development of the ground cover corresponding to the different phenological phases. At the four- and six-true leaf stages, the ground cover was greater in the third SD compared to the first SD by an average of 8 %. This is attributable to the faster plant growth due to the higher average temperatures in that period. This greater coverage of the terrain can certainly be useful for the crop, in order to

Table 3: Main growth stages, day length and Growing Degree Days (GDD) from emergence to flowering and from flowering to maturation

Lines	Date of sowing	Early panicle appearance (DAE)[1]	Flowering date (DAE)	Maturation date (DAE)	Day length from emergence to flowering (h)	Cumulative GDD[2] from emergence to flowering (°C)	Day length from flowering to maturation (h)	Cumulative GDD from flowering to maturation (°C)
A-61	March 27	67.5 a	85.3 a	150.0 a	12.3 – 15.0	957	15.2 – 12.9	1272
	April 20	52.0 bc	70.5 b	134.0 b	13.5 – 15.2	682	15.0 – 12.5	1305
	June 4	56.0 b	69.5 b	114.1 c	14.1 – 13.5	1006	13.5 – 11.3	474
Kharkov	March 27	65.5 a	84.0 a	150.3 a	12.3 – 15.0	974	15.2 – 12.9	1379
	April 20	49.8 bc	66.8 b	134.0 b	13.5 – 15.2	649	15.1 – 12.5	1353
	June 4	56.5 b	69.5 b	114.0 c	14.1 – 13.5	1029	13.5 – 11.3	451
A-67	March 27	66.0 a	85.8 a	150.0 a	12.3 – 15.0	992	15.2 – 12.9	1272
	April 20	52.0 bc	70.5 b	134.0 b	13.5 – 15.2	682	15.0 – 12.5	1305
	June 4	56.3 b	69.3 b	114.0 c	14.1 – 13.5	1029	13.5 – 11.3	451

Means followed by the same letter(s) are not different for $p \leq 0.05$; 1 DAE: Days After Emergence. 2 GDD: Growing Degree Days.

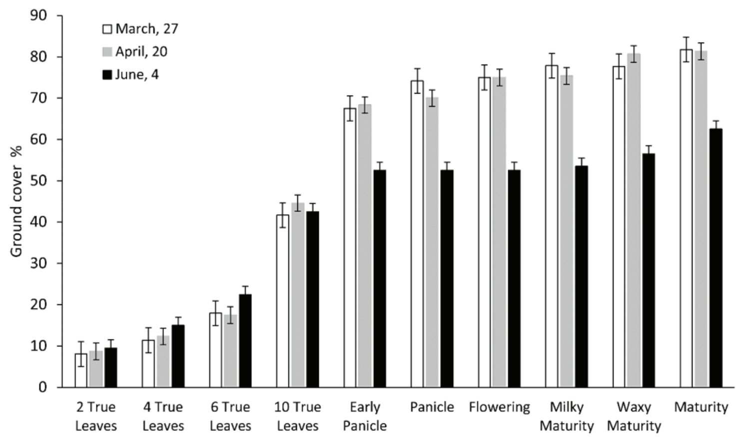

Figure 3: Ground cover according to the mean effect of the sowing date. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at $p \leq 0.05$.

exert a better and more precocious competition against weeds. However, starting from the formation of the panicle, ground cover is significantly lower for plants sown on the third SD. From the initial 52 % onto 63 % at full ripening, there is a corresponding 20 % less ground cover, compared to the mean of the first and second SD. The increasing temperatures, occurring in the last 5-6 phenological phases, reduced leaf development to permit the plants a tolerance to adverse climatic conditions. Similar results have been described by Zubillaga et al. (2019) in Argentina.

Particularly interesting are the data shown in Figure 4, pertaining to the presence of the sugar beet flea beetle in the early stages of development according to the SD. The trend regarding the presence of this insect, contrary to what one might think about a species equipped with masticatory apparatus, differs according to the varieties. Even though all varieties exhibit the same level of attack in the emergence phase, the incidence of insect attack increases drastically up to 80 % in the 'A-61' genotype coinciding with the four-true leaf phase, and up to 48 % in Kharkov. In the case of 'A-67', the incidence of

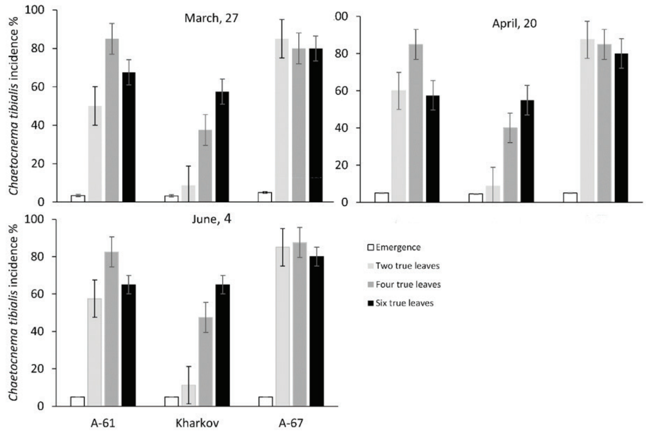

Figure 4: Incidence of sugar beet flea beetle (*Chaetocnema tibialis* Illiger) in different growth stages and date of sowing. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at $p \leq 0.05$

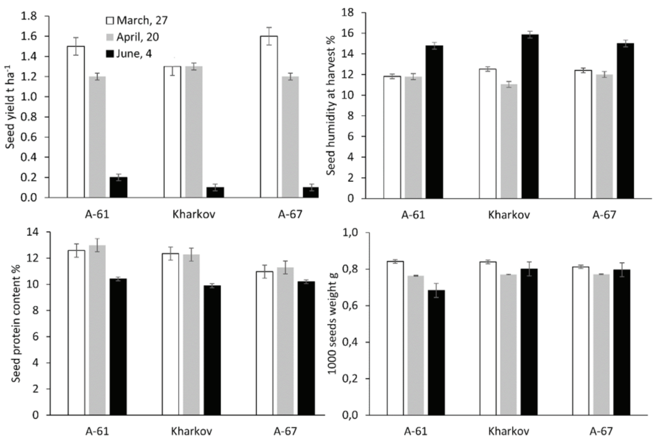

Figure 5: Seed yield, seed humidity at harvest and seed protein content of the varieties according to sowing date. Error bars represent the interval of the variability of the Tukey test. If the bars do not overlap, the difference between averages is significant at $p \leq 0.05$

the insect attack reaches 85 % at the two-true leaf phase, maintaining this level until the six-true leaf phase, after which treatment with deltamethrine was implemented. Genotype 'A-67' was more "palatable" to the insect from the earliest stages of growth. In contrast, 'Kharkov', was significantly less attacked by the insect over all three SD. This data is important, as amaranth is characterized by displaying a slow growth in the initial phenological phases. Hence, in the case of 'Kharkov', being less "palatable" for sugar beet flea beetle is an advantage in the early more delicate phases of the cultivation, even towards overcoming weed competition.

Amaranth cultivated in the Mediterranean area is generally characterized by a higher humidity levels at harvest, compared to the standard humidity of 12 %, necessitating drying to permit storage under safe conditions. Due to the climatic conditions in the present study the problem was reduced. However, Figure 5 showed significant (for $p \leq 0.01$) differences in seed moisture at the time of harvesting. Humidity was significantly higher in the third SD (15.5 %), compared to an average of 12 % for the remaining two SD. Higher humidity levels that exceed the standard, can be attributed to two concomitant factors. The first is the natural scalar maturation of the crop, also within the panicle of a single plant (acropetal trend). The second concomitant factor, relevant to the present experiment, was attributable to the delayed sowing that in time that hindered the complete filling of the seeds and the progressive loss of water. Seeds sown on the third SD were noted with many units of a darker, translucent endosperm, a sign of incomplete maturation and, therefore, a higher water content.

The decrease in yield in relation to the delay in SD compared to the one considered most suitable, was also reported by other authors, and in environments other than the Mediterranean (Henderson et al., 1998; Yarnia, 2010; Troiani et al., 2004). The best yields were obtained with the first SD (for 'Kharkov' also for the second). This is attributable to the non-excessively high temperatures in the pre-flowering phase, as well as the increasing photoperiods that likely favored a higher rate of photosynthesis and plant development, resulting in a greater accumulation of reserve substances.

The data on seed protein content (Figure 5) highlighted a 2.5-3.0 % reduction in e 'A-61' and 'Kharkov', from the first, and second to the third SD, respectively. A less sensitive reduction was observed for 'A-67'.

The mass of 1000 seeds was significantly reduced in relation to the delay in SD, only in the line 'A-61' (Figure 5). From the first to the third SD, there was a decrease from 0.842 g to 0.684 g. The same effect was observed by Chaudari et al. (2009), for *Amaranthus hypochondriacus.*

4 CONCLUSIONS

Results of this experiment clearly highlighted the high sensitivity of grain amaranth, to different sowing periods. With regard to the vegetative phase, an excessive delay in sowing, impacted negatively on ground cover and, therefore, on the potential competition against weeds. This trend is particularly negative during the first 30-35 DAE, as amaranth has a very slow growth, and is subject to the effects of competition. Moreover, the different palatability of the sugar beet flea beetle to the different accessions can be useful in the early stages of development of the culture. In fact, in the area where the present experiment was performed, the presence of this insect was manifested very early from the emergence of the cotyledonous leaves. Therefore, if not promptly controlled, the insect is able to inflict serious damage. In some years it was necessary to repeat the treatment with deltamethrine, also at the six-eight-true leaf stage.

The present research reported for the first time, the necessary GDD values required to attain good production results for amaranth in Italy. In this context (Central Italy) we can affirm that a GDD between 2100 and 2300 produces the best productive potential in *A. cruentus,* with completely ripe seeds and a lower harvest moisture content in comparison to later SD. The higher moisture content of the seeds of the third SD, would force the farmer to incur additional costs to reduce the values back to the standard 11-12 % requirement to ensure safe storage.

In conclusion, *A. cruentus*, introduced into the Mediterranean environment of central Italy with a March-April SD, can be considered a viable alternative crop, even under conditions of non-irrigated cultivation.

5 ACKNOWLEDGMENTS

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Študij polarnosti verig različnih viroidov in njihovih kombinacij pri okuženih rastlinah hmelja

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Študij polarnosti verig različnih viroidov in njihovih kombinacij pri okuženih rastlinah hmelja

Izvleček: Hmelj (*Humulus lupulus* L.) je pomembna kmetijska rastlina, ki jo pridelujemo zaradi hmeljnih storžkov, ki so osnovna sestavina pri proizvodnji piva. Hmelj pa je tudi gostiteljska rastlina štirim različnim viroidom. V naši raziskavi, kjer smo želeli proučiti naravo okuževanja s strani viroidov, smo analizirali nivo akumulacije in polarnost verig molekul viroidov. S podatki RNK sekvenciranja smo pokazali, da se viroidi v rastlini razmnožijo do ravni zasičenja, kar kaže na biološko kapaciteto rastline. Negativne spremembe na ravni akumulacije posameznih viroidov med vzorci hmelja z enojno, dvojno ali trojno okužbo kažejo na to, da je med viroidi prisoten antagonizem, pri čemer je viroid razpokanosti skorje agrumov najmanj, viroid zakrnelosti hmelja pa najbolj občutljiv na ostala dva. Kar se tiče polarnosti viroidne RNK molekule pa podatki RNK sekvenciranja kažejo, da je v povprečju več (−) oblik kot (+) oblik viroida. Pri tem najbolj izstopa viroid razpokanosti skorje agrumov. Pri rezultatih obratne transkripcije in verižne reakcije s polimerazo v realnem času pa glede polarnosti zaznamo variabilnost med različnimi kombinacijami okužb, razen v primeru viroida razpokanosti skorje agrumov, kjer se podatki o polarnosti skladajo s podatki RNK sekvenciranja.

Ključne besede: hmelj; viroidi; RT-qPCR; NGS; RNK sekvenciranje

Studying strands polarity of different viroids and their combinations in infected hop plants

Abstract: Hop plant (*Humulus lupulus* L.) is an important industrial crop, grown for harvesting hop cones however, it is a host to four different viroids as well. The nature of viroid infections is not entirely clarified. In our work, we focused on analyzing viroid accumulation and their strands polarity through RNA sequencing and reverse transcription polymerase chain reaction in real time. RNA-seq data indicate that viroids amplify until saturation further demonstrating plant's biological capacity. Negative fold changes in accumulation of individual viroids between hop samples with single and multiple infections are suggesting an antagonistic relationship amongst viroids, where citrus bark cracking viroid seems to be the least and hop stunt viroid the most sensitive to the other two. RNA-seq data also show that on average (−) viroid strand is dominating over (+), especially for the citrus bark cracking viroid. Reverse transcription polymerase chain reaction in real time results from strand polarity analysis seem to be less consistent between different combinations of infection but are showing level of conformity with RNA-seq in the case of citrus bark cracking viroid.

Key words: hop plant; viroids; RT-qPCR; NGS; RNA sequencing

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1 UVOD

Hmelj (*Humulus lupulus* L.) je pomembna kmetijska rastlina, ki jo gojimo zaradi hmeljnih storžkov. Ti se najpogosteje uporabljajo v pivovarstvu, učinkovine pa imajo tudi zanimive farmacevtske učinke. Hmelj napadajo različni patogeni mikroorganizmi, med njimi tudi štirje različni viroidi: viroid zakrnelosti hmelja (angl. hop stunt viroid, HSVd), hmeljev latentni viroid (angl. hop latent viroid, HLVd), viroid grbavosti jabolk (angl. apple fruit crinkle viroid, AFCVd) in nedavno potrjen viroid razpokanosti skorje agrumov (angl. citrus bark cracking viroid, CBCVd). Slednji na hmelju povzroča zakrnelost in odmiranje rastlin, zaradi česar so ukrepi na področju obvladovanja viroidnih okužb še toliko nujnejši. Poznavanje molekularnih mehanizmov rastlinske patologije je ključno za razvoj obrambnih strategij rastlin, zato smo v delu raziskovali naravo različnih kombinacij okužb viroidov HLVd, HSVd in CBCVd, pri čemer smo se osredotočili na analizo na ravni pomnoževanja viroidov v rastlinah in polarnosti molekul viroidov.

Viroidi so majhne, enoverižne molekule RNK z izrazito sekundarno strukturo, ki ne kodirajo nobenih proteinov (Diener, 1971), zaradi česar morajo za svoje biološke funkcije izkoristiti gostiteljeve molekule (Steger & Riesner, 2003). Njihov genom je dolg med 246 in 401 nukleotidi (Flores et al., 2005). Viroidi hmelja so pripadniki družine Pospiviroidae, za katere je značilna paličasta struktura molekule RNK in to, da podvojevanje poteka v jedru rastlinske celice (Flores et al., 2017). Po rastlini se širijo sistemsko in povzročajo bolezenska znamenja, ki so značilna tudi za virusne bolezni (Góra-Sochacka, 2004). Viroidi iz družine Pospiviroidae se podvojujejo na način kotalečega se kroga, po asimetrični poti, kjer se tvorijo dolgi multimeri negativnih (–) verig, ki se prepišejo v dolge multimere pozitivnih (+) verig. Monomerne oblike viroidov pa posledično nastanejo s cepitvijo dolgih multimernih (+) verig. Sledi tvorba zrele krožne monomerne molekule, ki je zadnji korak v podvojevanju.

Viroid zakrnelost hmelja (HSVd) so prvič odkrili pri hmelju na Japonskem (Sasaki & Shikata, 1977). Poleg hmelja okužuje tudi ostale rastline predvsem iz rodov *Vitis, Citrus* in *Prunus* (Kofalvi et al., 1997). O hmeljevem latentnem viroidu (HLVd) so prvi poročali Puchta et al. (1988). Za okužbo hmelja s tem viroidom je značilno, da ne povzroča hudih bolezenskih znamenj, vpliva pa na vsebnost nekaterih metabolitov v hmeljnih storžkih (Barbara & Adams, 2003). V slovenskih nasadih hmelja so Jakše et al. (2015) z uporabo tehnologije sekvenciranja naslednje generacije nedavno potrdili še okužbo z viroidom razpokanosti skorje agrumov (CBCVd). Duran-Vila & Semancik (2003) sta ga opisala kot himernega rekombinanta med vrstama viroid agrumov eksokortis (angl. citrus exocortis viroid, CEVd) in HSVd, kar bi tudi pojasnjevalo njegovo sposobnost okužbe hmelja (Jakše et al., 2015). Za omenjene viroide še ni bilo pokazano, da se prenašajo z vektorjem (Duran-Vila & Semancik, 2003), kar pomeni, da je v njihovo širjenje najverjetneje vpleten človek oz. agrotehnični ukrepi. Zaradi tega je za njihovo obvladovanje potrebno sledenje dobri kmetijski praksi, kar največkrat vključuje razkuževanje delovne opreme in orodja ter prilagojena agrotehnika pridelave. Za preprečevanje in omejevanje širjenja okužbe med rastlinami pa je nujno odstranjevati okužene rastline ali okužene dele nasadov in ponovno saditi zdrav sadilni material.

2 MATERIAL IN METODE

2.1 BIOINFORMATSKA ANALIZA

V raziskavi smo izvedli bioinformatsko analizo podatkov RNK sekvenciranja, ki smo jih pridobili v sklopu projekta »Analiza odziva rastlin ob hkratnih okužbah viroidov in identifikacija odpornosti – L4-6809«, ki sta ga izvedli sodelujoči organizaciji Inštitut za hmeljarstvo in pivovarstvo Slovenije in Oddelek za agronomijo, Biotehniška fakulteta, Univerza v Ljubljani. Sekvenciranje RNK je bilo izvedeno s tehnologijo Ion Torrent. Sekvenčne podatke, ki so dostopni preko SRA arhiva (bioprojekt s pristopno številko PRJNA528793) smo obdelali s programoma CLC Genomics Workbench (različica 12) in CLC Genomics Server (različica 11), kjer smo sledili standardni obravnavi sekvenčnih podatkov RNK sekvenciranja, začenši s kontrolo kakovosti odčitkov, s katero smo preverili skupno število odčitkov, njihovo dolžinsko razporeditev, vsebnost GC, morebitno nedoločitev baz (angl. ambiguity) in razporeditev kakovosti (angl. PHRED quality score). Za izvedbo smo uporabili orodje »QC for Sequencing Reads«. V drugem koraku smo filtrirali (angl. trimming) surove odčitke po dolžini tako, da smo zavrgli vse odčitke, ki so bili krajši od 35 bp. Izvedli smo tudi filtriranje po kakovosti z vrednostjo 0,05 in odstranili adapterska zaporedja tehnologije sekvenciranja Ion Torrent. V ta namen smo uporabili orodje »Trim Reads«. Na očiščenih zaporedjih smo ponovili kontrolo kakovosti in s tem dobili kakovostne odčitke, ki smo jih v nadaljevanju uporabili za kartiranje na referenčne genome viroidov, ki imajo v podatkovni zbirki NCBI naslednje pristopne številke: HLVd – NC_003611, CBCVd – KM211547 in HSVd – NC_001351. Uporabljena zaporedja so v analizi popolnoma enaka zaporedjem uporabljenim pri okuževanju rastlin z umetnimi kloniranimi konstrukti viroidov. Za kartiranje smo uporabili orodje »RNA-Seq Analysis«, kjer smo izpostavili tudi pozitivno orientiranost odčitkov. Na koncu smo izvedli še statistični test »Empirical Analysis of DGE«, ki temelji na negativni binomski porazdelitvi podatkov RNK sekvenciranja. Za grafični prikaz rezultatov pa smo odčitke normalizirali z metodo RPKM (angl. Reads Per Kilobase of transcript, per Million mapped reads) (Mortazavi et al., 2008).

2.2 ANALIZA Z OBRATNO TRANSKRIPCIJO IN VERIŽNO REAKCIJO S POLIMERAZO V REALNEM ČASU

Vzorce hmelja okuženega z različnimi kombinacijami viroidov smo ravno tako pridobili iz prej omenjenega projekta, rastline so bile vzorčene 28 mesecev po inokulaciji. Različni biološki vzorci pomenijo različna obravnavanja hmelja oz. okužbo z različnimi kombinacijami viroidov. Te so [1] = HLVd, [2] = CBCVd, [3] = HSVd, [4] = HLVd + CBCVd, [5] = HLVd + HSVd, [6] = CBCVd + HSVd in [7] = HLVd + CBCVd + HSVd. Vsako biološko obravnavanje smo testirali v treh bioloških ponovitvah. Izolacijo skupne RNK smo izvedli z uporabo komercialnega kita Spectrum™ Plant Total RNA Kit (Sigma-Aldrich), po navodilih proizvajalca. Po izolaciji smo z napravo NanoVue spectrophotometer (GE Healthcare Life Sciences) izmerili koncentracijo RNK in ocenili njeno čistoto. Razgradnjo molekul RNK smo ocenili na podlagi vrednosti RIN, ki smo jih določili z napravo Agilent Bioanalyzer 2100 (Agilent Technologies). Za oceno ravni pomnoževanja viroidov smo izvedli RT-qPCR v dveh korakih. Za oba koraka smo uporabili začetne oligonukleotide, ki so jih zasnovali Matoušek et al. (2017) in so prikazani v Preglednici 1, njihovo zaporedje pa je zapisano v orientaciji od 5' proti 3'. V prvem koraku smo izvedli obratno transkripcijo molekul viroidov tako, da smo specifično prepisali ali (+) ali (−) verige viroidov. Uporabili smo komercialni kit SuperScript™ IV First-Strand cDNA Synthesis Reaction (ThermoFischer) in sledili priloženemu protokolu. Pred izvedbo protokola smo izvedli denaturacijo sekundarnih struktur viroidov s 4 minutno inkubacijo pri temperaturi 98 °C. Z namenom relativne kvantifikacije okužbe smo pri vseh bioloških ponovitvah izmerili še nivo izražanja gena s konstantnim izražanjem, DRH1 (Štajner et al., 2013). V tem primeru smo za sintezo cDNK uporabili komercialni kit High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

V drugem koraku pa smo izvedli qPCR z uporabo Fast SYBR® Green Master Mix-a (Applied Biosystems) na napravi Applied Biosystems 7500 Fast Real-Time PCR System, pri čemer smo uporabili par začetnih oligonukleotidov specifičnih za prepisani (+) ali (−) verigi posameznega viroida oz. za gen DRH1. Program pomnoževanja je bil sestavljen iz več faz. Začetni denaturaciji 20 s pri temperaturi 95 °C, je sledilo 40 ciklov 3 s pri temperaturi 95 °C in 30 s pri temperaturi 60 °C. Na koncu je sledila še analiza talilnih krivulj, pri čemer je prvi korak trajal 15 s pri temperaturi 95 °C, drugi korak 60 s pri temperaturi 60 °C, tretji korak 15 s pri temperaturi 95 °C in zadnji 15 s pri temperaturi 60 °C. Na podlagi matematičnega modela (Pfaffl, 2012) smo izračunali relativne nivoje izražanja tarčnih genov z namenom, da smo lahko med seboj primerjali ravni akumulacije viroidov med različnimi biološkimi obravnavanji. Vrednosti Cq smo izmerili na istih vzorcih za posamezne viroide in za gen DRH1 s konstantnim izražanjem. Najprej smo iz dobljenih vrednosti Cq izračunali povprečje bioloških ponovitev, nato pa nadaljevali izračun relativnega izražanja z metodo ΔΔCq. Dobljene vrednosti smo uporabili za grafični prikaz. Glede na lastnosti pridobljenih podatkov poskusa smo za statistično testiranje uporabili robusten test ANOVA, ki na podlagi t-testa preverja ali sta povprečij dveh ali več populacij enaki. Zanj smo uporabili programski paket R (paket Rcmdr, različica 2.4-x).

3 REZULTATI IN DISKUSIJA

3.1 NIVO POMNOŽEVANJA VIROIDOV V VZORCIH HMELJA

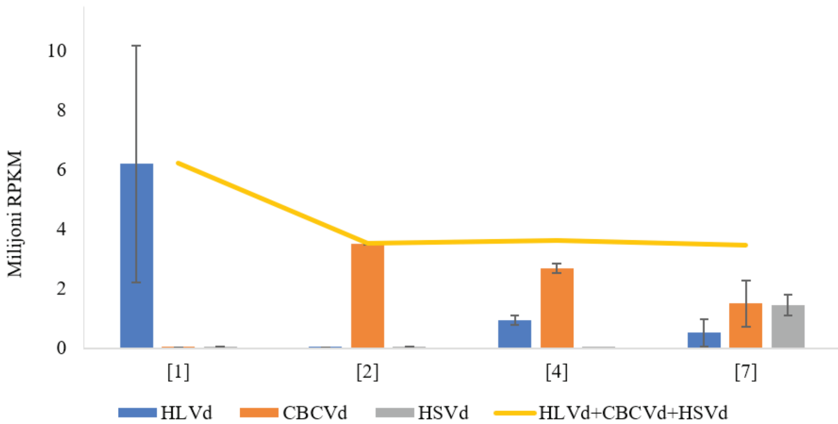
Rezultati filtriranja odčitkov so predstavljeni v Preglednici 2. Analiza je pokazala veliko kakovost surovih

Preglednica 1: Zaporedja začetnih oligonukleotidov uporabljenih v RT-qPCR v orientaciji 5'-3'
Table 1: Primer sequences used in RT-qPCR in 5'-3' orientation

	Viroid	Vodilni začetni oligonukleotid	Komplementarni začetni oligonukleotid
RT prepis	HLVd	ATCCCTCTTCGAGCCCTTGCCAC	GGATCCCCGGGGAAACCTACTCG
	CBCVd	GATCCCTCTTCAGGTATGTTCCCTCCTC	GGATCCCCGGGGAAATCTCTTCAG
	HSVd	GGCTCCTTTCTCAGGTAAGTCTCCTCCC	GGAGCCCCGGGGCAACTCTTCT
qPCR	HLVd	CGGCGACCTGAAGTTGCT	TTCCAACTCCGGCTGGTGT
	CBCVd	TCACTGGCGTCCAGCACC	AGGAAGAAGCGACGATCGG
	HSVd	CGGTGCTCTGGAGTAGAGGC	GTGATGCCACCGGTCGC

Preglednica 2: Povzetek rezultatov filtriranja NGS podatkov. Prikazani so podatki za povprečno število odčitkov, povprečno število nukleotidov in povprečno dolžino odčitkov na vzorec, pred in po filtriranju. Podatki so urejeni po bioloških obravnavanjih
Table 2: Summary of NGS data filtering showing average number of reads, average number of nucleotides and average read lengths per sample before and after trimming. Data are summarized according to biological treatments

Vzorec	Število odčitkov	Število nukleotidov [Gb]	Povprečna dolžina odčitkov [bp]	Število odčitkov	Število nukleotidov [Gb]	Povprečna dolžina odčitkov [bp]
	Pred filtriranjem			Po filtriranju		
[1] = HLVd	30.054.574	3,2	105,6	27.398.618	3,1	111,4
[2] = CBCVd	31.566.534	3,5	110,9	29.511.262	3,4	115,9
[4] = HLVd+CBCVd	21.152.456	2,1	99,5	20.288.798	2,0	100,8
[7] = HLVd+CBCVd+HSVd	23.860.218	2,6	109,7	23.339.936	2,6	110,4



Slika 1: Raven akumulacije viroidov na osnovi NGS analize pri različnih bioloških vzorcih. [1] = HLVd; [2] = CBCVd; [4] = HLVd in CBCVd; [7] = HLVd, CBCVd in HSVd. Rumena linija predstavlja seštevek vrednosti RPKM vseh treh viroidov. Intervali na stolpcih predstavljajo standardne odklone
Figure 1: NGS data showing viroid accumulation within different biological samples. [1] = HLVd; [2] = CBCVd; [4] = HLVd and CBCVd; [7] = HLVd, CBCVd and HSVd. Yellow line represents the sum of RPKM values of all three viroids. Intervals on the columns indicate standard deviation

Preglednica 3: Povzetek rezultatov testa EDGE za raven akumulacije posameznih viroidov
Table 3: Summary of EDGE test results for individual viroid accumulation

Viroid	Kombinacija okužbe 1	Kombinacija okužbe 2	Faktor spremembe	p-vrednost
HLVd	HLVd	HLVd+CBCVd	-1,29	0,11
	HLVd	HLVd+CBCVd+HSVd	-3,11	0,61
	HLVd+CBCVd	HLVd+CBCVd+HSVd	-1,28	0,84
CBCVd	CBCVd	HLVd+CBCVd	1,02	0,9
	CBCVd	HLVd+CBCVd+HSVd	-1,11	0,35
	HLVd+CBCVd	HLVd+CBCVd+HSVd	-1,4	0,1

podatkov. Vrednosti RPKM so prikazane na Sliki 1 in odražajo raven akumulacije viroidov pri različnih vzorcih analiziranih z NGS pristopom. Rezultate qPCR analize vzorcev hmelja pa smo pretvorili v relativne vrednosti tako, da lahko različne ravni akumulacije primerjamo med različnimi vzorci. Relativne vrednosti qPCR analize za različne vzorce hmelja so predstavljene na Sliki 2.

Iz grafa na Sliki 1 (NGS analiza) vidimo, da se skupna količina viroidov (rumena linija) skoraj ne spreminja med različnimi vzorci. To smo potrdili tudi s stati-

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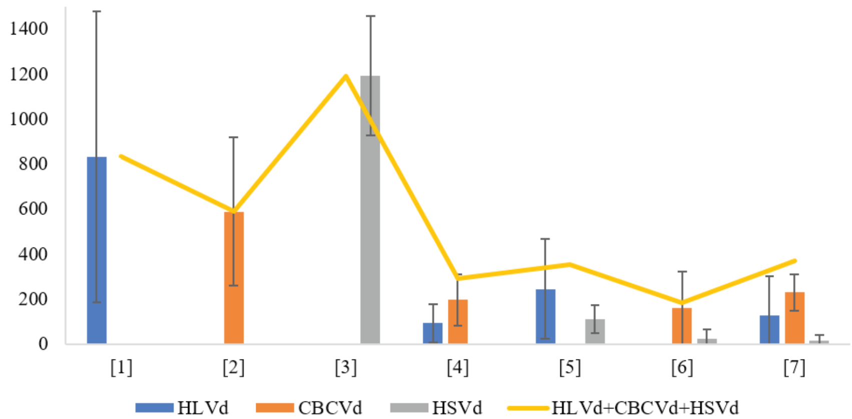


Slika 2: Raven akumulacije viroidov na osnovi RT-qPCR analize pri različnih bioloških vzorcih. [1] = HLVd; [2] = CBCVd; [3] = HSVd; [4] = HLVd in CBCVd; [5] = HLVd in HSVd; [6] = CBCVd in HSVd; [7] = HLVd, CBCVd in HSVd. Rumena linija predstavlja seštevek relativnih vrednosti ravni akumulacije vseh treh viroidov. Intervali na stolpcih predstavljajo standardne odklone
Figure 2: RT-qPCR data showing viroid accumulation within different biological samples. [1] = HLVd; [2] = CBCVd; [3] = HSVd; [4] = HLVd and CBCVd; [5] = HLVd and HSVd; [6] = CBCVd and HSVd; [7] = HLVd, CBCVd and HSVd. Yellow line represents the sum of relative quantities of all three viroids. Intervals on the columns indicate standard deviations

Preglednica 4: Povzetek rezultatov testa ANOVA za raven akumulacije posameznih viroidov
Table 4: Summary of ANOVA test results for individual viroid accumulation

Viroid	Kombinacija okužbe 1	Kombinacijaokužbe 2	Faktor spremembe	p-vrednost
HLVd	HLVd	HLVd+CBCVd	-9,04	0,15
	HLVd	HLVd+HSVd	-3,41	0,89
	HLVd	HLVd+CBCVd+HSVd	-6,66	0,01*
	HLVd+CBCVd	HLVd+HSVd	2,65	0,68
	HLVd+CBCVd	HLVd+CBCVd+HSVd	1,36	0,9
	HLVd+HSVd	HLVd+CBCVd+HSVd	-1,95	0,12
CBCVd	CBCVd	HLVd+CBCVd	-2,98	0,88
	CBCVd	CBCVd+HSVd	-3,72	0,12
	CBCVd	HLVd+CBCVd+HSVd	-2,57	0,97
	HLVd+CBCVd	CBCVd+HSVd	-1,25	0,77
	HLVd+CBCVd	HLVd+CBCVd+HSVd	1,16	0,1
	CBCVd+HSVd	HLVd+CBCVd+HSVd	1,45	0,59
HSVd	HSVd	HLVd+HSVd	-10,83	0,01*
	HSVd	CBCVd+HSVd	-49,63	<0,001***
	HSVd	HLVd+CBCVd+HSVd	-74,44	<0,001***
	HLVd+HSVd	CBCVd+HSVd	-4,58	0,03*
	HLVd+HSVd	HLVd+CBCVd+HSVd	-6,88	0,01*
	CBCVd+HSVd	HLVd+CBCVd+HSVd	-1,5	0,99

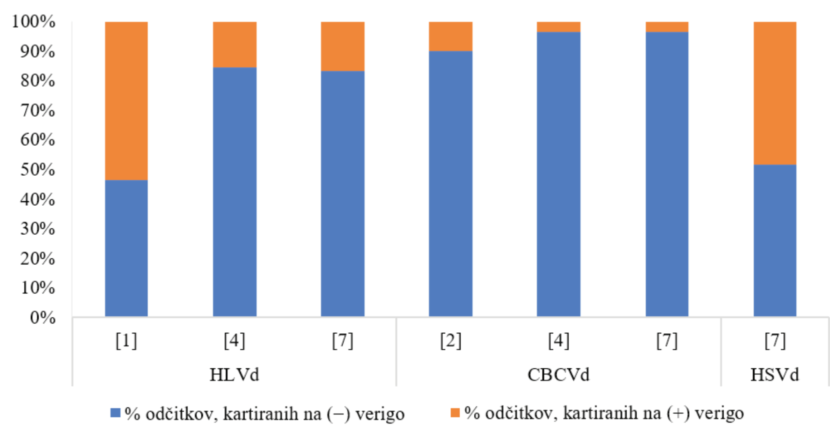
Zvezdica (*) je standardni prikaz programa R oz. tudi nekaterih drugih programov za statistično obdelavo podatkov. Predstavlja vizualno vrednost, saj nam v tabeli p-vrednosti pomaga, da hitreje opazimo tiste vrednosti, ki so statistično značilne. Število * (1, 2 ali 3) je vezano na število decimalnih mest, kjer se pojavi številsko mesto. 0,01 = *; 0,001 = **; 0 = ***
Asterisk (*) is a standard sign of R and other softwares for statistical analysis. It enables the user to quickly distinguish between the results that are either statistically significant or not. The number of * (1, 2 or 3) represents the number of decimal places before a numeric place

stičnim testom EDGE (angl. empirycal analysis of DGE) (Robinson & Smyth, 2008), ki je pokazal, da med vzorci ni statistično značilnih razlik na ravni kopičenja skupne količine viroidov. Ne glede na kombinacijo okužbe se viroidi razmnožijo do ravni zasičenja, kar kaže na omejeno biološko kapaciteto rastline. Opazimo tudi, da se med vzorci spreminja raven kopičenja posameznih viroidov. Tudi te razlike smo statistično ovrednotili, povzetek rezultatov pa je prikazan v Preglednici 3. Intervali na stolpcih predstavljajo standardne odklone in s tem biološko variabilnost med enakimi biološkimi obravnavanji.

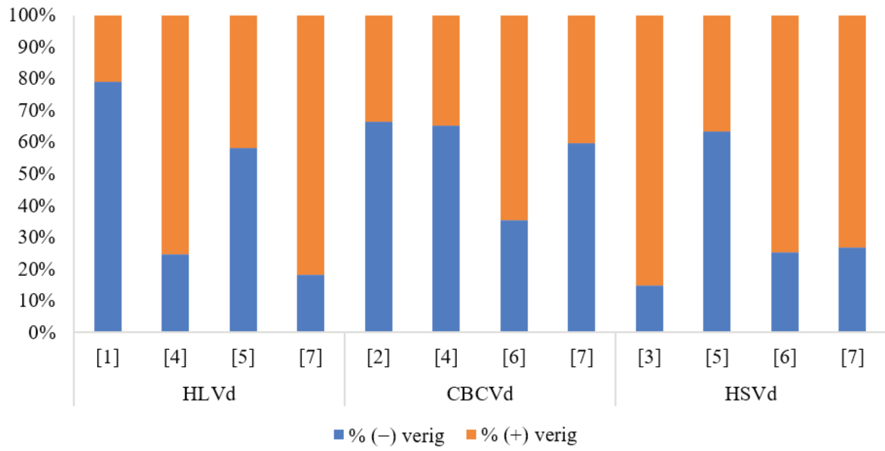
Kot lahko vidimo iz Preglednice 3, so skoraj vse spremembe v akumulaciji posameznih viroidov negativne, a v nobenem primeru ne gre za statistično značilno zmanjšanje. Na podlagi teh podatkov ne moremo zaključiti, da je med viroidi prisoten antagonizem, čeprav obstaja velika verjetnost, da so molekule viroidov v medsebojnih interakcijah. Glede na znane podatke iz literature domnevamo, da je vanje vpleten tudi rastlinski mehanizem RNK interference (RNKi) kot obrambe proti dvoverižnim RNK molekulam (Rahman et al., 2008; Pokorn et al., 2017).

Graf na Sliki 2 prikazuje rezultate pridobljene z RT-qPCR metodo, kjer smo preverjali raven količine viroidov v rastlini. Stolpci in rumena linija predstavljajo povprečno raven akumulacije posameznih oz. vseh treh viroidov pri različnih bioloških obravnavanjih. Glede na to, da smo izvedli relativno kvantifikacijo ravni pomnoževanja viroidov tako, da smo izmerili vrednosti Cq tudi za referenčni gen DRH1 s konstantnim izražanjem, imajo vrednosti na y-osi enoto 1. Skupna količina viroidov (rumena linija) med posameznimi biološkimi obravnavanji bolj variira kot pri NGS rezultatih (Slika 1). Koeficient variacije pri NGS analizi je bil samo 0,05, medtem ko pri metodi RT-qPCR 0,66. Iz grafa na Sliki 2 opazimo tudi, da se skupna količina viroidov zmanjša, kadar primerjamo okužbe s posameznim viroidom z okužbami z dvema ali tremi viroidi. S testom ANOVA smo najprej preverili ali so razlike med skupno količino viroidov med različnimi biološkimi obravnavanji statistično značilne. Izkazalo se je, da je prisotna mejno statistično značilna razlika samo med biološkima obravnavama HSVd ter CBCVd in HSVd (na sliki 2 z oznako [3] in [6]), pri vrednosti p = 0,0658. Pri vseh ostalih parnih kombinacijah okužbe pa razlika v skupni količini viroidov tudi ni statistično značilna. Po drugi strani pa statističnih razlik ne potrdimo že zaradi velike variabilnosti med biološkimi ponovitvami, kar je razvidno iz prikazanih intervalov na posameznih stolpcih. Da bi podprli to razlago, da viroidi dosežejo nivo zasičenja v rastlini ne glede na kombinacijo okužbe, bi morali analizo izvesti ponovno in sicer tako, da bi najprej izmerili količine viroidov v vzorcih ter v nadaljnjo analizo vključili le tiste, ki imajo med seboj primerljive količine virodov. S statističnim tesom ANOVA smo preverili tudi, kako se med biološkimi obravnavanji spreminja nivo akumulacije posameznih viroidov. Povzetek rezultatov testa ANOVA je prikazan v Preglednici 4.

Iz rezultatov testa ANOVA (Preglednica 4) vidimo, da niso vse spremembe ravni akumulacije viroidov negativne. Prisotne so tudi pozitivne spremembe, ki so sicer bistveno manjše, niso pa statistično značilne. V nasprotju z rezultati testa EDGE, ki je temeljil na NGS podatkih (Preglednica 3) smo pri viroidu HLVd pokazali na statistično značilno zmanjšanje samo v primerjavi okužbe HLVd z okužbo z vsemi tremi viroidi (HLVd, CBCVd in



Slika 3: Odstotek odčitkov kartiranih na (–) in (+) verigo pripadajočega viroida pri različnih bioloških vzorcih na osnovi NGS analize. [1] = HLVd; [2] = CBCVd; [3] = HSVd; [4] = HLVd in CBCVd; [7] = HLVd, CBCVd in HSVd
Figure 3: NGS data indicating the percentage of reads mapped to either (–) or (+) strand of each viroid within different biological samples. [1] = HLVd; [2] = CBCVd; [3] = HSVd; [4] = HLVd and CBCVd; [7] = HLVd, CBCVd and HSVd



Slika 4: Odstotek (–) in (+) verig pripadajočih viroidov pri različnih bioloških vzorcih na osnovi RT-qPCR analize. [1] = HLVd; [2] = CBCVd; [3] = HSVd; [4] = HLVd in CBCVd; [5] = HLVd in HSVd; [6] = CBCVd in HSVd; [7] = HLVd, CBCVd in HSVd
Figure 4: RT-qPCR data indicating the percentage of either (–) and (+) strands of each viroid within different biological samples. [1] = HLVd; [2] = CBCVd; [3] = HSVd; [4] = HLVd and CBCVd; [5] = HLVd and HSVd; [6] = CBCVd and HSVd; [7] = HLVd, CBCVd and HSVd

HSVd). To je posledica antagonizma, saj so viroidi v isti ekološki niši in uporabljajo iste omejene vire rastline. Antagonizem pa v tem primeru nastopi šele, kadar so v hmelju prisotni vsi trije viroidi. Pri viroidu CBCVd v nobeni parni kombinaciji nismo potrdili statistično značilne razlike, kar kaže na to, da je ta viroid najmanj občutljiv na prisotnost drugih dveh in s tem najbolj uspešen antagonist, kar morda razlaga tudi njegovo simptomatsko agresivnost (Jakše et al., 2015). Podobno kot v primeru viroida HLVd smo pri viroidu HSVd v skoraj vseh parnih kombinacijah potrdili statistično značilno zmanjšanje, kar ponovno kaže na interferenco med viroidi. Izjema je primerjava biološkega obravnavanja z viroidoma CBCVd in HSVd s trojno okužbo, kjer pa se raven akumulacije viroida HSVd praktično ne spremeni. Na podlagi tega sklepamo, da je viroid HSVd najbolj občutljiv, zlasti na viroid CBCVd.

Pri bioloških obravnavanjih s posameznim viroidom (na Sliki 2 z oznako [1], [2] in [3]) rezultati RT-qPCR kažejo, da je viroida HSVd največ, kar je zanimivo, saj je ta najmanj zastopan viroid v pridelavi hmelja v Sloveniji (Radišek et al., 2012). Glede na nekatere raziskave o medsebojnem vplivu patogenov v rastlini in podatke iz literature menimo, da igra rastlinska RNKi in ostali mehanizmi, ki vključujejo male RNK dinamično vlogo pri interakcijah med različnimi viroidi in hmeljem.

3.2 POLARNOST MOLEKUL VIROIDOV

Polarnost molekul nam med drugim podaja informacijo o aktivnosti podvojevanja viroidov v rastlinskih celicah. Pravzaprav prisotnost (–) oblik viroidov potrjuje aktivnost njihovega podvojevanja (Flores et al., 2017). Na Sliki 3 so prikazani sekvenčni odčitki, ki so se kartirali na (–) oz. (+) referenčne genome pripadajočih viroidov. Na podlagi tega lahko ocenimo razmerje med (–) in (+) polarnostjo molekul viroidov.

Iz Slike 3 najprej opazimo, da je v povprečju med vzorci razmerje med verigama pomaknjeno v smeri (–) verige, kar je zanimivo, saj je (+) veriga zrela oblika viroida in (–) veriga matrica za njen nastanek (Flores et al., 2017). Kot rečeno (–) verige kažejo na aktivnost podvojevanja, iz česar bi sklepali, da se viroid CBCVd najbolj aktivno podvojuje pri vseh vzorcih. Naši podatki pa podpirajo tudi rezultate dela Matoušek et al. (2017), ki so prvi pokazali na povečano količino (–) verige viroida CBCVd v primerjavi s (+) verigo pri okuženem hmelju. Pri viroidu HLVd opazimo spremembo razmerja polarnosti, če primerjamo enojno okužbo z dvojno ali trojno, kar bi lahko bila posledica povečanja aktivnosti podvojevanja.

Na Sliki 4 so prikazani rezultati RT-qPCR, kjer smo pomnoževali specifično (–) ali (+) verige viroidov.

Razmerje verig najbolj variira pri viroidu HLVd, saj dva vzorca kažeta na večji delež (–) verig, dva pa na večji delež (+) verig. Pri viroidih CBCVd in HSVd so trije od štirih vzorcev taki, ki kažejo na večjo količino ene od verig v primerjavi z drugo. Kar se tiče viroida CBCVd rezultati sovpadajo s podatki RNK sekvenciranja (Slika 3), čeprav (–) oblika ni tako močno prevladujoča nad (+) obliko. Rezultati kažejo, da metoda RT-qPCR ni najbolj ustrezna za tovrstni analizi, saj temelji na obratni transkripciji verig (Faggioli et al., 2017). Začetni oligonukleotidi za prepis (−) in (+) pa tudi ne diskriminirajo med monomernimi in multimernimi enotami viroidov, kar doprinese k variabilnosti rezultatov tako pri analizi akumulacije, kot tudi pri polarnosti. Zanimivo je, da nekateri podatki iz literature (Dadami et al., 2017) kažejo, da je pri viroidnih okužbah količina (+) verige večja od (−) oz. da je slednjo celo zelo težko detektirati. Feldstein et al. (1998) so v svoji raziskavi pokazali tudi, da je (+) oblika viroida vretenatosti krompirjevih gomoljev (angl. potato spindle tuber viroid, PSTVd) infektivna oblika viroida, saj z (−) oblikami niso uspeli okužiti paradižnika, zaradi česar bi pričakovali, da bo prisotna večja količina v (+) obliki v primerjavi z (−) obliko. To se kaže le v primeru viroida HSVd, kjer so bili 3 izmed 4 vzorcev taki, kjer se je pomnožilo več (+) kot (−) verig. Rastlinski obrambni sistem je morda bolj aktiven proti (+) oblikam, saj (−) ne predstavljajo neposredne grožnje, kar bi pri viroidu CBCVd pojasnilo to, da je razmerje pomaknjeno v smeri (−) oblike. Znano je, da dvoverižna RNK v celici vzbudi proces RNKi, v kateri sodeluje več proteinov (Rahman et al., 2008). Med njimi so pomembne endonukleaze oz. proteini podobni proteinom »Dicer« (angl. dicer-like proteins), ki med drugim delujejo tudi na molekule viroidov, pri čemer nastajajo od viroidov pridobljene male RNK (angl. viroid derived sRNA, vd-sRNA). Ti proteini pa delujejo tako na zrele molekule viroidov kot tudi tudi na intermediate podvojevanja (+) in (−) polarnosti. Glede na to, da so viroidi kljub aktivirani RNKi še vedno prisotni, pomeni, da so nanjo vselej vsaj delno odporni (Dadami et al., 2017). Možnost nekonstantnosti (+) in (−) verig pri mešanih okužbah bi lahko bila posledica mešanih viroidnih okužb. Le-te so s stališča polarnosti verig zelo slabo raziskane.

4 SKLEPI

Raziskave hkratnih okužb rastlin z viroidi so v začetkih, zato naše delo predstavlja izhodišče za nadaljnje raziskovanje rastlinske virologije. V našem delu smo analizirali nivo pomnoževanja in polarnost molekul viroidov pri različnih kombinacijah okužb hmelja. Na podlagi podatkov RNK sekvenciranja smo pokazali, da se viroidi podvojujejo do ravni zasičenja, kar kaže na biološko kapaciteto rastline. Žal tega na podlagi rezultatov qPCR nismo potrdili zaradi prevelike variabilnosti med biološkimi ponovitvami. V nadaljevanju priporočamo izvedbo analize RT-qPCR, tako da najprej izmerimo količino viroidov v vzorcih, nato pa v nadaljevanju vključimo samo tiste s primerljivimi količinami. Zmanjšanje količine posameznih viroidov kaže na prisotnost antagonizma med viroidi. Molekularni mehanizmi antagonizma ostajajo še nepojasnjeni. Menimo, da viroidi pridejo v interferenco tudi preko rastlinskih obrambnih sistemov, še posebej RNKi. Rezultati qPCR nam kažejo, da je viroid CBCVd najmanj občutljiv, viroid HSVd pa najbolj na prisotnost drugih viroidov. V sklopu analize polarnosti molekul pa podatki RNK sekvenciranja kažejo, da je v povprečju (−) verige več kot (+). Pri tem izstopa viroid CBCVd za katerega smo to potrdili tudi z analizo RT-qPCR. Za viroida HLVd in HSVd pa je značilna večja nekonsistentnost kar se tiče razmerja med (−) in (+) verigo, zaradi česar ostaja vprašanje polarnosti nepojasnjeno. V literaturi pa tudi ni podatka o dolžini (+) ali (−) intermediatov med podvojevanjem viroida. Menimo, da bi bilo potrebno uporabiti alternativno metodo, ki ne temelji na obratni transkripciji, ampak na neposrednem štetju molekul RNK. Tak princip uporablja tehnologija sekvenciranja Nanopore (Feng et al., 2015), kjer je možno izjemno dolgim odčitkom določiti nukleotidno zaporedje v enem koraku. S to metodo bi v teoriji lahko tudi določili dolžino intermediatov viroidov, ki nastanejo med podvojevanjem in odgovorili na vprašanje koliko monomerov sestavlja en multimerni intermediat.

5 ZAHVALA

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Cows Save the Planet: And Other Improbable Ways of Restoring Soil to Heal the Earth", by Judith Schwartz. Chelsea Green Publishing. ISBN: 9781603584326. Pp 240; USD 17.95

Paulina G. FLORES [1, 2]

In this first edition, the author Judith D. Schwartz brings a global vision to soil rehabilitation, based on her experiences of visiting farmers, ranchers, researches, and environmentalists. These groups all have a clear focus in common – they all believe in the importance of the organic matter in the soil to solve an ecological crisis.

The first chapter describes the excess of atmospheric carbon dioxide. The tagline of the communities working on soil carbon is: "Put the carbon back where it belongs". The chapter also exposes the fact that since 1850, twice as much atmospheric carbon dioxide has been derived from the burning of fossil fuels. The solution, in order to mitigate these emissions, is regenerative agriculture: building topsoil, encouraging the growth of deep-rooted plants, and increasing biodiversity. A key point is made concerning the "myth" of cattle methane emission. Methane is one of the biological consequences of plant growth while carbon dioxide is mainly a consequence of the combustion of fossil fuels.

Chapter 2 describes the importance of soil nutrient status for conventional agriculture in the United States and Australia. Macronutrients such as nitrogen, potassium, and phosphorus are part of soil improvement practices to increase crop productivity. However, leading Australian soil researcher Christine Jones says "it is all about building soil and soil carbon", which needs to be done via biological processes. According to Jones, 30 – 40 % of carbon fixed in green leaves can be transferred to soil and rapidly humified, resulting in rates of soil carbon sequestration in the order of 5 to 20 tonnes of CO_2 per hectare per year. This chapter also describes the global dependency on nitrogen fertilizers with the beginning of the green revolution in 1960.

Chapter 3 gives an overview of Drylands; the arid, semi-arid, and sub-humid areas with seasonal and unpredictable rains. Drylands are complex, delicate and vulnerable ecosystems. It is suggested that half of the armed conflict in the world can be in part attributed to environmental strains associated with dryland degradation. This chapter provides a framework for desertification driven by actions that have disturbed the life cycles of plant and animal species. Scientist Allan Savory's "Holistic Management" idea is also presented, which describes how grazing animals can serve as a tool for preventing or reversing the desertification process. The main topics covered include wildlife habitat, animal stock, selective grazing, herd behaviour, soil improvement, grass fires, and agroforestry.

In Chapter 4, the author explains how human activities have altered the ecological conditions that govern climate. There is a group of hydrologists and scientists in Slovakia and the Czech Republic who have written a "New Water" paradigm; to return carbon to the soil we need to return water to our soils. Their vision emphasises the importance of keeping water in the land. They have also tried to get international climate leadership attuned

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to water's role in addressing climate change. The chapter continues with the role of solar radiation on soil moisture depletion.

Chapter 5 describes the connection between soil and human health. Levels of key mineral nutrients – zinc, calcium, manganese, iron, and copper – in our food crops have declined by more than 50 percent over the last century. One of the major reasons for the diminishment of nutrients is declining soil quality, although the way we process, prepare, and transport food also plays a determinant role. French biochemist and farmer Andre Voisin proposes that any living cell, plant or animal, is a "biochemical photograph" of its environment. This theme is further discussed with soil microbiology, the chemical fertilizer role, and plant metabolites.

The importance of soil life biodiversity is discussed in the next chapter (6). The author uses the perspective of fertile soil and plant diversity to keep cattle, native fauna and pollinators happily sheltered and fed. This includes livestock and people as part of the diversity. As an example, there is a description in the chapter about how a farmer in North Dakota manages his farm using holistic planned grazing. He manages diversity by observing natural prairie. This chapter adds the perspective of ecologists and soil microbiologists regarding soil biota and all the energy transfers between the communities of organisms. There is reference to studies suggesting that the diversity of soil organisms in monocultures has declined, but on the other hand, how replacing annual crops with deep-rooted perennials tends to rebuild the organic matter, soil structure, and biological life. Following that, the author describes the soil megafauna, earthworms, and dung beetle. The chapter ends with a review of the negative effect of glyphosate over crops and soil life.

Chapter 7 gives an overview of commercially sold agricultural inputs and how the way these are applied may exacerbate soil micronutrient deficiencies. The use of chemical fertilizers and other treatments has increased worldwide as large tracts of agricultural land are turned over to commodity mono-crops. The author also discusses different points of view about toxicity and uses of glyphosate, and how it can affect soil life, wildlife and humans. The theme is continued with the question: "Who owns the soil? Many countries are finding it more economical to buy cheap agricultural land in places like Mozambique or Ethiopia. They can get more land, with cheap water and exporting in return. These agricultural land grabs are implicated in the increase of hunger in the third world, population displacement, broken communities and political corruption. This chapter also analyses the introduction of bio-fortified crops, human nutrient deficiencies, and chemical inputs.

Chapter 8 reviews Holistic Management (HM) implemented with reference to the scientist Allan Savory, who has inspired several farmers in the United States to implement HM principles. There is explanation of how in HM it is the livestock, through their behavior (grazing, trampling, feces, and urine) who set off multiple biological processes. Animal grazing has several impacts: grazing exposes the growth points of different plants to sunlight; trampling helps to decompose litter and break hardened soil, and the feces spread seeds. Chapter 8 also describes the pressure to make land profitable in the face of floods, drought periods, and human impact on the water cycle.

The ninth and last chapter of this book describes soil standards, where the economy and the environment are connected based on a set of principles such as energy, geographic expansion, rising population, natural resources, insurance programs, and subsidies. To improve soil health, it is necessary to keep livestock grazing on the land, regenerate resources and reduce soil fuel bills. Finally, the author offers a vision of forms of currency in nature, quoting Christine Jones: "Carbon is the currency for most transactions within and between living things", which is key for photosynthesis.

This book presents an overview of soil health, biodiversity, grazing, and climate change. It also shows the role of soil in the ecology and economy of the farm. The book will be very helpful to researchers, practitioners, and students working in agriculture and on environmental issues. In every chapter, the author reflects on her efforts dedicated to producing this outstanding work.

"Krave rešite planet: in drugi neverjetni načini za ohranitev tal pri zdravljenju Zemlje", Judith Schwartz. Chelsea Green Publishing. ISBN: 9781603584326. Pp 240; USD 17.95

V prvi izdaji avtorica Judith D. Schwartz prikaže globalno videnje za obnovo tal, ki temelji na njenih izkušnjah pri obiskih kmetov, raziskovalcev in okoljevarstvenikov. Te skupine imajo isti skupni cilj – vsi verjamejo v pomen organske snovi v tleh pri reševanju ekološke krize.

Prvo poglavje opisuje višek atmosferskega ogljikovega dioksida. Vodilo skupnosti, ki delajo na talnem ogljiku je: "Dajte ogljik tja kamor spada". Poglavlje izpostavlja dejstvo, da se je od leta 1850, vsebnost ogljikovega dioksida v zraku podvojila zaradi sežiganja fosilnih goriv. Rešitev za odpravo teh emisij je obnovitveno kmetijstvo, ki obnavlja vrhnjo plast tal, z vzpodbujanjem rasti rastlin, ki globoko koreninijo in povečevanjem biodiverzitete. Ključna točka je bila napravljena v zvezi z mitom o emisijah metana zaradi gojenja prežvekovalcev. Metan je ena izmed bioloških posledic rasti rastlin medtem, ko je ogljikov dioksid v glavnem posledica izgorevanja fosilnih goriv.

Poglavje 2 opisuje pomen talnih hranil za konvencionalno kmetijsko pridelavo v Združenih državah Amerike in Avstraliji. Makrohranila v dušikovih, kalijevih in fosforjevih gnojilih so del praks izboljšanja tal za povečanje produktivnosti kmetijskih rastlin. Vendar, kot pravi vodilni avstralski pedolog, Christine Jones, "vse je v gradnji tal in talnega ogljika", kar mora biti narejeno z biološkimi procesi. Po Jonesu, bi se lahko 30 – 40 % ogljika, ki ga vežejo zeleni listi premestilo v tla in hitro humificiralo, kar bi rezultiralo v sekvestraciji ogljika v tla v velikosti 5 do 20 ton CO_2 na hektar na leto. To poglavje opisuje tudi globalno odvisnost od dušikovih gnojil od začetka zelene revolucije v letu 1960.

Poglavje tri daje pregled suhih območij, aridnih, polaridnih, subhumidnih območij s sezonskim in nepredvedljivim deževjem. Predvideva se, da je polovica oboroženih spopadov na svetu posledica okoljskih razmer povezanih z degradacijo teh sušnih območij. Poglavje daje vpogled v potek desertifikacije, ki je posledica dejavnikov v motnjah življenskih ciklov rastlin in živali. Predstavljena je tudi ideja raziskovalca Allana Savoryja, avtorja dela "Holistično upravljanje " v katerem opisuje, kako lahko pašne živali služijo kot orodje za preprečevanje procesa desertifikacije ali celo povratnih procesov. Glavne teme, ki jih obravnava obsegajo habitate divjih živali, stalež živali, selektivno pašo, obnašanje čred, izboljšavo tal, travnate požare in kmetijsko gozdarstvo.

V poglavju štiri avtorica razloži kako so dejavnosti človeka spremenile ekološke razmere, ki uravnavajo podnebje. Skupina hidrologov in drugih znanstvenikov iz Slovaške in Češke ja napisala paradigmo "Nova voda" v kateri razložijo, da je za povratek ogljika v tla tlem potrebno vrniti vodo. Njihova vizija poudarja pomen zadrževanja vode v pokrajini. Skupina je poskušala prepričati mednarodno vodstvo o podnebnih spremembah o pomenu vode v njih. Poglavje se nadaljuje v pomenu sončevega sevanja na zmanjšanje vode v tleh.

Poglavje 5 opisuje pevazavo med zdravjem tal in zdravjem ljudi. Ravni ključnih mineralnih hranil kot so zink, kalcij, mangan, železo in baker so se v kmetijskih rastlinah za hrano zmanjšale v zadnjem stoletju za več kot za 50 odstotkov. Glavni razlog za upad teh hranil je zmajševanje kakovosti tal, čeprav imajo priprava, sestava in transport hrane tudi veliko vlogo. Francoski biokemik in kmet Andre Voisin je mnenja, da je vsaka živa celica, rastlinska ali živalska "biokemična fotografija" njenega okolja. Ta tema je nadalje obrvnavana glede na mikrobiologijo tal, vlogo mineralnih gnojil in rastlinskih metabolitov.

Pomen biodiverzitete tal je obdelan v poglavju 6. Avtor vidi perspektivo rodovitnih tal in bogastva rastlin v skupnem vzdrževanju domačih živali, samoniklega živalstva in med njimi opraševalcev. Ta princip vključuje vse rastline, živali in ljudi kot del raznolikosti (diverzitete). Kot primer je v poglavju opisan kmet iz Severne Dakote, ki upravlja svojo kmetijo s holistično zasnovano pašo. Raznolikost upravlja z opazovanjem naravne prerije. Poglavje dodaja perspektivo ekologov in talnih mikrobiologov glede talnih organizmov in vse prenose energije med združbami teh organizmov. Opisana je študija, ki kaže, da raznolikost talnih organizmov v monokulturah zmanjšana, vendar lahko z zamenjavo enoletnic s trajnicami, ki globoko koreninijo ponovno vgradimo v tla organsko snov in s tem izboljšamo strukturo tal in življenje v tleh. V povezavi s tem avtor opisuje talno megafavno, deževnike in hrošče govnače. Poglavje se konča s pregledom negativnih učinkov glifosata na posevke in življenje v tleh.

Poglavje 7 podaja pregled komercialno prodajanih kmetijskih sredstev in kako ta in na kakšen način njihova uporaba poveča pomanjkanje mikrohranil. Poraba mineralnih gnojil in drugih kemičnih pripravkov se je v svetovnem merilu močno povečala in velike površine kmetijske krajine so bile spremenjene v lahko upravljane monokulture. Avtor razpravlja o različnih vidikih toksičnosti uporabe glifosata, kako to vpliva na življenje v tleh, na druge divje živali in rastline ter človeka. Tema se nadaljuje s vprašanjem: "Kdo je lastnik tal? Mnoge države vidijo ekonomski interes v nakupu poceni kmetijskih zemljišč v državak kot sta Mozambik in Etiopija. Tako dobijo več zemlje s poceni vodo, kar si poplačajo z izvozom pridelkov. Te kraje kmetijske zemlje povzročajo lakoto v tretjem svetu, razseljevanje ljudi, razpad skupnosti in politično korupcijo. To poglavje analizira tudi uvajanje biološko izboljšanih kmetijskih rastlin, pomanjkanja hranil v prehrani ljudi in vnosu kemikalij.

Poglavje 8 daje pregled o holističnem upravljanju (Holistic Management, HM), ki je bilo uvedeno glede na dosežke znanstvenika Allana Savoryja,ki je navdušil številne kmete v Združenih državah, da prevzamejo ta način kmetovanja. Razloženo je, kako pri holističnem upravljanju živali s svojim obnašanjem (pašo, hojo, blatom in urinom) vplivajo na številne biološke procese. Paša živali ima številne vplive: objedanje izpostavlja rastne dele rastlin svetlobi; hoja živali pomaga k razgradnji opada in rahlja otrdela tla, z blatom se razširjajo semena. Poglavje 8 opisuje tudi napore, da bi naredili zemljišča bolj donosna v primeru poplav, sušnih obdobij in glede vpliva človeka na vodni cikel

Osmo in zadnje poglavje knjige opisuje standarde za tla, v katerih sta povezana ekonomija in okolje na osnovi nabora parametrov kot so energija, geografska ekspanzija, rast populacij, naravni viri, zavarovalniški programi in nadomestila. Za izboljšanje zdravja tal je potrebno na zemljiščih ohranjati pašo in s tem regenerirati resurse in s tem zmanjšati stroške za vzdrževanje tal. Na koncu ponuja avtorica vizijo v obliki valute v naravi, navajajoč Christine Jones: "Ogljik je valuta za večino transakcij v živih bitjih in med njimi", je tudi ključ za fotosintezo.

Knjiga predstavlja pregled o zdravju tal, biodiverziteti, paši in klimatskih spremembah. Pokaže tudi vlogo tal v ekologiji in ekonomiki kmetije. Knjiga bo v veliko pomoč raziskovalcem, praktikom in študentom, ki delajo na področjih kmetijstva in okolja. Vsa poglavja izražajo namen avtorice, da izpostavi pomen vseh obravnavanih tem.

Prevedel: prof. dr. Franc Batič

NAVODILA AVTORJEM

UVOD

Acta agriculturae Slovenica je četrtletna odprtodostopna znanstvena revija z recenzentskim sistemom, ki jo izdaja Biotehniška fakulteta Univerze v Ljubljani. Revija sprejema izvirne in še neobjavljene znanstvene članke v slovenskem ali angleškem jeziku, ki se vsebinsko nanašajo na širše področje rastlinske pridelave in živalske prireje in predelave. Zajema sledeče teme: agronomija, hortikultura, biotehnologija, fiziologija rastlin in živali, pedologija, ekologija in okoljske študije, agrarna ekonomika in politika, razvoj podeželja, sociologija podeželja, genetika, mikrobiologija, imunologija, etologija, mlekarstvo, živilska tehnologija, prehrana, bioinformatika, informacijske znanosti in ostala področja, povezana s kmetijstvom. Pregledne znanstvene članke sprejemamo v objavo samo po poprejšnjem dogovoru z uredniškim odborom. Objavljamo tudi izbrane razširjene znanstvene prispevke s posvetovanj, vendar morajo taki prispevki zajeti najmanj 30 % dodatnih izvirnih vsebin, ki še niso bile objavljene. O tovrstni predhodni objavi mora avtor obvestiti uredniški odbor. Če je prispevek del diplomske naloge, magistrskega ali doktorskega dela, navedemo to in tudi mentorja na dnu prve strani. Avtorji omenjenih del morajo biti tudi soavtorji članka, ki podaja izsledke dela. Navedbe morajo biti v slovenskem in angleškem jeziku, kadar so prispevki v slovenščini. V primeru člankov v slovenščini so dolžni avtorji priskrbeti angleški prevod naslova, izvlečka s ključnimi besedami in naslovov slik in tabel. Uredništvo revije zagotovi prevode izbranih bibliografskih elementov (naslova, izvlečka, opomb in ključnih besed) v primeru tujih avtorjev. Prispevke sprejemamo skozi celo leto samo preko spleta v OJS sistemu.

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Avtorji lektorirane prispevke oddajo v elektronski obliki na spletni strani OJS Acta agriculturae Slovenica.

AUTHOR GUIDELINES

INTRODUCTION

Acta agriculturae Slovenica is an open access peer-reviewed scientific journal published quarterly by the Biotechnical Faculty of the University of Ljubljana, Slovenia. The journal accepts original scientific articles from the fields of plant production (agronomy, horticulture, plant biotechnology, plant-related food-and-nutrition research, agricultural economics, information-science, ecology, environmental studies, plant physiology & ecology, rural development & sociology, soil sciences, genetics, microbiology, food processing) and animal production(genetics, microbiology, immunology, nutrition, physiology, ecology, ethology, dairy science, economics, bioinformatics, animal production and food processing, technology and information science) in the Slovenian or English languages. Review articles are published upon agreement with the editor. Reports presented at conferences that were not published entirely in the conference reports can be published. Extended versions of selected proceedings-papers can also be considered for acceptance, provided they include at least 30 % new original content, but the editorial board must be notified beforehand. If the paper is part of a BSc, MSc or PhD thesis, this should be indicated together with the name of the mentor at the bottom of the front page and will appear as footnote. Authors of mentioned thesises should be also coauthors of manuscript. Slovenian-language translation of selected bibliographic elements, for example the title, abstract, and key words, will be provided by the editorial board. Manuscripts are accepted throughout the year. Only online submissions are accepted..

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