121 • 1
2025

ACTA AGRICULTURAE SLOVENICA

Acta agriculturae Slovenica • eISSN 1854-1941 • 121 – 1 • Ljubljana, marec 2025

UNIVERZA V LJUBLJANI
BF Biotehniška fakulteta

Acta agriculturae Slovenica eISSN 1854-1941

Letnik / Volume 121 · Številka / Number 1 · marec / March 2025

Jezikovni pregled / *Proofreading*
Avtorji v celoti odgovarjajo za vsebino in jezik prispevkov / *The authors are responsible for the content and for the language of their contributions.*

Založnik in izdajatelj / *Publisher and Issuer*
Založila / Published by: Založba Univerze v Ljubljani / University of Ljubljana Press
Za založbo / For the Publisher: Gregor MAJDIČ, rektor Univerze v Ljubljani / the Rector of the University of Ljubljana
Izdala / Issued by: Univerza v Ljubljani, Biotehniška fakulteta / University of Ljubljana, Biotehnical Faculty
Za izdajatelja / For the Issuer: Marina PINTAR, dekanja Biotehniške fakultete UL / the Dean of the Biotehnical Faculty UL

Naslov Uredništva / *Editorial Office Address*
Univerza v Ljubljani, Biotehniška fakulteta, Acta agriculturae Slovenica
Jamnikarjeva ulica 101, SI-1000 Ljubljana

E-naslov / *E-mail*
Acta.Agriculturae.Slovenica@bf.uni-lj.si

Spletni naslov / *Web address*
https://journals.uni-lj.si/aas/

Acta agriculturae Slovenica izhaja samo kot spletna revija, skupni letnik pa praviloma obsega štiri številke. / *Acta agriculturae Slovenica is published only as an online journal with four issues per year in one common volume.*

Trenutno revija ne zaračunava stroškov za predložitev in obdelavo vključenih prispevkov. / *The journal does not charge APCs or submission charges.*

Acta agriculturae Slovenica izhaja s finančno pomočjo / *is published with the financial support:* Javne agencije za znanstvenoraziskovalno in inovacijsko dejavnost Republike Slovenije / *Slovenian Research and Innovation Agency*.

Acta agriculturae Slovenica je vključena v / *is included into*: COBISS, dLib, Scopus, DOAJ, CAB Abstracts, Google Scholar, Zoological Records.

Ovitek: Odrasel osebek japonskega hrošča (*Popillia japonica* Newman) v primerjavi s kovancem za 1 evro (Foto: Š. Modic)
*Cover: Adult Japanese beetle (*Popillia japonica* Newman) compared to a 1 € coin. (Photo: Š. Modic)*

Acta agriculturae Slovenica
Volume / Letnik **121** · Number / Številka **1 · 2025**

Table of Contents / Kazalo

Original Scientific Article / Izvirni znanstveni članek

Acta agriculturae Slovenica
Volume / Letnik **121** · Number / Številka **1** · **2025**

doi:10.14720/aas.2025.121.1.12524 Original research article / izvirni znanstveni članek

Adaptiveness, selection and nutritional value of clonal populations of *Allium sativum* L. ssp. *sativum* in the forest steppe of Ukraine

Viacheslav YATSENKO [1, 2], Nataliia YATSENKO [1], Serhii POLTORETSKYI [1], Ivan MOSTOVIAK [1], Nataliia POLTORETSKA [1], Oleh LAZARIEV, Vitalii LIUBYCH [1]

Received February 26, 2023; accepted December 16, 2024.
Delo je prispelo 26. februar 2023, sprejeto 16. december 2024

Adaptiveness, selection and nutritional value of clonal populations of *Allium sativum* L. ssp. *sativum* in the forest steppe of Ukraine

Abstract: The purpose of this study was to identify the degree of emergence and impact on the yield of weakened shoots of softneck forms of garlic and comprehensive study of the parameters of adaptive variability and selection value. During 2020–2022, nine local and introduced samples of garlic were studied in the field conditions. Research has established that the formation of a reduced flower-bearing shoot reduces the mass of the bulb by 7.6–31.1 %, and the yield by 6.1–38.6 %. The results indicate that the higher the relationship between the genetic and environmental coefficient of variation, the higher the heritability value. The following samples were selected as the starting material for further selection based on the yield: according to adaptability and ecological plasticity – Nos. 'A.s.16/16' and 'A.s.44/17'; in terms of stability – Nos. 'A.s.19/16', 'A.s.35/16' and 'A.s.43/17' and samples of the intensive type – 'A.s.16/16', 'A.s.27/16', 'A.s.33/16' and 'A.s.44/17', which will ensure high yields in optimal cultivation conditions. The obtained data will serve as the basis for the selection research scheme in the conditions of introduction in Ukraine. As a result of the research, a working collection of raw material was created for the selection of garlic.

Key words:: bulb mass, ecological variation, genetic variation, reduced scape, stability, yield

Prilagodljivost, selekcija in prehranska vrednost klonskih populacij česna (*Allium sativum* L. ssp. *sativum*) v lesostepju Ukrajine

Izvleček: Namen te raziskave je bil določiti velikost vznika in njegov vpliv na pridelek česna brez cvetočih poganjkov kot tudi obširnejša raziskava parametrov prilagodljivosti s selekcijsko vrednostjo. V rastnih sezonah 2020–2022 je bilo preučevanih devet lokalnih in tujih vzorcev česna v poljskih razmerah. V razsikavi je bilo ugotovljeno, da tvorba cvetočih poganjkov zmanjša maso čebulic za 7,6–31,1 % in pridelek za 6,1–38,6 %. Rezultati nakazujejo, da večje kot je razmerje med koeficientoma genetske in okoljske variabilnosti, večja je vrednost dedovanja. Naslednji vzorci so bili izbrani kot začetni material za bodočo selekcijo na osnovi pridelka: glede na prilagodljivost in ekološko plastičnost vzorca Nos. 'A.s.16/16' in 'A.s.44/17'; glede na stabilnost vzorci Nos. 'A.s.19/16', 'A.s.35/16' in 'A.s.43/17' ter vzorci intenzivnega tipa 'A.s.16/16', 'A.s.27/16', 'A.s.33/16' in 'A.s.44/17', ki zagotavljajo velike pridelke in optimalne pridelovalne razmere. Pridobljeni podatki bodo služili kot osnova za načrt selekcije pri uvajanju v razmere pridelovanja v Ukrajini. Kot rezultat raziskave je bila osnovana zbirka izhodiščnega materiala za selekcijo česna.

Ključne besede: masa čebulic, ekološka spremenljivost, genetska spremenljivost, zmanjšani cvetoči poganjki, stabilnost, pridelek

1 Uman National University of Horticulture, Instytutska, 1, 20301, Uman, Ukraine
2 corresponding author: slaviksklavin16@gmail.com

1 INTRODUCTION

The Alliaceae family, which includes staple crops such as onions, garlic, leeks, and chives, is the second most important family of monocots after the Poaceae. Garlic (*Allium sativum* L.) is the main crop of the family after onion (*Allium cepa* L.) (Benke et al., 2020; Khandagale et al., 2020) and was thought to have originated in Central Asia (Seung-Hyun et al., 2021). It is well known that garlic (*Allium sativum* L.) is one of the most important bulb vegetables and is mainly used as a spice or flavoring agent for food products. It is used in several types of products such as garlic oil, powder, salt, paste and flakes.

Garlic is less effective in genetic improvement than onions due to sexual sterility, and as a result does not produce true seeds, so bulbs are used for vegetative propagation (Tesfaye, 2021). The vast majority of the world's garlic genetic resources are non-flowering (Etoh and Simon, 2002). Garlic clones that do not produce scape are considered softneck, but hardneck types of garlic flower on rare occasions, but did not form seed ovaries due to underdeveloped gametophytes, which cause male and female sterility (Singh et al., 2018). As a result, garlic propagates only by cloves or air bulbils and has great difficulties with classical breeding methods (Benke et al., 2020). A study by Hirata et al. (Hirata et al., 2016), on garlic cultivars worldwide, finds a diversity of garlic phenotypes expressing a wide variety of traits such as bulb mass, number of bulbils per plant, bulb integuments, leaf length, false diameter stems, the number of leaves on a plant, ability to bloom, resistance to biotic stress and abiotic stress. The diversity of garlic varieties is an important basis for the creation and introduction of new garlic varieties for the efficient use of genetic resources and for the improvement of breeding programs. Garlic varieties often have specific physiological compatibility with specific agro-climatic conditions, resulting in a large number of different varieties (Mario et al., 2008). Accordingly, it seems necessary to choose more compatible and high-yielding varieties of garlic for the climatic conditions of Ukraine. The low productivity of garlic is a problem for all parties to develop cultivation technology in order to improve quality. The use of garlic cultivation technology that can increase productivity includes the selection of plant population varieties. At the moment, there is a selection of garlic to obtain high-yielding varieties that meet the existing requirements. Garlic breeding is necessary to obtain improved cultivars that are well adapted to local environmental conditions (Zheng et al., 2007).

One of the most important areas of selection of any crop, including winter garlic, is the detection of the reaction of plants to the environment, to its stressful conditions, determination of the level of reaction of plants to biotic and abiotic factors. In the process of growth and development, plants constantly interact with the environment, resulting in the process of adaptation of the organism. The process of adaptation never ends and takes place throughout the life of the plant. The basis of adaptation is variability, a property of the organism, reflecting the mechanisms of its interaction with the environment, it is the most important factor of evolution, which ensures the suitability of species and populations to changing environmental conditions. (Filipchenko, 1923). Variability characterizes the rate of reaction of a species to the influence of environmental factors, its ability to adapt. Hence the purpose of selection, according to E.H. Pivovarov. and Dobrutskaya M., consists in the creation of genotypes that possess the desired rate of variability (Pivovarov and Dobrutskaya, 2000). Currently, several forms of variability are distinguished: genetic (varietal), environmental, geographic, phenotypic. Many scientists note the primary importance for selection of the study of patterns of phenotypic variability. This is not an inherited variability, but it must be taken into account when obtaining varieties, since it makes it difficult to recognize valuable genotypes. The study of population composition of varieties and ontogenesis of plants, different morphobiotypes should be observed on different ecological backgrounds. Sudden changes in environmental factors, for example, photoperiodic or temperature regime, leading to the splitting of the population, which reveals variability in a number of characteristics and the possibility of isolating plant morphobiotypes within it, i.e., conducting selection (Sinskaya, 1963).

This study is devoted to the manifestation of reduced scape in garlic clonal populations, as it is known that this phenomenon significantly reduces the marketability of bulbs and garlic yield. Today, softneck varieties of garlic are of great interest among industrial producers, since the technology of their cultivation excludes a rather expensive item of expenditure – the removal of the scape, regardless of whether this technological operation is carried out manually or mechanized. Therefore, the main goal of this study was to evaluate the adaptive and productive potential of softneck collection samples of winter garlic and the prospects of their use in breeding programs for the climatic conditions of the Forest Steppe of Ukraine.

2 MATERIALS AND METHODS

In the course of 2020–2022, in the soil and climatic conditions of the Right Bank Forest Steppe of Ukraine, a study was conducted on the study of the adaptive

Table 1: Origin of research clonal populations of A*llium sativum* ssp. *sativum*

No. samples	country	district
A.s.1/16	Spain	Catalonia
Hloria	Ukraine	Zbarag
A.s.16/16	France	Cadours
A.s.19/16	Ukraine	Uman
A.s.27/16	Ukraine	Mankivka
A.s.33/16	Ukraine	Uman
A.s.35/16	Azerbaijan	Ağstafa
A.s.43/17	Ukraine	Uman
A.s.44/17	Ukraine	Uman

variability of the collection forms (clonal populations) of garlic on the experimental field of the educational and production department of Uman National University of Horticulture.

Collected specimens of different ecological and geographical origin with distinctive morphological features were used for the study which were selected by the expedition method, when surveying crops of landraces in peasant farms in the respective regions of Ukraine and Europe.

The establishment of experiments was performed by systemically design. Repetition of the experiment – four times. The accounting area one variant of the research land is 100 m^2. Garlic planting was carried out on October, 10–15 according to the 45 × 6 cm scheme. Garlic was harvested on June 20–July 2.

Data from Uman weather station served as the information base for the analysis of meteorological conditions during the years of the study (2020–2022). The course of agrometeorological factors over the years of research created suitable conditions for the growth and development of garlic plants.

The analysis of given data on air temperature and amount of atmospheric precipitation during the research period was generally characterized as favorable for the growth and development of garlic. A characteristic feature of the 2019–2020 agricultural year was the elevated temperature background, insufficient precipitation in the summer and autumn periods. A characteristic feature of the 2020–2021 agricultural year was a favorable temperature background and a sufficient amount of precipitation. The total amount of precipitation for the year was 655.7 mm, which exceeded the long-term average by 69 mm. The weather conditions of the 2021–2022 agricultural year were characterized by a significantly lower level of precipitation compared to previous years and multi-year average data, and the temperature regime was close to the multi-year average data (Fig. 1).

The weather conditions of the growing season of winter garlic in 2020–2022 were not the same, so the results of the study were evaluated objectively.

Biometric measurements and indicators of individual productivity were performed on 100 typical plants without repetitions.

The experimental design was a systematic design with four replicates.

2.1 GENETIC AND STATISTICAL PROCESSING OF THE RESULTS.

A large number of methods are used to assess adaptability. Most of them are based on the method of regression analysis, the mathematical model of which for determining the stability and plasticity of varieties was calculated according to Eberhart and Russell, and is also based on the principles of combining and transforming the effects of the environment and the interaction of the genotype with growing conditions. The coefficient of linear regression of yield of a variety shows its reaction to changes in growing conditions. The higher the value of the coefficient ($bi > 1$), the better the response of the variety. In the case of $bi < 1$, the variety reacts weakly to changes in environmental conditions. Under the condition that $bi = 1$, there is a complete correspondence of the change in the yield of the variety in accordance with the change in growing conditions. Nonlinear deviations from the regression line ($\sigma^2 d$ – stability). The lower the stability coefficient, the more stable the variety (Eberhart and Russell, 1966).

The general homeostaticity of varieties (H_{om}) was calculated according to the formula (Khangildin, 1984).

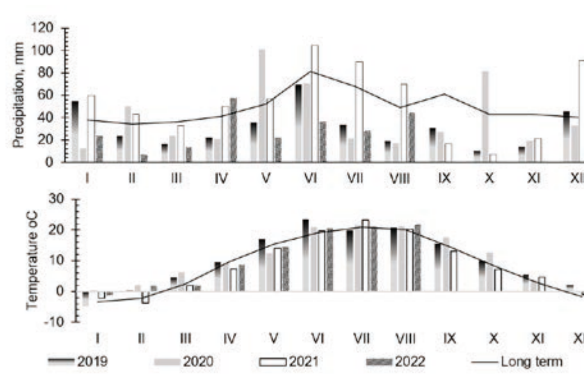


Figure 1: Climate chart for the study period (2020–2022)

$$H_{om} = \frac{\overline{X}^2}{\sigma},$$

where: x̄ – arithmetic average by grade;
σ – generalized root mean square deviation.
Breeding value of the variety:

$$(S_c) = \overline{X} \times \frac{\bar{x}_{lim}}{\bar{x}_{opt}},$$

where: $\overline{X}$ – arithmetic average by grade;
$\overline{X}_{lim}$ – limited arithmetic mean;
$\overline{X}_{opt}$ – optimal arithmetic mean.

Coefficient of multiplicity (CM). To avoid the linear artifact of the regression coefficient, V. A. Dragavtsev in 1981 introduced a new parameter - the coefficient of multiplicity, which allows comparing the variability of the trait. The higher the numerical value of this coefficient, the stronger the sign changes:

$$C_M = \frac{\overline{X}i + bi \cdot yi}{Xi},$$

where $\overline{X}$i – average value of the studied characteristic in the *i* variety;
bi – linear regression coefficient of *i* variety;
yi –average value for all averages for all grades *yi* for each *j* point of the experiment.

According to the method of A. O. Gryaznov, the average index of ecological plasticity is calculated

$$IEP = \frac{\left(\frac{YC_1}{AYC_1} + \frac{YC_2}{AYC_2} + \dots + \frac{YC_n}{AYC_n}\right)}{n},$$

where – YC_1, YC_2, YC_n value of trait (yield) in the variety in different years of trials; AYC_1, AYC_2, AYC_n –average value of quality of the varieties in each of variants of the experiment (Gryaznov, 1996).

Coefficient of adaptability (CA). To determine the adaptive capacity, the coefficient of adaptability of the variety (CA) was used.

The annual adaptability coefficient (CA) is calculated for the variety according to the formula (Zhivotkov et al., 1994):

$$CA = \frac{(Xij) \times 100 \times X)}{100},$$

where Xij – yield of a certain variety in the year of testing; X –average variety yield of the year.
The absolute average coefficient of adaptability (CAA) is calculated for the variety according to the formula:

$$CAA = \frac{(XiA) \times 100 \times Xm)}{100},$$

where XiA – average yield of the variety over years of testing, Xm –multi-year average variety yield.

Stress resistance and compensatory ability of varieties were determined by Rossielle and Hemblin (1981):

$$SR = Y_{min} - Y_{max};$$

$$CA = \frac{Y_{min} + Y_{max}}{2},$$

where Y_{min} and Y_{max} –minimum and maximum value of the variety characteristic.

The coefficient of variation is a relative value used to characterize the dispersion (variability) of a feature. It is the ratio of SD mean square deviation to the arithmetic mean, expressed as a percentage:

$$CV = \frac{SD}{X}.$$

Coefficient of variation on the following ratio scale:
CV < 10 % – variation is weak; CV 11–25 % – variation is average; CV > 25 % –variation is significant.

In the experiments, phenotypic, genotypic and ecological variability of varieties was determined (Burton et al., 1953; Shing, et al., 1993) according to the following formulas:

Genetic variance:

$$\sigma_G^2 = \frac{CM_p - CM_e}{r};$$

Environmental variance:

$$\sigma_A^2 = CM_e;$$

Phenotypic variance:

$$\sigma_F^2 = \sigma_G^2 + \sigma_A^2.$$

Coefficient of genotypic variation:

$$\frac{\sqrt{\sigma_G^2}}{\overline{X}} \times 100;$$

Coefficient of phenotypic variation:

$$\frac{\sqrt{\sigma_P^2}}{\overline{X}} \times 100;$$

Coefficient of ecological variation:

$$\frac{\sqrt{\sigma_A^2}}{\overline{X}} \times 100,$$

where CM_p –generalized root mean square value of the population trait; CM_e –generalized root mean square error, r – number of repetitions.

Heritability (h^2) narrow sense was calculated according to the following equation:

$$\frac{\sigma_G^2}{\sigma_A^2}$$

Heritability (according to Falconer, 1989) in a broad sense:

$$H^2_{Falconer} = \frac{\sigma_A^2}{\sigma_P^2}$$

The nutritional value. Proteins, fats, carbohydrates and ash content were determined by using standard methods described in the procedures of the American Organization of Analytical Chemists (International Organization of International, AOAC International) (Horwitz, Latimer, 2005). The crude fat was determined using a Soxhlet apparatus (Behr R 106 S, Germany) with petroleum ether, according to the AOAC 920.85 methodology (Horwitz, Latimer, 2016). The content of ash was determined by burning at 600 °C to constant mass following procedures AOAS 923.03 (Horwitz, Latimer, 2016). The energy was calculated by the formula:

The statistical processing of obtained results was carried out with the calculation of arithmetic mean (X) of the standard deviation (SD), calculated using Microsoft Excel 2019. Correlation dependencies were determined by using Statistica 10 Software.

To assess the quality of connection between dependent variable and factors in the correlation-regression model, we used the value of coefficient of the determination based on Chaddock scale.

3 RESULTS AND DISCUSSION

The coefficient of variation (CV) of the bulb mass in plants that formed a reduced peduncle and those that did not was at an average level – 16.9 and 17.7 %; the coefficient of variation of the environment (CVA) in the same variants was within high limits – 33.5 and 26.6 % (Table 2).

For bulb mass, the relationship between the coefficient of genetic and environmental variation (CVG/CVA) was significant (0.43 and 0.50) both in plants without a reduced scape and with its formation. For the yield trait, the relationship between the coefficient of genetic and environmental variation (CVG/CVA) was also noticeable (0.44 and 0.53), however, the coefficients of variation in garlic plants that formed a reduced scape were insignificantly higher (in terms of bulb mass and yield). The absence of a statistical error in samples No. 'A.s.19/16' and No. 'A.s.44/17' is explained by the fact that some of their plants formed a reduced scape only in 2020 (data not provided), Table 2).

According to Vencovsky (1992), high performance requires a CVG/CVA ratio close to unity or greater than unity, because in these cases, genetic variation is greater than environmental variation, indicating that selection for a given trait will have the best conditions with point of view of clonal selection.

The results shown in Table 1 indicate a low heritability of garlic, it is higher only in the case of shoot, which is caused by adverse environmental conditions in a specific year of testing. The given results indicate that the higher the relationship between the genetic and environmental coefficient of variation, the higher the value of heritability.

Samples No. 'A.s.16/16' and 'A.s.44/17' were characterized by a high mass of the bulb – 57.22 and 52.24, respectively, but they were unstable – $\sigma^2 d$ = 3.99 and 3.03. Samples numbered 'A.s.35/16' ($\sigma^2 d$ = 2.02), 'A.s.43/17' ($\sigma^2 d$ = 2.06) and 'A.s.19/16' ($\sigma^2 d$ = 2.18) with a bulb mass of 34.88–42.33 g were relatively stable. Cultivar 'Hloria' with a bulb mass of 38.15 g and trait stability of 1.93 (data not shown). Collection samples of softneck garlic were divided into three groups: I) – with a large bulb mass (<50 g) – samples numbered 'A.s.16/16' and 'A.s.44/17'; II) – average bulb mass (35–49 g) – cultivar

Table 2: Bulb mass and yield of softneck forms of clonal populations of garlic (X ± SD)

Sample	Mass of the bulb, g		Yield, t ha^{-1}		Number of cloves pcs. per bulb	
	WRS	RS	WRS	RS	WRS	RS
A.s.1/16	40.97 ± 7.31	37.83 ± 7.16	15.62 ± 2.00	14.28 ± 0.81	17 ± 4.1	8 ± 1.6
Hloria	38.15 ± 3.72	31.23 ± 9.57	14.68 ± 1.63	9.01 ± 7.17	13 ± 2.5	8 ± 0.8
A.s.16/16	57.22 ± 15.90	51.80 ± 19.92	19.09 ± 3.09	13.29 ± 3.11	16 ± 2.5	10 ± 0.9
A.s.19/16	42.33 ± 4.74	34.00 ± 0.00	14.83 ± 1.11	12.00 ± 0.0	19 ± 2.2	8 ± 0.9
A.s.27/16	34.87 ± 8.97	33.87 ± 8.49	14.71 ± 3.47	11.89 ± 1.84	14 ± 0.9	10 ± 1.6
A.s.33/16	36.72 ± 8.85	33.63 ± 7.53	14.63 ± 2.45	13.54 ± 1.94	13 ± 1.7	9 ± 1.2
A.s.35/16	38.42 ± 4.10	30.27 ± 9.43	14.82 ± 0.37	13.06 ± 3.99	18 ± 1.9	8 ± 1.7
A.s.43/17	34.88 ± 4.26	33.55 ± 2.57	14.63 ± 1.01	13.73 ± 0.51	22 ± 1.2	9 ± 1.2
A.s.44/17	52.24 ± 9.15	36.00 ± 0.0	19.11 ± 2.31	13.50 ± 0.0	19 ± 1.7	9 ± 0.9
$\bar{X}$	41.76 ± 6.97	35.80 ± 8.53	15.79 ± 1.13	12.7 ± 1.96	16.7	8.9
σ_G^2	123.8	143.4	7.9	13.6	1.7	0.5
σ_F^2	23.0	35.6	1.6	3.8	16.0	3.0
σ_A^2	146.7	179.0	9.4	17.4	14.3	2.4
h2	0.19	0.20	0.16	0.28	0.12	0.22
$H^2_{falconer}$	0.84	0.80	0.84	0.10	0.89	0.82
CVG, %	11.5	16.7	7.9	15.3	7.8	8.3
CVF, %	29.0	37.4	19.4	32.9	23.9	19.3
CVA, %	26.6	33.5	17.8	29.1	22.6	17.5
CVG/CVA	0.43	0.50	0.44	0.53	0.34	0.47

Note: WRS – plants that did NOT form a reduced scape; RS – plants that formed a reduced scape.

Hloria and samples numbered ‘A.s.19/16’, ‘A.s.33/16’, ‘A.s.35/16’; III) – with a small bulb mass (> 35 g) – samples numbered ‘A.s.27/16’ and ‘A.s.43/17’ (Table 2).

Research revealed a significant decrease in the number of clove in the bulb for plants that formed a reduced scape. On average, this indicator decreased from 16.7 pcs. to 8.9 pcs./bulb, which also affected the average mass of the clove, which on average increased in plants with a reduced scape to 4.08 g. However, statistical analysis showed that in plants that formed a reduced scape, the dependence of the number of clove on the genotype (CVG, %) increased, and on the contrary, it decreased on environmental conditions (CVA, %), compared to varieties that did not form a reduced scape. From which we can make an assumption that the researched garlic varieties will form a full-fledged scape in wild conditions. A strong linear dependence of the average tooth mass on their number in the bulb was also found, where $r^2 = 0.7285$ (Figure 2).

An increase (by 24–137 %) in the mass of the tooth was noted in plants that formed a reduced scape. The smallest difference in the change of this indicator was found in cultivar ‘Hloria’ and samples No. ‘A.s.16/16’, ‘A.s.33/16’ and ‘A.s.44/17’. The dependence between the coefficient of genetic and environmental variation (CVG/CVA) was noticeable (0.51), but too small to obtain high productivity (Figure 3).

Analyzing the adaptive capacity in terms of yield, samples numbered ‘A.s.16/16’ (19.09 t ha^{-1}, CA = 1.21) and ‘A.s.19/16’ (19.11 t ha^{-1}, CAA = 1.21) turned out to be high-yielding and adaptive, however they were unstable – σ^2d = 1.76 and 1.52 and were characterized as samples of the intensive type (bi = 15.4 and 1.71), that is, only with optimal provision of all factors, these samples will provide high productivity. As a result of the genetic and statistical

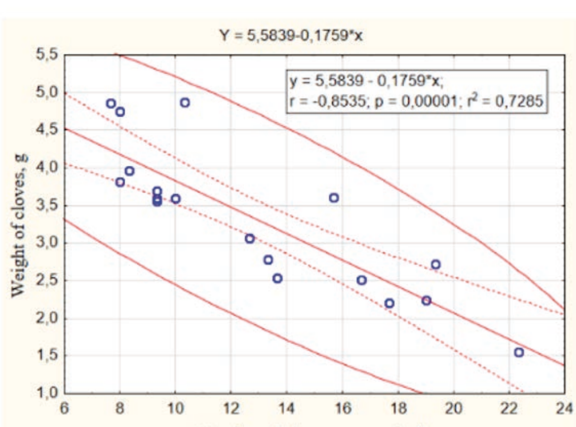
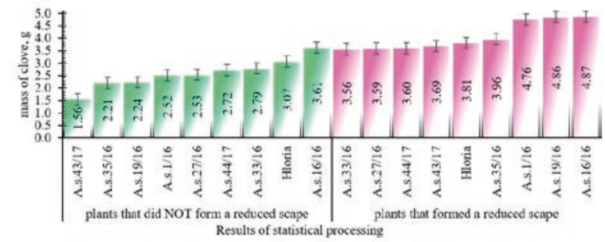


Figure 2: Point graphs and theoretical regression line for the linear correlation between clove weight and number of cloves



Results of statistical processing

	$\bar{X} \pm SD$	σ^2_G	σ^2_F	σ^2_A	CVG, %	CVF, %	CVA, %	CVG/CVA	h^2	$H^2_{Falconer}$
WRS	2.58 ± 0,54	0.075	0.595	0.520	10.6	29.9	27.9	0.38	0.30	*0.87*
RS	4.08 ± 0,54	0.373	1.788	1.415	15.0	32.8	29.2	0.51	0.44	*0.79*

Figure 3: The average mass of tof clonal populations of *Allium sativum* L. subsp. *vulgare* that did not form and formed a reduced scape (2020-2022)
Note: WRS – plants that did NOT form a reduced scape; RS – plants that formed a reduced scape.

analysis, the two most stable samples (σ^2d = 0.61 and 1.00) were selected – Nos. ‘A.s.35/16’ and ‘A.s.43/17’ with a yield of 14.82 and 14.63 t ha^{-1}. However, the ecological regression coefficient indicates their negative reaction to changes in external environmental factors (bi = 0.33 and 0.89) and weak adaptive capacity – CAA = 0.94 and 0.93.

According to yield, collection varieties of winter garlic were grouped as follows: high-yielding – Nos. ‘A.s.16/16’ and ‘A.s.44/17’; medium-yielding – cultivar Hloria and Nos. ‘A.s.1/16’, ‘A.s.19/16’, ‘A.s.27/16’, ‘A.s.33/16’, ‘A.s.35/16’ and ‘A.s.43/17’; stable yielders – Nos. ‘’ A.s.35/16, ‘A.s.43/17’ and cultivar Hloria (Table 3).

Conducting a regression analysis, the results of which are shown in Figure 4, showed a change in the dependence of the yield on the mass of the bulb. According to the obtained data, the relationship between the above indicators (according to the Chaddock scale) in plants that did not shoot was very strong – $r^2 = 0.8814$ and decreased to the level of «no connection» in plants that formed a reduced scape – $r^2 = 0,0772$.

With the introduction of local varieties (specimens) of garlic, the genotype is transferred from one zone to another, approaching or moving away from the center of origin, which can manifest itself in the emergence of full or weakened shoots or, conversely, the absence of scape in varieties that previously formed a full-fledged scape. For the most part, weakened shooting in softneck forms of garlic manifests itself under adverse weather conditions, in particular drought. The results of the research on the emergence of weakened shooting of garlic are shown in Table 4, indicating a significant differentiation of the samples according to this feature. Thus, among the researched collection samples of winter garlic, cultivar Hloria and samples No. ‘A.s.19/16’ and ‘A.s.44/17’ stand out with the lowest percentage of plants that formed reduced scape – from 0 to 2 % (by year)

Table 3: Parameters of adaptive capacity and breeding value of garlic plants that did not form a scape according to the trait "yield"

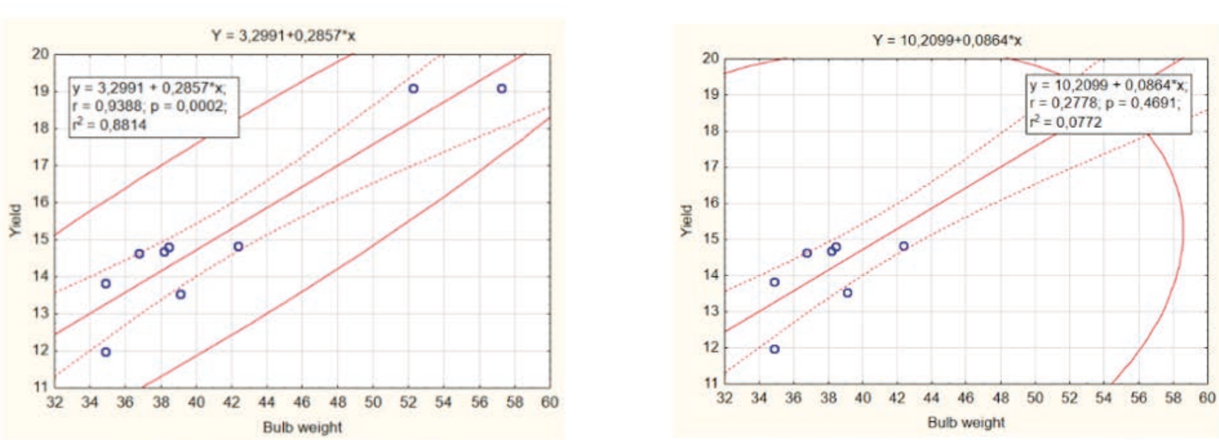
Sample	$\bar{X}$	CV, %	σ^2d	bi	Hom	Sc	CM	IEP	SR	CA	CAA
A.s.1/16	15.62	4	1.42	0.76	87.0	14.6	1.76	0.99	-5	31	0.99
Hloria	14.68	11	1.28	-0.23	76.8	13.7	0.75	0.94	-4	28	0.93
A.s.16/16	19.09	16	1.76	1.54	129.9	17.8	2.27	1.21	-8	38	1.21
A.s.19/16	14.83	7	1.05	-0.95	78.4	13.8	-0.01	0.95	-3	29	0.94
A.s.27/16	14.71	15	1.86	2.83	77.1	13.7	4.04	0.92	-7	27	0.93
A.s.33/16	14.63	17	1.57	2.12	76.3	13.6	3.29	0.92	-6	28	0.93
A.s.35/16	14.82	3	0.61	0.33	78.2	13.8	1.35	0.94	-1	30	0.94
A.s.43/17	14.63	4	1.00	0.89	76.3	13.6	1.97	0.93	-1	28	0.93
A.s.44/17	19.11	12	1.52	1.71	130.1	17.8	2.41	1.21	-5	40	1.21

Note: CV, % – coefficient of variation; σ^2d – stability; bi – coefficient of linear regression; Hom – homeostaticity; Sc – breeding value; CM – coefficient of multiplicity; *IEP* – index of ecological plasticity; SR – stress resistance; CA – compensatory ability; CAA – absolute average coefficient of adaptability.

and the weakest emergence of shoots – cultivar Hloria formed a reduced scape, which broke the pseudostem of the plant at a height of 2 to 4 cm and No. 'A.s.44/17', in which an underdeveloped inflorescence was visible at a level of 0 to 15 cm. Level 0 was taken to be the placement of an underdeveloped inflorescence under the covering scales of the garlic bulb, which is shown in Figure 5.

In general, based on the weather conditions of a specific growing year, it can be seen that in the years with less moisture supply and higher temperatures (2020 and 2022), the percentage of shoot plants and the degree of emergence of a reduced scape was the highest, which is confirmed by a higher level of environmental variation relative to genetic (CVG = 47.7 %; CVA = 154.2 %). Also, the results of statistical processing indicate the independence of this trait from the genotype, that is, a low level of inheritance (h^2 = 0.10), from which it can be concluded that the degree of emergence of a reduced scape with the formation of air bulbyls does not depend on varietal characteristics, only on the degree of selection of the variety and environmental conditions in which the phenotype was formed.

Analyzing the number of bulbils in the inflorescence, their strong variation by year is noticeable (CV = 58–73 %, data not shown). The obtained results also confirm that environmental conditions have a greater influence (CVA = 154.2 %) than genetic ones (CVG = 47.7 %) on the formation of this trait.. Samples Nos. 'A.s.33/16', 'A.s.35/16', and 'A.s.43/17' (5.6–6.4 pieces) formed a larger number of bulbils than the average indicator by 7.5–24.1 %. All other samples formed 0.8–23.0 % less bulbils



a) garlic plants without scape

b) garlic plants that formed reduced scape

Figure 4: Point graphs and theoretical regression line for the linear correlation between bulb mass and yield of garlic

Figure 5: Zero value of the emergence of weakened shoot of garlic plants, which formed a reduced scape

Table 4: The degree of emergence of weakened scape of clonal populations of garlic

Sample	Number of plants that formed r educed scape, %	Height at which of a reduced scape is formed, min–max, cm	Number of bulbils pcs. per plant (X ± SD)	Mass of 1000 pcs. of bulbils, g,
A.s.1/16	3	0–9	5.0 ± 1.41	1151.6
Hloria	1	2–4	2.7 ± 2.05	1042.0
A.s.16/16	8	0–15	4.3 ± 0.54	1225.7
A.s.19/16	1	0–6	0.0 ± 0.00	–
A.s.27/16	35	0–15	5.2 ± 0.64	1638.0
A.s.33/16	14	0–8	5.6 ± 1.23	1127.7
A.s.35/16	5	0–6	5.9 ± 0.66	966.3
A.s.43/17	4	3–11	6.4 ± 0.42	946.0
A.s.44/17	1	–	0.0 ± 0.00	–
$\bar{X}$	8.0	0–8	3.89	1156.8
σ_G^2	151.4		0.3	62994.1
σ_F^2	14.5		6.7	5232.6
σ_A^2	165.8		6.3	68226.7
h2	0.10		0.05	0.08
$H^2_{falconer}$	0.91	–	0.84	0.92
CVG, %	47.7		14.9	6.3
CVF, %	161.4		66.4	22.6
CVA, %	154.2		64.7	21.7
CVG/CVA	0.31		0.23	0.29

than the average value, and samples No. 'A.s.19/16' and 'A.s.44/17' did not form bulbils at all.

For a mass of 1000 bulbils, CV and CVA were at an average level. Based on the mass of 1000 bulbils, the correlation between the coefficient of genetic and environmental variation (CVG/CVA) was weak – 0.29. Regarding the mass of 1000 bulbils garlic plants presented a very low heritability – h^2 = 0.08, but the heritability in a broad sense is reliable for the purposes of comparing characteristics and the degree of emergence of the trait and for predicting the results of breeding studies (Vencovsky and Barriga, 1992). According to Stanfield (1971), traits are considered highly heritable at a level of heritability (h^2) greater than 0.50, medium heritability – 0.20–0.50, and low heritability – less than 0.20.

From the author's previously published data, it can be seen that the mass of 1000 bulbils depends on their number in the inflorescence. The number of bulbils of softneck samples depended to a greater extent on environmental conditions than on varietal characteristics, which, accordingly, affected the formation of the mass of 1000 pcs. (CVG = 6.3 %; CVA = 21.7 %). A high coefficient of ecological variation indicates the dependence of this indicator on the conditions of the environment in which it was formed.

Nutritional value of the studied garlic genotypes is presented in Table 5. The results show that, it can be seen that according to the set of indicators of the nutritional value , sample cultivar 'Hloria' stood out with an elevated content of protein, carbohydrates, with a low content of fat (including essential oil), which characterizes it as a table variety. The highest concentration of essential oil was noted in sample No. 'A.s.1/16', where this indicator prevailed over other varieties by 6.31–58.36 %, that is, according to this feature, it can be classified as a technical variety. The minimum accumulation of essential oil was in cultivar 'Hloria' – 0.26 ± 0.02 mg 100 g f. m.–1, which is 39.17 % less than the average value for all varieties, which allows it to be classified as a table variety – fresh consumption. Statistical processing of the data showed a strong influence of ecological growing conditions on the formation of this indicator. Yes, significant changes in the dynamics of essential oil accumulation were observed over the years, but intervarietal withdrawal was stable - if a variety had a high concentration of essential oil, it always had it. According to CVG and CVA, the dependence of indicators of the nutritional value on environmental growing conditions was revealed, which confirms the high plasticity of garlic culture.

Table 5: Content of essential oil and the nutritional value of clonal populations of garlic (X ± SD)

Sample	Essential oil, mg 100 g f. m.$^{-1}$	Protein, g 100 g f. m.$^{-1}$	Carbohydrates, g 100 g f.m.$^{-1}$	Fat, g 100 g f. m.$^{-1}$	Energy, kcal 100 g f. m.$^{-1}$
A.s.1/16	0.63 ± 0.02	5.28 ± 0.25	23.90 ± 0.29	0.58 ± 0.03	121.90 ± 1.74
Hloria	0.26 ± 0.02	5.88 ± 0.27	27.39 ± 0.39	0.29 ± 0.03	135.69 ± 1.71
A.s.16/16	0.37 ± 0.03	5.10 ± 0.41	22.90 ± 0.78	0.52 ± 0.01	116.64 ± 4.76
A.s.19/16	0.39 ± 0.02	5.30 ± 0.19	25.52 ± 0.95	0.28 ± 0.02	125.76 ± 4.55
A.s.27/16	0.32 ± 0.01	5.33 ± 0.09	26.53 ± 1.09	0.36 ± 0.02	130.70 ± 4.39
A.s.33/16	0.48 ± 0.03	5.38 ± 0.17	25.67 ± 0.41	0.30 ± 0.01	126.87 ± 1.97
A.s.35/16	0.56 ± 0.03	5.40 ± 0.08	24.70 ± 0.42	0.36 ± 0.02	123.68 ± 1.88
A.s.43/17	0.44 ± 0.02	5.50 ± 0.18	25.53 ± 1.28	0.46 ± 0.01	128.25 ± 5.88
A.s.44/17	0.59 ± 0.02	5.24 ± 0.33	24.80 ± 0.91	0.52 ± 0.05	124.82 ± 4.91
$\bar{X}$	0.43	5.38	25.22	0.41	126
σ_G^2	0.0002	0.10	2.2	0.01	
σ_F^2	0.0150	0.02	0.2	0.00	
σ_A^2	0.0148	0.12	2.5	0.01	
h2	0.01	0.04	0.05	0.26	
$H^2_{falconer}$	0.99	0.84	0.98	0.91	
CVG, %	3.1	2.59	1.8	3.5	
CVF, %	27.4	6.45	6.2	27.2	
CVA, %	27.2	5.90	5.9	26.9	
CVG/CVA,	0.12	0.44	0.31	0.13	

The presence of genetic variability in a culture is of key importance for sustainable agriculture, as the improvement of any culture is directly proportional to the value of its genotypic variability, the assessment of which is primarily necessary for its effective use, especially when old varieties are replaced by new ones. In general, garlic shows a good genetic dispersion in terms of the number and quality of traits, although it reproduces vegetatively (through cloves), taking into account the future threat of genetic erosion and the uncontrolled introduction of new varieties, an assessment of the adaptive variability of promising samples of softneck garlic was carried out according to the degree of emergence of a reduced scape, mass of 1000 bulbils, bulb mass, "yield". Prior to this study, many scientists (Kumar et al. 2019; Singh et al. 2014) investigated collections of varieties, local or elite lines separately, but the number of accessions was very much less.

The difference between garlic ecotypes was significant for all vegetative characteristics, which indicates their high diversity. Many studies (Stavělíková, 2008, Polyzos et al., 2019, Anderson et al., 2014; Kıraç et al., 2022) reported a large variation in the morphological characters of garlic, which partially or fully agreed with the results obtained in this study. Bahadur et al., (2018), Valter et al., (2019), Sánchez-Virosta et al., (2021) showed that the qualitative and quantitative characteristics of garlic directly depend on the location of the plants in the field, which, in turn, depends on the genotype (G), the environment (E) and the interaction of these two factors (G × E). Different ecotypes of garlic showed great diversity in morphological characters, including leaf size, plant height, bulb size, color and shape. Differences between researchers' results are explained by the large variation between groups of garlic, as well as the different effects of climatic conditions of the study site, especially temperature and length of daylight (Kamenetsky et al., 2004; Yatsenko, 2021).

Other researchers have reported significant differences in vegetative and reproductive characteristics of different garlic ecotypes (Figliuolo et al., 2001; Gvozdanovic-Varga et al., 2002; Zahedi et al., 2007; Sandhu et al., 2015; Sho et al., 2016). The difference in garlic bulb mass may be related to genetic diversity and the ability to adapt to environmental conditions (Abdel-Razzak and El-Sharkawy, 2012, Ganesh et al., 2022).

4 CONCLUSIONS

In general, after conducting a genetic and statistical evaluation of softneck collection samples of garlic, it was found that up to 21 % of plants at a height of up to 15 cm can form a reduced scape (obviously depending on the degree of selection). As a result of the evaluation of garlic samples, high-yielding (Nos 'A.s.16/16' and 'A.s.44/17'), stable yielding (numbers 'A.s.35/16' and 'A.s.43/17'), intensive (with bi >1 – 'A.s.16/16', 'A.s.27/16', 'A.s.33/16' and 'A.s.44/17'), selectively valuable (Nos. 'A.s.16/16' and 'A.s.44/17') and highly adaptive (Nos. 'A.s.16/16' and 'A.s.44/17'). The obtained results will serve as the starting material for further selection work with promising samples selected for a complex of economically valuable traits and the creation of domestic softneck forms of garlic

5 REFERENCES

Abdel-Razzak, H. S., & El-Sharkawy, G. A. (2012). Effect of biofertilizer and humic acid applications on growth, yield, quality and storability of two garlic (*Allium sativum* L.) cultivars. *Asian Journal of Crop Science*, *5*(1), 48–64.

Al-Otayk, S. (2008). Variation in productive characteristics and diversity assessment of garlic cultivars and lines using DNA markers. *Journal of King Abdulaziz University-Meteorology, Environment and Arid Land Agriculture Sciences*, *20*(1), 63–79.

Anderson, S., Cecon, P., Dias, C., Puiatti, M., Finger, F., & Carneiro, A. (2014). Morphological phenotypic dispersion of garlic cultivars by cluster analysis and multidimensional scaling. *Scientia Agricola*, *71*(1), 38-43. doi: 10.1590/S0103-90162014000100005.

Bahadur, B., Shree, S., Verma, R., Kumar, R., & Verma, R. (2018). Polygenic Variations and Character Association Studies in Garlic. *Current Journal of Applied Science and Technology*, *31*(3), 1–8. doi: 10.9734/CJAST/2018/45901.

Benke, A. P., Khar, A., Mahajan, V., Gupta, A., & Singh, M. (2020). Study on dispersion of genetic variation among Indian garlic ecotypes using agro morphological traits. *Indian Journal of Genetics and Plant Breeding*. *80*(1), 94–102. doi: 10.31742/IJGPB.80.1.12

Burton, G.W., & De Vane, R.W. (1953). Estimating heritability in tall Fescue (*Festuca arundinacea*) from replicated clonal material. *Agronomy Journal*, *45*, 478-481. http://dx.doi.org/10.2134/agronj1953.00021962004500100005x

Dragavtsev, V. A., Tsilke, V. A., & Reiter, B. G. (1984). *Genetics of traits of spring wheat productivity in Western Siberia.* Novosibirsk: Nauka. 229 p.

Eberhart, S. A., & Russell, W. A. (1966). Stability parameters for comparing varieties. *Crop Science*, *6*(1), 36–40.

Etoh, T., & Simon, P. W. (2002). Diversity, fertility, and seed production of garlic. In: Rabinowvitch, H.D., Currah, L. (Eds.), *Allium crop science: Recent advances* (pp. 101–117). Wallingford, UK: CABI Publishing.

Falconer, D.S. (1989). *Introduction to Quantitative Genetics, 3nd edn*. Longman Inc., New York. 448 p.

Figliuolo, G., Candido, V., Miccolis, V., & Zeuli, P.S. (2001). Genetic evaluation of cultivated garlic germplasm (*Allium* *sativum* L. and A. *ampeloprasum* L.). *Euphytica*, *121*(3), 325–334.

Filipchenko, Yu. A. (1923). *Variability and methods of its study*. Moscow: SPb, 235 p.

Ganesh, C., Hedau, N., Ram, H., Khade, Y., Kant, L., & Khar, A. (2022). Garlic: retrospect, status quo and dimensions. *Genetic Resources and Crop Evolution*, *69*(2), 1–16. doi: 10.1007/s10722-022-01439-x.

Gryaznov, A. A. (1996). *Karabalsky barley. Kustanai: Pechatnyy dvor*. 448 p.

Gvozdanović-Varga, J., Vasić, M., & Cervenski, J. (2002). Variability of characteristics of garlic (*Allium sativum* L.) ecotypes. *Acta Horticulturae*, *579*, 171–175. doi: 10.17660/ActaHortic.2002.579.26

Hirata, S., Abdelrahman, M., Yamauchi, N., & Shigyo, M. (2016). Diversity evaluation based on morphological, physiological and isozyme variation in genetic resources of garlic (*Allium sativum* L.) collected worldwide. *Genes & Genetic Systems*, *91*(3), 161–173. doi: 10.1266/ggs.15-00004.

Horwitz, W., & Latimer, G. (2005). Association of Official Agricultural Chemists (AOAC). *Official Methods of Analysis of AOAC International* (18th ed.). AOAC International, USA.

Horwitz, W., & Latimer, G. (2016). Association of Official Agricultural Chemists (AOAC). *Official Methods of Analysis of AOAC International* (20th ed.). AOAC International, USA.

Kamenetsky R., Shafir I. L., Zemah H., Barzilay A., & Rabinowitch H. D. 2004. Environmental control of garlic growth and florogenesis. *Journal of the American Society for Horticultural Science*, *129*(2), 144–151.

Karklelienė, R., Juskeviciene, D., Radzevičius, A., & Sasnauskas, A. (2018). Productivity and adaptability of the new carrot and garlic cultivars in Lithuania. *Zemdirbyste-Agriculture*. *105*(2), 165-170. 10.13080/z-a.2018.105.021.

Khandagale K., Krishna R., Roylawar P., Ade A. B., Benke A., Shinde B., & Rai A. (2020). Omics approaches in *Allium* research: Progress and wa ahead. *PeerJ*, *8*, e9824. DOI: 10.1266/ggs.15-00004.

Khangildin, V. V. (1984). Problems of selection for homeostasis and questions of the theory of the selection process in plants. *Breeding, Seed Production and Varietal AgriculturalTtechnology in Bashkiria. 1*, 102-123.

Kiraç, H., Dalda, Ş. A., & Coşkun, Ö.F. (2022). Morphological and molecular characterization of garlic (*Allium sativum* L.) genotypes sampled from Turkey. *Genetic Resources and Crop Evolution*, *69*, 1833–1841. https://doi.org/10.1007/s10722-022-01343-4.

Kumar, M., Sharma, V. R., Kumar, V., Sirohi, U., Chaudhary, V., Sharma, S., … Sharma, S. (2019). Genetic diversity and population structure analysis of Indian garlic (*Allium sativum* L.) collection using SSR markers. *Physiology and Molecular Biology of Plants*, *25*(2), 377-386. doi: 10.1007/s12298-018-0628-y.

Mario, P.C., Viviana, B.V., & María, G.A. (2008). Low genetic diversity among garlic (*Allium sativum* L.) accessions detected using random amplified polymorphic DNA (RAPD). *Chilean Journal of Agricultural Research*, *68*(1), 3–12.

Pivovarov, V. F., & Dobrutskaya, E. G. (2000). *Ecological bases of selection and seed production of vegetable crops*. Moscow. 591 p.

Polyzos, N., Papasotiropoulos, V., Lamari, F., Petropoulos, S., & Bebeli, P. (2019). Phenotypic characterization and quality traits of Greek garlic (*Allium sativum* L.) germplasm cultivated at two different locations. *Genetic Resources and Crop Evolution*, *66*(8), 10.1007/s10722-019-00831-4.

Rossielle, A. A., & Hemblin, J. (1981). Theoretical aspects of selection for yield in stress and non- stress environvents. *Crop Science, 21*(6), 27–29.

Sánchez-Virosta, Á., Sadras, V., & Sánchez-Gómez, D. (2021). Phenotypic plasticity in relation to inter-cultivar variation of garlic (*Allium sativum* L.) functional performance and yield-stability in response to water availability. *Scientia Horticulturae*, *285*, 110128. doi: 10.1016/j.scienta.2021.110128.

Sandhu, S. S., Brar, P. S., & Dhall, R. K. (2015). Variability of agronomic and quality characteristics of garlic (*Allium sativum* L.) ecotypes. *Sabrao Journal of Breeding and Genetics*, *47*(2), 133–142.

Seung-Hyun, C., Woo-Jin, S., Yeon-Sik, B., & Kwang-Sik, L. (2021). Determination of the geographic origin of garlic using the bioelement content and isotope signatures. *Food Control*, *130*, 108339. doi: 10.1016/j.foodcont.2021.108339.

Shing, M., Ceccarelli, S., & Hambling, J. (1993). Estimation of heretability from varietal trials data. *Theorical and Applied Genetics*, *86*, 437–441.

Singh, G., Ram, C.N., Singh, A., Shrivastav, S., Maurya, P., Kumar, P., & Om, S. (2018). Genetic variability, heritability and genetic advance for yield and its contributing traits in garlic (*Allium sativum* L.). *International Journal of Current Microbiology and Applied Sciences*, *7*(2), 1362-1372. doi: 10.20546/ijcmas.2018.702.165.

Singh, L., Koul, G., & Gohil, R. (2014). Analysis of morphological variability in the Indian germplasm of *Allium sativum* L. *Plant Systematics and Evolution*, *300*(2), 245–254.

Sinskaya, E.N. (1963). *The problem of populations in higher plants*. *Leningrad*: Sel'khozizdat Publ., *1963*, *124 p.*

Stanfield, W.D. (1971). *Genética. Teoría y 440 problemas resueltos*. Segunda ed. Serie Schaum, México: McGraw-Hill, 405 p.

Stavělíková, H. (2008). Morphological characteristics of garlic (*Allium sativum* L.) genetic resources collection - Information. *Horticultural Science*, *35*(3), 130–135.

Tesfaye, A. (2021). Genetic variability, heritability, and genetic advance estimates in garlic (*Allium sativum*) from the Gamo Highlands of Southern Ethiopia. *International Journal of Agronomy*. Article ID 3171642, 8 pages. https://doi.org/10.1155/2021/3171642

UPOV. (2001). Union for the protection of new varieties of plants. Guidelines for the conduct of tests for distinctness, uniformity and stability. *Garlic (Allium sativum* L.*)*, Geneva. 24 p.

Valter, C. de A. J., Guimarães, A. G., & Tiago, D. (2019). Associations between morphological and agronomic characteristics in garlic crop. *Horticultura Brasileira*, *37*(2), 204–209. doi: 10.1590/s0102-053620190211

Vencovsky, R., & Barriga, P. (1992). Genética Biométrica no fi tomelhoramento, Ribeirao Preto: *Revista Brasileira de Genética*, 496 p.

Yatsenko, V. V. Adaptive variability of winter garlic and biologization of growing technology. Dnipro: Serednyak T.K. 2021. 179 p.

Zahedi, B., Kashi, A., Zamani, Z., Mosahebi, G., & Hassani, H. (2007). Evaluation of Iranian garlic (*Allium sativum* L.) genotype using multivariate analysis methods based on morphological characters. *Biotechnology*, *6*(3), 353–356.

Zheng, S. J., Kamenetsky, R., Féréol, L., Barandiaran, X., Rabinowitch, H. D. Chovelon, V., & Kik, C. (2007). Garlic breeding system innovations. *Medicinal and Aromatic Plant Science and Biotechnology, 1*(1), 6–15.

Zhivotkov, L.A., Morozova, Z.A., & Sekatueva, L.I. (1994). Methodology for identifying the potential productivity and adaptability of varieties and breeding forms of winter wheat in terms of yield. *Selection and Seed Production, 2*, 3–6.

doi:10.14720/aas.2025.121.1.16386 Original research article / izvirni znanstveni članek

Combining ability of parental forms, inheritance of the trait of lycopene content in fruits of F1 tomato hybrids

Luidmila RUDAS [1], Mariia TORBANIUK [1, 2]

Received May 28, 2023, accepted December 31, 2024
Delo je prispelo 23. maj 2023, sprejeto 31. december 2024

Combining ability of parental forms, inheritance of the trait of lycopene content in fruits of F1 tomato hybrids

Abstract: Tomatoes contain many vitamins and minerals, and the antioxidant potential is provided by the carotenoid pigment lycopene. The main direction of our research is the improvement of the quality of tomato fruits, the assessment of the general and specific combining ability of five parental forms of tomato, and the establishment of the character of inheritance. Three medium-ripe lines (№492; №494; LK490) and two genotypes (Dark Green, T-3627) with mutant genes for increased lycopene content in fruits ($hp\text{-}2^{dg}$, B^{c}) were selected for diallel crossing (5 x 5). The conducted genetic analysis showed that the content of lycopene in tomato fruits is controlled by an additive-dominant genetic system. The main role in the genetic control of traits is played by the additive effects of genes, which allows selection by phenotype, starting from the second hybrid generation. Inheritance of lycopene content in tomato fruits occurs by the type of incomplete dominance. A high (positive assessment of the effects) of the general combining ability (GCA) according to the content of lycopene in tomato fruits over the three years of research had the Dark Green genotype (0.48-0.54), the LK 490 line (0.26-1.68), genotype T-3627 (0.38-1.09)–for two years.

Key words: tomato, combining ability, lycopene content in fruits, hybrid, breeding

Kombinacijska sposobnost starševskih oblik F1 križancev paradižnika za dedovanje lastnosti vsebnosti likopena vplodovih

Izvleček: Paradižnik vsebuje mnogo vitaminov in mineralov, njegov antioksidacijski potencial daje vsebnost karotenoida likopena. Glavni namen raziskave je bil preučiti možnost izboljšanja kakovosti plodov, oceniti sposobnost splošne in posebne kombinacijske sposobnosti petih starševskih oblik paradižnika in ugotoviti način dedovanja. Tri linije s srednjo zgodnostjo zorenja (№492; №494; LK490) in dva genotipa (Dark Green, T-3627) s spremenjenimi geni za povečanje vsebnosti likopena v plodovih ($hp\text{-}2^{dg}$, B^{c}) so bili izbrani za dialelno križanje (5 x 5). Genetska analiza je pokazala, da je vsebnost likopena v plodovih paradižnika uravnavana z aditivno-dominantnim sistemom dedovanja. Glavno vlogo pri uravnavanja te lastnosti imajo aditivni učinki genov, kar omogoča izbor fenotipov z začetkom v drugi generaciji križancev. Dedovanje vsebnosti likopena v plodovih paradižnika popteka z nepopolno dominanco. Veliko splošno kombinacijsko sposobnost (GCA), ocenjeno po pozitivnih učinkih glede na vsebnost likopena v plodovih je imel v treh letih poskusa genotip Dark Green (0,48-0,54), linija LK 490 (0,26-1,68) in genotip T-3627 (0,38-1,09) sta to izkazala v dveh letih.

Ključne besede: paradižnik, kombinacijska sposobnost, vsebnost likopena v plodovih, križanec, žlahtnenje

1 Cherkasy research station of the National Scientific Center «Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine», Dokuchaeva street, 13, Kholodnyanske, Cherkasy district, Cherkasy region, UA20731
2 Corresponding author, e-mail: mariyatr@ukr.net

1 INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most popular fresh vegetables on the world market and occupies a leading position in industrial production (Costa & Heuvelink, 2018; Selvaggi et al., 2021). The health benefits of tomatoes are confirmed by scientific research and human experience. They are the main source of the antioxidant lycopene, which helps in the treatment of many diseases, and also contain easily digestible carbohydrates, pectin substances, rich in vitamins (Poobalan et al., 2019; Madia et al., 2021; Akbari et al., 2022). They have antibacterial and anti-inflammatory effects, regulate the work of the nervous system, they contain a certain amount of phytoncids (Raiola et al., 2014; Sachdeva et al., 2020). Not only this, but they are the main raw material for the cannery industry, as well as active consumption in fresh form for salads and other dishes (Wang et al., 2022; Meng et al., 2022).

Lycopene is a powerful antioxidant and phytonutrient which plays a major role in human life (Arain et al., 2018; Liang et al., 2019; Imran et al., 2020; Li et al., 2021). It has a positive effect on the body's metabolic processes, activates brain activity, normalizes digestive tract activity, improves liver, kidney and genital functions (Sun et al., 2021; Arballo et al., 2021; Abenavoli et al., 2022). According to research, the bioavailability of lycopene in heat-treated tomatoes (tomato pastas, sauces, ketchups) increases compared to fresh tomatoes, which makes it particularly useful for processing (Vitucci et al., 2021; Wu et al., 2022; Tchonkouang et al., 2022).

Currently, there is a worldwide trend towards a healthy diet that is increasing demand for quality products, including tomatoes. The creation of varieties and hybrids with an increased content of lycopene is important to ensuring consumer demand. Our research aims to involve accessions with *hp-2*^{dg}, *B*^{c} mutant genes in the breeding process schema, that provides high-value genotypes for future work. The prospects of this direction of research are determined by the successful expansion by scientists of the gene pool of tomato varieties and hybrids with an increased content of lycopene in the fruits.

2 MATERIALS AND METHODS

The basis of our research was the identification of parental forms with high combining ability and carrying out genetic analysis to establish the parameters and character of inheritance of lycopene content in tomato fruits.

The research was carried out in 2017-2019 at the experimental field of the Cherkasy research station of the National Scientific Center «Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine» (Kholodnyanske) (49^{0} N at 31^{0} 52' E), Ukraine.

The basic material for the study was five parent forms: lines №492, №494, LK490, genotypes Dark Green, T-3627 and twenty F_1 hybrids obtained according to the complete diallel scheme. Experiments were conducted according to the one-factor method of Dospekhov (1985). Analysis of data on lycopene content in fruits was carried out using diallel and genetic analysis (Yates, 1947; Hayman, 1954; Jinks, 1954; Fedin, 1970). Combining ability (general and specific) was determined according to the first scheme of Griffing (1956).

Determination of lycopene content in tomato fruits was carried out according to the simplified method of determination of lycopene by I.K. Murry "Methods of biochemical research of plants" (Ermakov et al., 1952). Tomato fruits for research were selected in the phase of full ripeness. Powdered anhydrous sodium sulfate Na_2SO_4 was used as an adsorbent. Extraction of lycopene from plant material was carried out with a mixture of hexane-acetone (96:4). Indicators were determined on a spectrophotometer using two scales, 451 and 503. Statistical analysis of the results was performed according to the methodology formula in three repetitions (Barrett & Anthony, 2000; Brandt at al., 2003; Anthony & Barrett, 2006; Alda at al., 2009).

3 RESULTS AND DISCUSSION

Over three years of research, significant differences between the options have been established, one can expect unequal content of lycopene in fruits, differences in the combining ability (general or specific) of the studied parental components (Table 1).

It was established that high lycopene content was in LK490 line (4.7-11.0 mg 100 g^{-1}), Dark Green genotype (6.6-10.6) and T-3627 (5.6-8.1 mg 100 g^{-1}). The lowest content in line №492 (1.7-4.0) and №494 (2.1-4.2 mg 100 g^{-1}) (Table 2).

Average indicators of lycopene content in hybrids with the participation of the LK490 line (3.2-8.2 mg 100 g^{-1}), Dark Green genotype (3.5-7.1) and T-3627 (3.4-6.7 mg 100 g^{-1}) exceed the average group indicators for parental components. At the same time, hybrids with the participation of lines №492 and №494 showed lower indicators compared to the average group. The difference between the average indicator of F_1 and the average indicator of the original forms is negative (during the three years of research), that is, the lower expression of the trait dominates.

The conducted analysis of variance of combining ability (Table 3) indicates reliable differences in general

Table 1: Analysis of variance of lycopene content in tomato fruit

Years	Type of scattering	Sum of squares	Degree of freedom	Middle square	F calc.	F табl.
2017	General	342.7	74			
	Repetitions	0.2	2			
	Options	333.7	24	13.9*	76.4	1.74
	Residual	8.7	48	0.2		
	LSD_{05}			0.7		
2018	General	142.3	74			
	Repetitions	1.1	2			
	Options	124.4	24	5.2*	14.8	1.74
	Residual	16.8	48	0.4		
	LSD_{05}			1.0		
2019	General	169.5	74			
	Repetitions	2.1	2			
	Options	142.7	24	6.0*	11.5	1.74
	Residual	24.7	48	0.5		
	LSD_{05}			1.2		

* Significant at 5 % level

Table 2: Average value of lycopene content in tomato fruits in the parental lines, genotypes, ($^{\bar{x}}P$) and hybrids ($^{\bar{x}}F_1$), mg 100 g^{-1}

Lines, genotypes	Years of research					
	2017		2018		2019	
	P	F1	P	F1	P	F1
№492	4.0	5.5	1.7	2.6	4.0	4.5
№494	4.2	5.6	2.1	2.2	3.3	4.6
LK490	11.0	8.2	4.7	3.2	7.6	6.2
Dark Green	10.6	7.1	6.6	3.5	6.9	6.1
T-3627	8.1	6.2	5.6	3.4	7.4	6.7
$\bar{x}$	7.6	6.5	4.1	3.0	5.9	5.6
LSD05	0.71		0.99		1.20	

and specific combining ability. In addition, a significant reciprocal effect was found in 2017 and 2018.

The genotype Dark Green (0.48-0.54) and the line LK490 (0.26-1.68) had a high (reliable positive assessment of effects) general combining ability (GCA) for lycopene content in fruits over three years of research. Genotype T-3627 had high positive values of GCA for two years of research (0.38-1.09) (Table 4).

Lines №492 and №494 had low (significant negative effect) GCA values–(from minus 1.13 to minus 0.33) and (from minus 1.08 to minus 0.79), respectively, so it is undesirable to use them for creating heterotic hybrids.

As a result of the conducted research, reliable differences in specific combining ability (SCA) were established. To identify lines and genotypes with high or low SCA, the variance for each parental component was calculated for comparison with the overall average value (Table 5).

High reliable values of SCA were observed in two years of research in Dark Green and T-3627 genotypes, and in one year in line LK490.

The variances of the effects of general (δ_{gi}^2) and specific (δ_{Si}^2) combining ability were compared. It was established that in line №494 for three years of research, line №492 for two years of research, genotypes Dark Green, T-3627, line LK490 for one year, the variance of GCA is greater than the variance of SCA, which indicates the superiority of additive effects of genes in the genetic control of the trait "lycopene content in fruits". This makes it possible to recommend selection in the breeding process by phenotype. At the same time, a significant contribution of non-additive effects was revealed, as evidenced by the superiority of SCA variance over GCA in genotypes

Table 3: Analysis of variance of the combining ability of lycopene content in tomato fruits

Years	Type of scattering	Sum of squares	Degrees of freedom	Middle square	F calc.	F tabl.
2017	Hybrids	333.7	24	13.9*	76.4	1.79
	GCA	50.4	4	12.6*	208.0	2.61
	SCA	25.6	10	2.6*	42.3	2.08
	Reciprocals	35.2	10	3.5*	58.0	2.08
	Residual	2.9	48	0.1*		
2018	Hybrids	124.4	24	5.2*	14.7	1.79
	GCA	11.8	4	3.0*	25.3	2.61
	SCA	21.2	10	2.1*	18.1	2.08
	Reciprocals	8.5	10	0.9*	7.3	2.08
	Residual	5.6	48	0.1*		
2019	Hybrids	142.7	24	5.9*	11.8	1.79
	GCA	42.4	4	10.6*	63.2	2.61
	SCA	2.4	10	0.2*	1.4	2.08
	Reciprocals	2.7	10	0.3*	1.6	2.08
	Residual	8.1	48	0.2*		

* Significant at 5 % level

Table 4: Evaluation of effects of general combining ability (GCA) of lycopene content in tomato fruits

Lines,	Years		
genotypes	2017	2018	2019
№492	-1.01*	-0.33*	-1.13*
№494	-0.90*	-0.79*	-1.08*
LK490	1.68*	0,26*	0.60*
Dark Green	0.54*	0,48*	0.52*
T-3627	-0.30*	0.38*	1.09*
LSD05	0.14	0.19	0.23

* Significant at 5 % level

of Dark Green, T-3627, line LK490 over two years of research.

Genetic analysis by Hayman (1954), Jinks (1954) did not reveal an epistatic interaction of genes, the content of lycopene in fruits is determined by an additive-dominant genetic system (Fig. 1-3).

This is confirmed by the high significance of indicators *D* and *H1*, which characterize the variability of lycopene content in tomato fruits (Table 6). The values *H1* and *H2* are unequal, which indicates the presence of alleles that are unevenly distributed among the parental components. The value of *H2/4H1* indicator deviates from the level of 0.25 and is 0.17-0.18, which indicates an unequal number of genes with dominant and recessive alleles in the parental components. The parameter

$\frac{\sqrt{4DH\ 1+F}}{\sqrt{4DH\ 1-F}}$ ranged from 1.03-2.90, which indicates a more pronounced effect of dominant gene alleles in the studied lines and samples.

The value of parameter *D*, which measures additive variability in the population, was higher compared to values of *H1*, which measures the dominant variability over two years of research. Values *H1* > *H2*, which indicates an unequal ratio of positive and negative effects.

Positive reliable values of *F (F > 0)* indicate a more pronounced influence of dominant alleles in the set of studied lines and samples.

Over two years of research, the average degree of dominance was 0.33-0.82. The regression line crosses the positive part of *Wr* axis, so it is possible to claim incomplete dominance at all loci. In 2018, there was a tendency to dominate, but it is unreliable.

The parameter *h2/H2* is determined in case of a significant difference. Such a difference was observed in 2017 and 2018. This made it possible to calculate the value of this indicator, which is 7.96 and 11.56, respectively.

Table 5: Estimation of variances of general combining ability (GCA) and specific combining ability (SCA) for lines and genotypes of lycopene content in tomato fruits of 2017-2019

Lines, genotypes	Years	№494	LK 490	Dark Green	T-3627	δ_{Si}^2	δ_{gi}^2
№492	2017	0.18	0.68*	-0.23	-0.15	0.10	1.01
	2018	0.04	0.23	-0.45*	0.85*	0.16	0.09
	2019	-0.09	-0.27	-0.02	-0.23	-0.09	1.26
№494	2017		0.39*	-0.34*	0.34*	0.06	0.80
	2018		-0.01	-0.09	-0.63*	0.01	0.60
	2019		-0.11	-0.10	0.45	-0.07	1.15
LK490	2017			-1.10*	-1.06*	0.69*	2.81
	2018			-0.73*	-0.67*	0.17	0.05
	2019			-0.37	-0.10	-0.07	0.34
Dark Green	2017				-1.32*	0.74*	0.28
	2018				-1.38*	0.57*	0.21
	2019				0.22	-0.08	0.25
T-3627	2017					0.71*	0.08
	2018					0.78*	0.12
	2019					-0.05	1.17
Average value	2017					0.46	
	2018					0.34	
	2019					-0.07	

* Significant at 5 % level -Note. δ_{Si}^2 - variance of the effect of specific combining ability; δ_{gi}^2-variance of the effect of general combining ability.

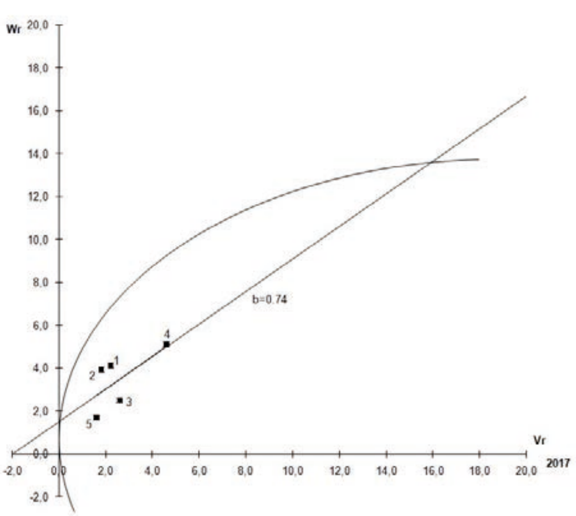


Figure 1: Graph of the dependence of Wr by Vr content of lycopene in tomato fruits, 2017 1-Line №492; 2-Line №494; 3 - Line LK490; 4 - Genotype Dark Green; 5 - Genotype T-3627.

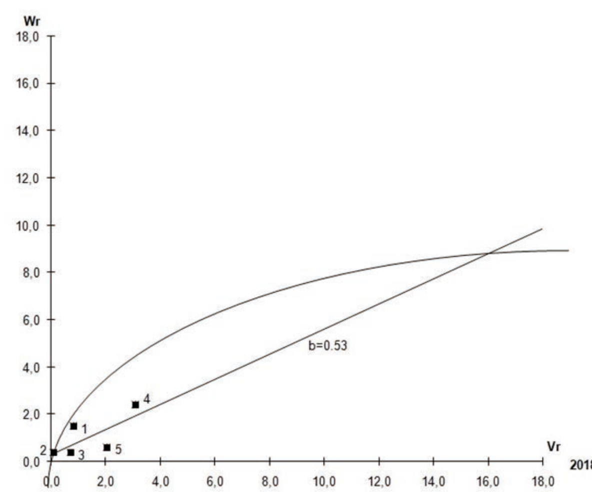


Figure 2: Graph of the dependence of *Wr* by *Vr* content of lycopene in tomato fruits, 2018 1-Line №492; 2-Line №494; 3-Line LK490; 4-Genotype Dark Green; 5-Genotype T-3627.

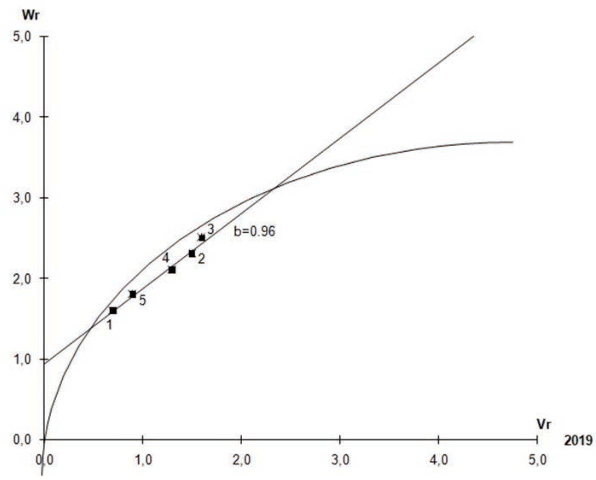


Figure 3: Graph of the dependence of Wr by Vr content of lycopene in tomato fruits, 2019 1 - Line №492; 2-Line №494; 3-Line LK490; 4 - Genotype Dark Green; 5-Genotype T-3627.

That is, the studied parental components differed among themselves from eight to twelve groups of genes, demonstrating the dominance effect.

The highest indicators of the *F* parameter, which reflects the relative contribution of additive and dominant gene action to the phenotypic manifestation of the trait in F_1 hybrids, were obtained in 2017, the lowest in 2019 (Table 7).

Lines №492 and №494, LK490, genotype T-3627 had reliable positive effects in two years of research, in one year – Dark Green genotype. This indicates the dominance of dominant alleles in the indicated lines and samples, one year recessive alleles prevailed.

The correlation coefficient between the average values of lycopene content in tomato fruits of parents ($\bar{x}p$) and the sum *(Wr + Vr)* in 2017 and 2018 was 0.21-0.67 (Table 8). Values of high positive correlation indicate the increasing of the trait by recessive genes. A negative implausible value of the correlation coefficient in 2019 indicates the absence of a dominant direction.

The theoretical values of $W_{dom} + V_{dom}$ and $W_{rec} + V_{rec}$ for lines and genotypes carrying all dominant and recessive alleles were determined on the basis of the correlation coefficient between the average values of parental component traits in parents ($\bar{x}p$) and the sum *(Wr + Vr)*. A line or sample that theoretically had all dominant alleles was characterized by the value $W_{dom} + V_{dom}$ – 6.13 (2017); 2.08 (2018) and 5.85 (2019). The theoretical value of $W_{rec} + V_{rec}$ of the parental component with the largest

Table 6: Genetic parameters of lycopene content in tomato fruits

	Years		
Parameters	2017	2018	2019
D	11.28 ± 0.81	4.57 ± 0.65	4.07 ± 0.03
F	8.73 ± 2.02	4.93 ± 1.63	0.04 ± 0.06
H_1	7.52 ± 2.06	5.58 ± 1.66	0.43 ± 0.06
H_2	5.00 ± 1.98	4.00 ± 1.60	0.14 ± 0.06
h^2	39.70 ± 1.34	46.25 ± 1.08	2.04 ± 0.04
E	0.06 ± 0.33	0.12 ± 0.27	0.17 ± 0.01
H_1/D	0.67	1.22	0.11
$\sqrt{H_1/D}$	0.82	1.11	0.33
$H_2/4H_1$	0.17	0.18	0.08
$\frac{\sqrt{4H\ 1+F}}{\sqrt{4H\ 1-F}}$	2.80	2.90	1.03
h^2/H_2	7.93	11.56	14.14
Conditionally dominant(CD)	6.13	2.08	5.85
Conditionally recessive(CR)	11.65	9.96	5.84

Table 7: Estimation of the direction of dominance (F) by lycopene content in tomato fruits for each parental forms and their hybrids

Lines, genotypes	Years		
	2017	2018	2019
№492	8.19 ± 2.75*	5.08 ± 2.22*	1.93 ± 0.09*
№494	9.35 ± 2.75*	8.78 ± 2.22*	-1.00 ± 0.09*
LK490	10.60 ± 2.75*	7.53 ± 2.22*	-1.69 ± 0.09*
Dark Green	1.33 ± 2.75*	-1.24 ± 2.22*	-0.28 ± 0.09*
T-3627	14.17 ± 2.75*	4.49 ± 2.22*	1.21 ± 0.09*

* Significant at 5 % level

number of recessive genes was 11.65 (2017); 9.96 (2018) and 5.84 (2019).

With the help of regression graphs (Fig. 1–3), we obtained more complete information about the manifestation of dominant and recessive effects.

According to the results of the F_1 evaluation, the point with the greatest recessiveness was approached by the Dark Green genotype, which had 75 percent recessive alleles in 2017. Parental components were located in the zone of dominance in 2018 and had 100 % dominant alleles. Line LK490, characterized by the highest lycopene content in fruit, was located closer to the zone with the greatest recessiveness in 2019. The Dark Green genotype point is in the middle of the regression line. The point of line №492 (with low lycopene content in fruits) lies closer to the zone with the greatest dominance.

4 DISCUSSION

The significance of the work is due to the need to solve the important task of expanding the genetic diversity of tomato source material with increased lycopene content in fruits, studying the inheritance of valuable economic traits and determining their breeding value in hybrid generations, to increase the effectiveness of breeding work to create new varieties and hybrids of tomatoes with improved nutritional properties. Highly pigmented forms with *hp-2*^{dg}, *B*^{c} genes are used in the breeding process to create valuable varietal diversity, which contribute to increasing the level of lycopene in fruits.

The work carried out on the study of the character of variability and inheritance in the future makes it possible to effectively forecast breeding work with minimal time expenditure. The created new assortment of tomatoes will expand market opportunities for the consumer and will serve as a basis for further breeding work.

Table 8: The results of correlation and regression analyses of lycopene content in tomato fruits

Indicator	2017	2018	2019
Correlation (*r*) between *Wr* and *Vr*	0.67	0.71	1.00
Regression (*b1*) between *Wr* and *Vr*	0.74	0.53	0.96
Correlation (*r*) between $\bar{x}p$ and *Wr* and *Vr*	0.21	0.67	-0.001
Regression (*b2*) between $\bar{x}p$ and *Wr* and *Vr*	0.30	0.76	-0.003

5 CONCLUSIONS

Five parental forms of tomato (*Solanum lycopersicum* L.) to determine the combining ability were studied. Twenty F_1 hybrids obtained according to the full diallel scheme (5 x 5) were used to study the inheritance of the trait "lycopene content in fruits". Lines №492, №494, LK490 and two collection genotypes with an increased content of lycopene in fruits were used as parental forms in the diallel crossing system according to Hayman (1954) and Jinks (1954): Dark green (*hp-2*^{dg}), T- 3627(*B*^{c}).

An assessment of the general and specific combining ability of five initial forms of tomato evaluated on the trait "lycopene content in fruits", the character of inheritance of the characteristic was determined. According to the research results, it was established that the lycopene content in fruits is controlled by an additive-dominant genetic system. The trait is inherited by the incomplete dominance. The direction of dominance changes – from the dominance of genes that reduce the manifestation of the trait to its absence.

Over the three years of research, the LK 490 line (0.26-1.68) and the Dark Green genotype (0.48-0.54) had a high (reliably positive assessment of effects) GCA, over two years – the T-3627 genotype (0.38-1.09). They can be recommended for creating heterotic hybrids and varieties.

6 REFERENCES

Abenavoli, L., Procopio, A. C., Paravati, M. R., Costa, G., Milić, N., Alcaro, S., & Luzza, F. (2022). Mediterranean diet: the beneficial effects of lycopene in non-alcoholic fatty liver disease. Journal of Clinical Medicine, 11(12), 3477. https://doi.org/10.3390/jcm11123477

Akbari, B., Baghaei Yazdi, N., Bahmaie, M., & Mahdavi Abhari, F. (2022). The role of plant derived natural antioxidants in reduction of oxidative stress. BioFactors, 48(3), 611-633. https://doi.org/10.1002/biof.1831

Alda, L. M., Gogoasa, I., Bordean, D. M., Gergen, I., Alda, S., Moldovan, C., & Nita, L. (2009). Lycopene content of tomatoes and tomato products. Journal of Agroalimentary Processes and Technologies, 15(4), 540-542.

Anthon, G., & Barrett, D. M. (2006, June). Standardization of a rapid spectrophotometric method for lycopene analysis. In X International Symposium on the Processing Tomato 75 (pp. 111-128).

Arain, M. A., Mei, Z., Hassan, F. U., Saeed, M., Alagawany, M., Shar, A. H., & Rajput, I. R. (2018). Lycopene: a natural antioxidant for prevention of heat-induced oxidative stress in poultry. World's Poultry Science Journal, 74(1), 89-100. https://doi.org/10.1017/S0043933917001040

Arballo, J., Amengual, J., & Erdman Jr, J. W. (2021). Lycopene: A critical review of digestion, absorption, metabolism, and excretion. Antioxidants, 10(3), 342. https://doi.org/10.3390/antiox10030342

Barrett, D. M., & Anthon, G. (2000, June). Lycopene content of California-grown tomato varieties. In VII International Symposium on the Processing Tomato 542 pp. 165-174).

Brandt, S., Lugasi, A., Barna, É., Hóvári, J., Pék, Z., & Helyes, L. (2003). Effects of the growing methods and conditions on the lycopene content of tomato fruits. Acta Alimentaria, 32(3), 269-278.

Costa, J. M., & Heuvelink, E. P. (2018). The global tomato industry. In Tomatoes (pp. 1-26). Wallingford UK: CABI.

Dospekhov, B.A. (1985). Methods of field experience. Moscow: Agropromizdat, 352 p. [in Russian]

Ermakov, A.I., Arasymovych, V.V., Smirnova-Ikonnikova, M.I., Murri, I.K. (1952). Methods of biochemical research of plants. Moscow, Leningrad: Selkhozgiz, 520 p. [in Russian]

Fedin, M.A. (1970). About heterosis of wheat. Moscow: Kolos, 240 p. [in Russian]

Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. Australian Journal of Biological Sciences, 9(4), 463-493. https://doi.org/10.1071/BI9560463

Hayman, B. I. (1954). The theory and analysis of diallel crosses. Genetics, 39(6), 789. https://doi.org/10.1093/genetics/39.6.789

Imran, M., Ghorat, F., Ul-Haq, I., Ur-Rehman, H., Aslam, F., Heydari, M. & Rebezov, M. (2020). Lycopene as a natural antioxidant used to prevent human health disorders. Antioxidants, 9(8), 706. https://doi.org/10.3390/antiox9080706

Jinks, J. L. (1954). The analysis of continuous variation in a diallel cross of Nicotiana rustica varieties. Genetics, 39(6), 767. https://doi.org/10.1093/genetics/39.6.767

Liang, X., Ma, C., Yan, X., Liu, X., & Liu, F. (2019). Advances in research on bioactivity, metabolism, stability and delivery systems of lycopene. Trends in Food Science & Technology, 93, 185-196. https://doi.org/10.1016/j.tifs.2019.08.019

Li, N., Wu, X., Zhuang, W., Xia, L., Chen, Y., Wu, C. & Zhou, Y. (2021). Tomato and lycopene and multiple health outcomes: Umbrella review. Food Chemistry, 343, 128396. https://doi: 10.1016/j.foodchem.2020.128396

Madia, V. N., De Vita, D., Ialongo, D., Tudino, V., De Leo, A., Scipione, L., ... & Messore, A. (2021). Recent advances in recovery of lycopene from tomato waste: A potent antioxidant with endless benefits Molecules, 26(15), 4495. https://doi.org/10.3390/molecules26154495

Meng, F., Li, Y., Li, S., Chen, H., Shao, Z., Jian, Y. & Wang, Q. (2022). Carotenoid biofortification in tomato products along whole agro-food chain from field to fork. Trends in Food Science & Technology. https://doi.org/10.1016/j.tifs.2022.04.023

Poobalan, V., Praneetha, S., Arumugam, T., Kumaravadivel, N., & Jeyakumar, P. (2019). Medicinal properties of vegetable crops. IJCS, 7(5), 1538-1542.

Raiola, A., Rigano, M. M., Calafiore, R., Frusciante, L., & Barone, A. (2014). Enhancing the health-promoting effects of tomato fruit for biofortified food. Mediators of Inflammation, 2014. https://doi.org/10.1155/2014/139873

Sachdeva, V., Roy, A., & Bharadvaja, N. (2020). Current prospects of nutraceuticals: A review. Current Pharmaceutical Biotechnology, 21(10), 884-896. https://doi.org/10.2174/1389201021666200130113441

Selvaggi, R., Valenti, F., Pecorino, B., & Porto, S. M. (2021). Assessment of tomato peels suitable for producing biomethane within the context of circular economy: A gis-based model analysis. Sustainability, 13(10), 5559. https://doi.org/10.3390/su13105559

Sun, W., Shahrajabian, M. H., & Cheng, Q. (2021). Natural dietary and medicinal plants with anti-obesity therapeutics activities for treatment and prevention of obesity during lock down and in post-COVID-19 era Applied Sciences, 11(17), 7889. https://doi.org/10.3390/app11177889

Tchonkouang, R. D. N., Antunes, M. D. C., & Vieira, M. M. C. (2022). Potential of Carotenoids from Fresh Tomatoes and Their Availability in Processed Tomato-Based Products. https://doi:10.5772/intechopen.103933

Vitucci, D., Amoresano, A., Nunziato, M., Muoio, S., Alfieri, A., Oriani, G. & Salvatore, F. (2021). Nutritional controlled preparation and administration of different tomato purées indicate increase of β-carotene and lycopene isoforms, and of antioxidant potential in human blood bioavailability: A pilot study. Nutrients, 13(4), 1336. https://doi.org/10.3390/nu13041336

Wang, C., Li, M., Duan, X., Abu-Izneid, T., Rauf, A., Khan, Z. & Suleria, H. A. (2022). Phytochemical and nutritional profiling of tomatoes; impact of processing on bioavailability-a comprehensive review. Food Reviews International, 1-25. https://doi.org/10.1080/87559129.2022.2097692

Wu, X., Yu, L., & Pehrsson, P. R. (2022). Are processed tomato products as nutritious as fresh tomatoes? Scoping review on the effects of industrial processing on nutrients and bioactive compounds in tomatoes. Advances in Nutrition, 13(1), 138-151. https://doi.org/10.1093/advances/nmab109

Yates, F. (1947). Analysis of data from all possible reciprocal crosses between a set of parental lines. Heredity, 1(3), 287-301. https://doi.org/10.1038/hdy.1947.19

doi:10.14720/aas.2025.121.1.16450 Original research article / izvirni znanstveni članek

Efficiency of labor in winter pruning of apple trees using technological innovations

Élio Fillipi dos SANTOS [1], Wendel Paulo SILVESTRE [2], [3], Lessandra Silva RODRIGUES [4], Carine COCCO [5]

Received October 25, 2023, accepted January 28, 2025.
Delo je prispelo 15. oktober 2023, sprejeto 28. januar 2025

Efficiency of labor in winter pruning of apple trees using technological innovations

Abstract: The increase of new technologies in fruit-growing crops has increased productivity in cultural practices and helped farmers expand their production areas. This work aimed to quantify labor performance in winter pruning of apple trees, using manual and electronic pruning shears associated with a pruning platform and ladders. The treatments consisted of two types of pruning shears and two support systems. A pruner carried out pruning for 16 days, with a journey of 10 h per day. The average yield in manual pruning with a ladder was 21.35 plants pruned per hour per man. Using a platform increased the yield to 46.25 plants pruned per hour per man. Pruning with electronic pruning shears with a ladder showed an average yield of 33.47 plants pruned per hour per man. With platform support, yield increased to 128.5 plants pruned per hour per man. Using electronic pruning shears reduced the time for carrying out the activity. Pruning performed with platform support was more efficient, regardless of the type of tool used. This way, the technologies available for apple tree pruning reduce costs, increasing operational profitability.

Key words: electronic pruning shear, fruit growing, pruning platform.

Učinkovitost dela pri zimskem obrezovanju jablan z uporabo tehnoloških inovacij

Izvleček: Povečanje novih tehnologij gojenja sadnih rastlin je povečalo učinkovitost načinov gojenja in pomagalo pridelovalcem pri razširitvi njihovih pridelovalnih območij. Namen raziskave je bil ovrednotiti učinkovitost dela pri zimski rezi jablan pri uporabi ročnih in električnih škarij s samohodnimi platformami in lestvami. Obravnavanja so obsegala dve vrsti škarij za rez (ročne in električne) in dva podporna sistema (samohodna platforma in lestev). Rezač je opravil delo v 16 dneh, če je rezal 10 ur na dan. Pri uporabi ročnih škarij in lestve je en rezač porezal 21,35 dreves na uro. Pri uporabi platforme in ročnih škarij se je učinkovitost rezi enega rezača povečala na 46,25 dreves na uro. Pri rezi z električnimi škarjami in lestvijo je en rezač v poprečju porezal 33,47 dreves na uro. Z uporabo električnih škarij in platforme se je učinkovitost rezi povečala na 128,5 dreves na uro. Uporaba električnih škarij je zmanjšala čas rezi. Rez z uporabo platforme je bila učinkovitejša ne glede na uporabo vrste škarij. Na takšen način dostopne tehnologije za rez jablan zmanjšujejo stroške rezi in povečajo donosnost.

Ključne besede: električne škarje za rez, sadjarstvo, samohodne platforme

1 University of Caxias do Sul, Course of Agronomy, Caxias do Sul, RS, Brazil. ORCID: https://orcid.org/0009-0002-0189-9540
2 University of Caxias do Sul, Course of Agronomy, Laboratory of Studies of the Soil, Plant, and Atmosphere System and Plant Metabolism (LESPA), and Postgraduate Program in Process Engineering and Technologies (PGEPROTEC), Caxias do Sul, RS, Brazil. ORCID: https://orcid.org/0000-0002-9376-6405
3 Corresponding author. E-mail: wpsilvestre@ucs.br
4 University of Caxias do Sul, Course of Agronomy, Caxias do Sul, RS, Brazil. ORCID: https://orcid.org/0000-0001-9467-7896
5 University of Texas Rio Grande Valley, Edinburg, TX, United States. ORCID: https://orcid.org/0000-0002-1850-2266

1 INTRODUCTION

Family farming is, in general, known as a backward sector from an economic, technological, and social point of view, fundamentally focused on producing primary food products with a logic of subsistence production (Souza et al., 2019). This image is far from reality since the universe of family farming in Brazil is highly heterogeneous and includes impoverished and wealthy families (Vieira et al., 2019). Family farmers differ not only in terms of property size and production capacity but also in access to technology, infrastructure, and level of organization (Souza Filho et al., 2011).

Technology is one of the most influential factors in the development and growth of various sectors of the economy at a national level. Specifically in agriculture, one of the most critical activities in the Brazilian economy, technology allows the use of innovations in rural properties. It contributes to raising the productivity of crops and labor income, creating differentiated opportunities for farmers. Furthermore, specifically in family farming, according to Souza Filho et al. (2011), adopting technology can contribute to supplying the lack of labor. It may be an alternative to family succession since one of the problems in the rural environment is the aging of farmers and, consequently, the aging process. of the workforce available on the properties due to the emigration of young people due to lack of opportunities in rural areas (Ribeiro Filho & Tahim, 2022).

The limitation on the size of the property, many times, ends up compromising the financial viability of the family establishments since the scale of production becomes a structural problem. The modernization of the property, which includes training family members, the use of quality inputs, machinery, and equipment appropriate to the segment and the conditions of family farmers, is a way of allowing sustainability and significant gains in productivity in the face of systems which are less technified (Bittencourt, 2018).

Fruit-growing crops play a vital role in Brazilian income distribution, expanding job opportunities and substantially improving the quality of life in communities. This activity often demonstrates remarkable profitability, enabling economic sustainability even for small properties. Given that it demands a considerable amount of highly specialized labor, fruit growing promotes a significant increase in the availability of jobs, conferring valuable benefits to the regions where it finds its development space (Klesener, 2020).

Among the various fruit species cultivated in Brazil, the apple tree (*Malus domestica* Borkh.) is one of the most important in production numbers. According to Beling (2022), the revenue of the productive sector was around US$ 360 million (R$ 1.73 billion) in the 2020/2021 harvest, while the total financial transaction in the area was US$ 1.24 billion (R$ 6.0 billion) in addition to offering job opportunities for 175,000 people in southern Brazil and 52,000 direct jobs in the region. The apple tree has great economic importance worldwide, being the fifth most produced fruit in the world (FAO, 2023) and the third most consumed in Brazil, behind only bananas and oranges (Beling, 2022).

In Brazil, the total apple area in general is estimated at around 32.90 thousand hectares, according to the IBGE (2022), with a production of about 1.30 million tons, with Santa Catarina contributing with 635,000 t per year, followed by Rio Grande do Sul with 628,000 t, Paraná with 30 t (Revista da Fruta, 2022), and the Southern region of the country was responsible for 98 % of the national production of this crop (IBGE, 2022). Adverse weather events, mainly frost and hail in the central producing regions of Rio Grande do Sul and Santa Catarina, may be responsible for significant production losses (CEPEA, 2018).

According to information from the Brazilian Institute of Geography and Statistics (IBGE, 2022), the State of Rio Grande do Sul is currently the most prominent national apple producer, followed by the State of Santa Catarina. Average production in the 2013/2021 period accounted for about 50 % of the Brazilian output, with the leading producers located in the northeast of the state, with emphasis on the municipalities of Vacaria, Caxias do Sul, and Bom Jesus, followed by the municipalities of Meus Capões, Monte Alegre dos Campos, Ipê, São Francisco de Paula, and São José dos Ausentes.

There is a great demand for labor in apple orchards, which requires optimization to carry out cultural practices and evaluate the possibility of mechanization. Machines that reduce manual labor, such as harvesting platforms, bin transport carts, seedling planters, shredders, and electric pruning shears, can increase the efficiency of management activities in orchards, such as pruning, thinning, and harvesting, reducing activities manuals (Petri et al., 2018).

The popularity of the apple means that its cultivation remains promising for small farmers since the number of properties dedicated to this activity in southern Brazil exceeds 4,500. Most of these properties are small, although larger crops account for at least 40 % of production (Anuário Brasileiro da Maçã, 2018). Apple cultivation is an excellent opportunity for less extensive, family-owned properties, which have this fruit as their main product and source of income in a diversified fruit culture. Incorporating new production technologies in apple tree cultivation has significantly increased the quality and harvested volume of the fruit (Mathias & Rufato, 2017).

During the apple tree pruning period, an activity essentially carried out by family members, the demand for labor is greater than that available. This causes the action to be prolonged if temporary workers are not hired. In addition, the available workforce is often poorly qualified to carry out the activity, which is considered exhausting, making the family farmer's challenge even greater. The delay in completing this agricultural practice can be detrimental to the profitability of the crop if it is considered the high investment required to produce with quality, not to mention economic fluctuations and climatic factors (Souza Filho et al., 2011).

Fruiting or production pruning is carried out during the winter. It aims to eliminate old branches, promote the formation of new shoots, and establish a balance between the production and vegetative growth of the orchard. Its intensity will determine the plant's growth and fruiting as needed (Duarte et al., 1992).

According to Bittencourt (2017), access to innovation allows the maintenance of family farmers in the countryside, creating conditions for the economic viability of family properties and their ability to develop as a family social unit, contributing to the sector's modernization. Due to the limited size of these properties, production capacity and sustainability may be impaired. One of the main benefits that the incorporation of innovations and technologies can offer is to improve the performance of the workforce, with economic return, as well as better ergonomics for the family farmer. Souza Filho et al. (2011) point out that technology plays a vital role in determining the economic performance of the property, as it allows for increased productivity and has an important effect on the sustainability of the activity. Thus, the workforce available on the property can be better used throughout the year.

Given the above, the present work aimed to quantify labor performance in winter pruning of apple trees, using manual and electronic pruning shears associated with a pruning platform and ladders.

2 MATERIALS AND METHODS

The study was carried out in an apple orchard on a family farm located in the Capela São Gotardo community, Vila Seca District, in Caxias do Sul, RS (geographical coordinates: 29°03' S and 51°03' W), at an altitude of 680 m above sea level. The production of apples was intended for fresh consumption.

The orchard was implanted in 2008 in a total area of 9.0 ha, conducted in a high-density system, with 4.0 m spacing between rows and 1.0 m between plants, totaling approximately 2,500 plants per hectare. The composition of the orchard was 75 % 'Maxxigala' plants and 25 % 'Fuji Suprema' cultivar plants, both on 'Marubakaido' rootstock.

The treatments consisted of two types of pruning shears (manual and electronic) and two support systems (ladder and platform). The pruning of the apple trees was carried out by a pruner, an employee of the property, between August 03 and 23, 2018, totaling 16 days, for 10 h daily, divided into two shifts, corresponding to 5:00 am and 5:00 pm. The correct use of the tools was demonstrated one day before data collection to verify the pruner's understanding of how the experiment would be carried out.

Initially, pruning was conducted with manual pruning shears supported by a ladder on August 03 and 04 and August 06 and 07, 2018. Then, manual pruning shears were used with platform support from 08 to August 11, 2018. Then, electronic pruning shears with ladder support were used from August 13 to 16, 2018. Afterward, pruning was performed with electronic pruning shears with platform support from August 20 to 23, 2018. During this period, 160 h of pruning was conducted, 40 h with each tool and support system. For each repetition, 5 h of pruning time were considered, totaling eight repetitions for each treatment.

The tools used were the Felco 621 pruning saw, the Felco 31 manual pruning shears, and the Felco 820 electronic pruning shears 420 mm, with a non-slip handle and steel blade. Felco 31 manual pruning shears were used to cut branches up to 25 mm, with a mass of 225 g and a length of 210 mm; the blade was made of tempered steel and the anvil of brass, and the handle was also made of non-slip material. Felco 820 electronic pruning shears were used to cut branches up to 45 mm, with a mass of 980 g and a length of 290 mm. It was controlled and powered by a battery, and its ergonomics sought to relieve the pressure on the arms' and shoulders' muscles during the entire pruning process, the blade being made of tempered steel.

A ladder and a pruning platform were used in the experiment in support of the tools. The ladder had a height of 3 m and a mass of 12 kg, consisting of six steps, and was made of aluminum. The pruning platform had a capacity for up to five people, requiring an operator. It was hitched to the third point of the tractor, and its structure was coupled to the bin transport carts. The platform had a height of 2.8 m and a length of 4.0 m, and its advancement system was pneumatic.

The number of plants pruned per hour per man was measured for the four treatments. A completely randomized, bifactorial design followed, considering the pruning shear (manual and electronic) and the support system (ladder and platform) as factors. The results were submitted to analysis of variance (ANOVA), and the means were compared using the Tukey test at a 5 % error probability using the AgroEstat software. Subsequently, based on the average number of plants pruned per hour per man for each pruning tool and each support system, the number of days needed to prune one hectare of apple orchards was calculated.

3 RESULTS AND DISCUSSION

Table 1 compiles the results of the average number of apple trees pruned per hour per man. The data indicate that when the activity was carried out using technological innovations (electronic pruning shears and platform), there was an increase in the number of plants pruned for the same period and the same operator.

The yield of using manual pruning shears with a ladder was 21.35 plants per hour per man. With platform support for pruning, an increase in the average number of plants pruned was observed, corresponding to 46.25 plants per hour per man, a rise of 116.63 % when compared to pruning with ladder support.

As for the number of plants pruned using electronic pruning shears and ladder support, an average of 33.47 plants were pruned per hour per man. With the pruning carried out with the platform's help, there was an increase of 283.93 % compared to the pruning with a ladder, with an average of 128.50 plants pruned per hour per man.

According to Batalha et al. (2005), family farmers' use of new technologies provides conditions for exploring new opportunities and practices that require a more sophisticated production management level. This significant difference between the support systems, ladders, and pruning platforms, even using the same tool type, is due to the ease of pruning using platforms. When using the platform, the pruner does not need to descend from the ladder and reposition it several times throughout the day, causing a loss of efficiency and worker fatigue.

The average yields obtained with the manual and electronic tools and using the ladder support system were 21.35 and 33.47 plants per hour per man, respectively (Table 1). This corresponds to a variation of 56.77 % between the two types of tools. For the results of plants pruned per hour per man using manual and electronic pruning shears, the highest average yields were obtained with platform support compared to the ladder support system. The average number of plants pruned per hour with manual pruning shears was 46.25, while with electronic pruning shears, the average was 128.50 plants, corresponding to a variation of 177.84 % between support systems.

Using the orchard in which the present study was carried out as a parameter, with an average of 2,500 plants per hectare and considering that the worker in charge of pruning works 10 h a day, it would take about 12 days to prune one hectare with the use of manual pruning shears and ladder support. On the other hand, using electronic pruning shears, pruning time would be reduced to approximately 5.5 days, an efficiency gain of roughly 6.5 days per hectare using an electronic tool (Figure 1).

ors (2023).

Figure 1 shows that in the support system that uses a pruning platform, it would take approximately 7.5 days to prune 1.0 ha using manual pruning shears and 1.9 days to prune the same area using electronic pruning shears. With this, there is an efficiency gain of approximately 5.6 days per hectare using an electronic tool. In percentage terms, this corresponds to a reduction in pruning time per hectare of 74.66 %.

Considering the four treatments used in this ex-

Table 1: Number of apple trees pruned per hour per man using manual pruning shears and electronic pruning shears, with ladder support and platform for pruning.

	Support system	
Pruning shears	Ladder	Platform
Manual	21.35 Bb	46.25 Ab
Electronic	33.47 Ba	128.50 Aa
Coefficient of variation (%)	2.5495	

Means followed by the same letter, lowercase in the column (pruning shear type) and uppercase in the line (support system type), do not differ by the Tukey's Test at a 5 % error probability.

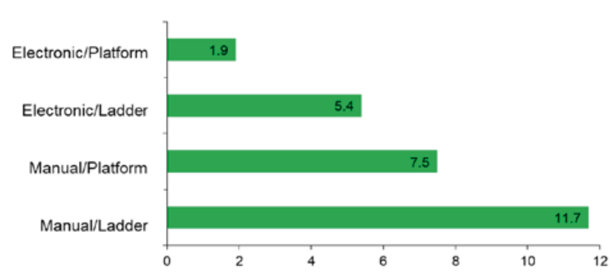


Figure 1: Number of days required to prune one hectare of apple trees considering the performance of an operator performing the pruning with manual pruning shears and electronic pruning shears, with a support ladder and platform for pruning. Source: Auth

periment, it can be noted that the highest yield gain occurred with electronic pruning shears associated with the platform, followed by the use of a manual tool and platform. The lowest yield was observed when using a combination of manual pruning shears and a ladder, a system traditionally used in orchards in the Serra Gaúcha for winter apple and other fruit tree pruning. These results demonstrate that, currently, the producer must be concerned with the planting density and conducting the narrow crown, which facilitates pruning and harvesting, improving the fruit quality and allowing mechanization in the orchard (Petri et al., 2018).

Managing a family farm is no longer based solely on mastering knowledge and traditional farming practices. Investing in education and training increases the ability to allocate resources better and make more effective decisions regarding adopting new technologies (Souza Filho et al., 2011). For the authors, the organization of the property is essential to sustainably incorporate innovations because innovation can create conditions for maintaining the economic viability of family properties and their ability to reproduce as a family social unit, contributing to their modernization.

The apple tree culture has recently undergone major transformations in Brazil, leading to important productivity increases. In addition to the rise in the number of technologies used in the crop, the planting density was of great importance for the rise in production, which went from 550 to 600 plants per hectare in the 1970s to 2,500 to 3,500 plants per hectare today. Despite this, the apple tree crop has seen its profit margin reduced (Petri et al., 2018).

Brazilian production also grew, leading to a greater supply of apples on the market. According to Petri et al. (2018), three points are essential to maintain the economic activity of the producer: production cost, productivity, and selling price. These three aspects could be improved depending on the technologies used, as the selling price is directly linked to the quality of the fruit. Furthermore, according to Souza Filho et al. (2011), the probability of investment in technology is greater when the orchard is managed by the owner, which is the reality in family farming.

Electronic pruning shears are currently one of the innovations available to facilitate the work of family farmers, who are the biggest consumers of this type of technology. Large apple-producing companies often end up not making this tool available to workers due to the low qualification of the hired labor. However, in family farming, family members are responsible for cultural practices in the orchards, including pruning. Therefore, they are more careful using and maintaining this tool.

For the production systems practiced by family farming, which are increasingly faced with the shortage and aging of the present workforce, the generation of information on the use of technologies available on the market is essential. Considering that this is the case of apple production orchards in Serra Gaúcha so that the acceptance and use of available technological innovations increase according to the farmers' resources, cultural barriers must be overcome with the generation and dissemination of information, improving the quality of life of farming families. In addition, using technologies can be an attraction for the continuity of young people in agricultural production, making them remain in rural areas.

4 CONCLUSIONS

Electronic pruning shears increased the number of plants pruned per hour per man, reducing the number of days for carrying out the activity in apple orchards. Apple tree pruning, performed with electronic pruning shears, was more efficient when compared to manual tools, regardless of the type of support used. Pruning performed with platform support was more efficient, even with manual pruning shears. Thus, the technologies available for apple tree pruning increase the efficiency of the process, reducing costs and enhancing farmer gains.

5 REFERENCES

Anuário Brasileiro da Maçã. (2018). Retrieved from: http://www.editoragazeta.com.br/sitewp/wp-content/uploads/2018/06/Ma%C3%A7%C3%A3_2018_Site-Editora.pdf

Batalha, M. O., Buainain, A. M., & Souza Filho, H. M. (2005). Tecnologia de gestão e agricultura familiar. In: Batalha, M. O. (Ed.). *Gestão do agronegócio: textos selecionados*. São Carlos, SP: EDUFSCAR.

Beling, R. R. (2022). *Anuário Brasileiro de Horti & Fruti 2022*. Santa Cruz do Sul, RS: Editora Gazeta Santa Cruz.

Bittencourt, D. (2017). *Agricultor familiar: entenda como inovações tecnológicas podem te ajudar*. Retrieved from: http://digital.agrishow.com.br/agricultor-familiar-entenda-como-inovacoes-tecnologicas-podem-te-ajudar/

Bittencourt, D. (2018). *Agricultura familiar, desafios e oportunidades rumo à inovação*. Brasília, DF: Embrapa. Retrieved from: https://www.embrapa.br/busca-de-noticias/-/noticia/31505030/artigo---agricultura-familiar-desafios-e-oportunidades-rumo-a--inovacao

Centro de Estudos Avançados em Economia Avançada (CEPEA). (2018). *Produção da safra 2017/18 deve ser menor*. Retrieved from: https://www.cepea.esalq.usp.br/br/diarias-de-mercado/maca-cepea-producao-da-safra-2017-18-deve-ser-menor.aspx

Duarte, J. H. S. et al. (1992). *Poda e condução em macieiras*. Porto Alegre, RS: Fundação Gaúcha do Trabalho e Ação Social.

Food and Agriculture Organization of the United Nations (FAO). (2023). *Faostat – FAO statistical database*. Retrieved from: https://www.fao.org/faostat/en/#home

Instituto Brasileiro de Geografia e Estatística (IBGE). (2022). *Produção de Maçã*. Retrieved from: https://www.ibge.gov.br/explica/producao-agropecuaria/maca/br

Klesener, H. M. (2020). *Para além da geração de renda: os significados da fruticultura para os agricultores familiares de Santa Helena–PR*. Thesis (Master's). Universidade Estadual do Oeste do Paraná, Programa de Pós-Graduação em Desenvolvimento Rural Sustentável, Marechal Cândido Rondon.

Mathias, J., & Rufato, A. R. (2017). *Como Plantar Maçã*. Retrieved from: https://revistagloborural.globo.com/vida-na-fazenda/como-plantar/noticia/2017/05/como-plantar-maca.html

Petri, J. L., Sezerino A. A., & Martin, M. S. (2018). *Artigo exclusivo Todafruta: estado atual da cultura da macieira*. Caçador, SC: EPAGRI. Retreived from: http://www.todafruta.com.br/artigo-exclusivo-estado-atual-da-cultura-da-macieira/

Revista da Fruta. (2022). *Satélite confirma: SC é o maior produtor nacional de maçã*. Retrieved from: http://banca.maven.com.br/pub/revistadafruta/?numero=32#page/1

Ribeiro Filho, J. R., & Tahim, E. F. (2022). Inovação e contingencialidade na agricultura familiar. *Revista Gestão & Conexões*, *11*, 87-107.

Souza Filho, H. M. S. *et al.* (2011). Condicionantes da Adoção de Inovações Tecnológicas na Agricultura. *Caderno de Ciências & Tecnologia*, *28*, 223-255.

Souza, P. M. *et al.* (2019). Diferenças regionais de tecnologia na agricultura familiar no Brasil. *Revista de Economia e Sociologia Rural*, *57*, 594-617.

Vieira, L. P. L., Bahiense, D. V., & Silva, S. M. (2019). Produção acadêmica sobre sucessão rural e agricultura familiar: uma análise do contexto brasileiro do período (2003-2018). *Extensão Rural*, *26*, 89-103.

doi:10.14720/aas.2025.121.1.16550 Original research article / izvirni znanstveni članek

Evaluation of salinity tolerance in seedlings of *Iris* × *germanica* L. hybrids

Mohammad Hossein AZIMI [1, 2], Asghar EBRAHIMI [3], Mohammadreza SHAFIEI [4], Zeynab HAMZEHEI [5]
Pegah SAYYAD-AMIN [6]

Received November 08, 2023; accepted December 14, 2024
Delo je prispelo 8. november 2023, sprejeto 14. december 2024

Evaluation of salinity tolerance in seedlings of *Iris* × *germanica* L. hybrids

Abstract: Salinity is an abiotic stress that primarily impacts plant development and agricultural productivity worldwide and typically occurs in arid and semi-arid areas. Less research has been done on the impact of salt irrigation on the growth and development of ornamental plants, particularly-plants with bulb . In order to identify salt-tolerant in *Iris* × *germanica* L. genotypes (four genotypes including OPRC 14,18, 23 and 54), an experiment was carried out with four NaCl levels (4 as control, 6, 8, and 12 dS m^{-1}). The variation among genotypes caused different responses to salinity conditions. The results showed that the morphological, physiological, and biochemical characteristics of OPRC23 genotype were superior to those of other genotypes. The highest peroxidase enzyme activity was observed at 8 dS m^{-1} salinity level. The highest content of chlorophyll a, b, and carotenoid was obtained at a salinity level of 6 dS m^{-1} (NaCl). The OPRC54 genotype had the highest levels of chlorophyll a, b, and proline content at 12 dS m^{-1} salinity. In conclusion, different levels of salinity can expose to different genotypes, which leads to the selection of specific salt tolerant genotypes. The genotypes examined in this experiment of Iris germanica were resistant to salinity stress and this trait is of interest to landscape designers.

Key words: kperoxidase enzyme, carotenoid, proline content, chlorophyll content

Ovrednotenje tolerance na slanost pri sejankah križancev nemške perunike (*Iris* × *germanica* L.)

Izvleček: Slanost je abiotski stres, ki prvenstveno prizadane razvoj rastlin in ogroža kmetijsko pridelavo širom po svetu in se značilno pojavlja v sušnih in polsušnih območjih. Relativno manj raziskav je bilo opravljenih o vplivu namakanja s slano vodo, še posebno tistih na rastlinah z gomolji, korenikami in čebulicami. Z namenom ugotoviti toleranco na slanost pri genotipih nemške perunike (*Iris* × *germanica* L.) (štiri genotipi ko so obsegali OPRC 14,18, 23 in 54) je bil izveden poskus s štirimi ravnmi NaCl (4 kot kontrola, 6, 8, in 12 dS m^{-1}). Variabilnost med genotipi je povzročila različen odziv na slanostne razmere. Rezultati so pokazali, da je bil genotip OPRC23 glede na mofološke, fiziološke in biokemijske lastnosti najboljši. Največja aktivnost peroksidaze je bila ugotovljena pri slanosti 8 dS m^{-1}. Največja vsebnost klorofilov a, b in karotenoidov je bila pri slanosti 6 dS m^{-1}(NaCl). Genotip OPRC54 je imel največjo vsebnost klorofilov a, b, in prolina pri slanost 12 dS m^{-1}. Zaključimo lahko, da ispostavitev različnih genotipov različnim nivojem slanosti vodi k selekciji genotipov s specifično odpornostjo na slanost. V tem poskusu preučevani genotipi nemške perunike so bili odporni na slanostni stres in bi lahko bili zanimivi v tem pogledu za krajinske arhitekte.

Ključne besede: peroksidaza, karotenoidi, vsebnost prolina, vsebnost klorofila

1 Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran
2 Corresponding author: .azimi58@gmail.com
3 Municipality of Mahallat, Iran
4 Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran
5 Horticultural Science, Department of Agricultural Organization, Qom, Iran.
6 Department of Horticultural Science and landscaping of Ferdowsi University of Mashhad, Iran.

1 INTRODUCTION

Iris × *germanica* L. is one of the most important varieties of the bearded iris (Azimi et al., 2017), which is cultivated as a perennial ornamental plant (Azimi et al., 2017). In previous studies, new genotypes of *Iris* × *germanica* with a broad color range and different shapes have been recognized in Iran (Azimi et al., 2017) and the genetic difference between 43 F1 new hybrids and introduced superior hybrids has been evaluated (Azimi et al., 2018). Colorful flowers, rhizome propagation, resistance to calcareous soils, tolerance to undesirable environmental conditions, and low water requirements of that species are the causes for their selection for landscape design (Azimi, 2015).

In arid and semi-arid areas, salinity is a common abiotic stressor that affects plant development and agricultural productivity (Evelin and Kapoor, 2009; Evelin et al., 2012; Porcel et al., 2012). Around the world, it has been estimated that salinity affects roughly 7 % of agricultural crops; it has also been anticipated that the increase in salinity will cause the loss of 36 % of arable land in the ensuing 27 years (Evelin and Kapoor, 2009; Procel et al., 2012; Ruiz-Lozano et al., 2012; Kapoor et al., 2013). When the plants are exposed to high salt concentrations, plants' growth is distrurbed because of toxicity of Na^+ and Cl^- ions (Fatma et al., 2016). The main cause of the declines in growth and yield under salinity is the significant accumulation of Na^+ ions in plant's tissues (Munns & Tester, 2008). Salt stress affects levels of growth regulators and metabolic enzyme activity, damages membranes, impairs enzyme performance, and disturbs the nutritional balance of minerals (Munns & Tester, 2008; Hasanuzzaman et al., 2013). Therefore, these physiological changes result in reduced cell division, expansion, or promotion of cell death and induce a decrease in growth rate and yield; they also destroy chlorophyll in leaves, which leads to leaf senescence (Rahemi et al., 2017). Physiological and biochemical processes like ion balance, seed germination, osmotic management, photosynthesis, respiration, and nitrogen metabolism are disrupted as soil salinity rises (Kaya et al., 2009; Porcel et al., 2012). The first effect of salinity is osmotic stress; further, oxidative stress occurs due to the accumulation of reactive oxygen species (ROS), including superoxide, hydroxyl radicals, and peroxide (Noctor et al., 2014) that have detrimental effects on normal cell growth and metabolism (Aroca et al., 2013). Increased Na^+ and Cl^- absorption leads to disruption in the potassium, phosphorus, calcium, and nitrogen ions' absorption (Ulczycka-Walorska et al., 2020).

Regarding the cultivation of ornamental plants in landscaping, it is necessary to use salinity-resistant ornamental species or induce resistance traits to these stresses through plant breeding and physiological methods (Bayat et al., 2013). Considering the effects of salinity stress on vegetative traits in ornamental plants including petunia, tagetes, scarlet sage, snapdragon, madagascar periwinkle, marigold, and cockscomb, it was shown that marigold is more tolerant to salinity and the sodium accumulation is less than the others (Saki, 2014). Bayat et al. (2013) reported that the flower number and diameter of *Gerbera aurantiaca* Sch. Bip. exposed to salinity decreased compared to control plants. In another study by Wen Yuan et al. (2012), root and leaf relative water content, stomatal conductivity, and the rates of net photosynthesis and evapotranspiration of *Iris lactea* Pall. var. chinensis seedlings declined under salt stress.

According to above references, *I.* x *germanica* as a perennial plant with a beautiful appearance and a high compatiblity with salinity, is an appropriate selection for cultivation in saline conditions. Less research has been done on the impact of salt irrigation on the growth and development of ornamental plants, particularly bulbous plants. Therefore, due to the drought and salinity crisis, which are considered limiting factors for landscape development, the physiological and morphological study of that species is important. The specific objective of the present study was to the effect of saline irrigation water or soil sanity on the performance of iris.

2 MATERIALS AND METHODS

This experiment was carried out at the municipality research farm of Mahallat, Iran; with latitude = 33°54'N, longitude = 50°27'E and 1600 m above sea level. The seeds of *Iris* × *germanica* L. genotypes were obtained from the "Ornamental Plants Research Center (OPRC) of Mahallat, Iran". The genotypes of *Iris germanica* were encoded as OPRC14, OPRC18, OPRC23, and OPRC54. For rapid and uniform germination, seeds were stratified in wet cocopeat at 4-6 °C for 45 days (Azimi et al., 2016). The seeds were cultivated in a cultivation tray and kept in a greenhouse with 70 ± 5 % relative humidity and 25 ± 5 °C conditions. The seedlings were transplanted at the three-leaf stage into the pots (in late April, 2017). Then, the uniform seedling genotypes were selected and transplanted into the pots with 20 cm in diameter and 40 g in mass filled with loamy soil, rotten animal manure, and compost with the ratio of 1:1:1, then transferred to open space. Amount of soil per pot was 2 kg. The NPK fertilizer was used with the ratio of 18:18:18.The single salt (NaCl) was

Table. 1: The chemical characteristics of water used in the salinity experiment.

pH	EC (dS m^{-1})	Nitrate (mg l^{-1})	Calcium (mg l^{-1})	Magnesium (mg l^{-1})	Bicarbonate (mg l^{-1})	Carbonate (mg l^{-1})	Sodium (mg l^{-1})	Chlorophyllorine (meq l^{-1})
7.5	363	20	32	12	70.15	0	16	24.85

Con. Table 1: The physical and biochemical characteristics of soil used in the experiment.

Depth (cm)	EC (Ec × 10 dS/cm)	Saturation (pH)	Organic carbon (%)	Total N (%)	Phosphorous (ppm)	Potassium (ppm)	Texture	Clay (%)	Silt (%)	Sand (%)
	7.44	7.5	4	0.4	300	3000	Loam	28.5	20	51.5

applied. The amount of water/ irrigation/pot was 400 ml.The experiment consisted of four salinity concentrations [EC = 4 dS m^{-1}, and electrical conductivity (EC) at 6, 8, and 12 dS m^{-1}] with three replicas (Table 1).

The salt for the salinity treatments was added gradually to the soil with irrigation water at 7-day intervals. To prevent salt accumulation, the pots were leached twice a week. The fresh water and soil characteristics are given in tables 1 and 2. Three months after the salinity application, the plants were harvested. Leaf freshness, leaf length and width, leaf area, plant height, water relative content, leaf cell membrane permeability (electrolyte leakage), proline, chlorophyll, and carotenoids content, and peroxidase enzyme activity were measured.

2.1 MEASUREMENTS OF PARAMETERS

The leaf length, width, area, and number, plant height and plant diameter of the *Iris × germanica* genotypes seedlings were measured at the beginning and end of the experiment. The rate of these changes was determined using the information gathered at the beginning and end of salt stress treatments. After the leaves were detached from the roots and thoroughly rinsed with deionized water, the mass of the freshly harvested leaves was ascertained. The plant samples were oven-dried for 24 hours at 60 °C, and the dry mass was recorded.

2.2 MEASUREMENT OF CHLOROPHYLL A, B, TOTAL CHLOROPHYLL AND CARTENOIDS CONTENTS

The chlorophyll content was determined by rinsing the leaves in an 80 % acetone solution and measuring absorbance at 645 and 663 nm with a spectrophotometer (Nazemi Rafi et al., 2019). The carotenoids content of petals and leaves was measured at 480 and 510 nm, and estimation values were calculated using the following formula:

Chlorophyll a mg g^{-1} = 12.7(A663) - 2.69 (A645) × V/1000 × 10

Chlorophyll b mg g^{-1} = 22.9(A645) - 4.68 (A663) × V/1000 × 10

Total Chlorophyll mg g^{-1} = 20.2(A645) - 8.02 (A663) × V/1000 × 10

Carotenoids mg g^{-1} = 7.6(OPRC5480) - 1.49 (A510) × V/1000 × 10

2.3 PEROXIDASE ACTIVITY

The Guaiacol technique was used to measure the peroxidase (POD) activity (Guan et al., 2015). For three minutes, the variations in 470 nm absorbance were used to follow how well guaiacol was being oxidised. 50 ml of 100 mM PBS (pH 6.0), 19 ml of 30 % H_2O_2, and 28 l of guaiacol comprised the reaction mixture solution. The enzyme extract was added to the reaction mixture solution to initiate the reaction.

POD activity was calculated using the following equation:

POD activity (ΔOPRC54$_{70}$ min·g FM^{-1}) = ΔOPRC54$_{70}$ × V_T M^{-1} × V_S × t. ΔOPRC54$_{70}$: the changes of absorption; were: V_T: total volume of the extracted solution; V_S: volume of enzyme solution for testing; M: the mass of samples."

2.4 PROLINE CONTENT

The method developed by Bates et al. (1973) was used to measure the proline content in the leaves.

Each of the four replications' fresh leaves (1.0 g) were homogenised in 10 ml of 30 ml l^{-1} sulfosalicylic acid. Proline was measured spectrophotometrically using the extract.

2.5 STATISTICAL ANALYSIS

The experiment was conducted as a factorial design based on complete randomized block design with three replications, and in each replicate, 8 seedling genotypes were planted.

Using the "SAS statistical programme", data were examined by variance mean comparison and the "Duncan multiple range test".

3 RESULTS AND DISCUSSION

3.1 ANALYSIS OF VARIANCE

Variance analysis of interaction salinity and genotypes had a significant impact ($p \le 0.01$) on leaf length

Table 2: Mean comparison of genotypes and salinity levels on *Iris* × *germanica* characteristics.

Genotypes	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	PH (cm)	Leaf number	Plant diameter (cm)	LDM (g)
OPRC14	14.58d	0.91d	13.43d	14.57d	5.14c	2.79d	0.83b
OPRC18	19.43b	1.77b	34.53b	19.43b	6.54a	3.55b	0.91ba
OPRC23	21.84a	1.87a	40.32a	21.90a	6.61a	3.85a	1.11a
OPRC54	16.09c	1.32c	21.43c	16.09c	6.18b	2.87c	1.10a
Salinity							
*EC= 4 (control)	17.58b	1.35d	24.69c	17.61b	5.54c	3.23b	0.90a
*EC = 6	17.89ab	1.43c	26.92b	17.89ab	5.97b	3.22b	0.94a
*EC = 8	18.27a	1.49b	28.39a	18.24a	6.43a	3.3a	1.128a
*EC = 12	18.22a	1.56a	29.71a	18.22a	6.41a	3.3a	0.99a

Con. Table 2: Mean comparison of genotypes and salinity levels on *Iris* × *germanica* characteristics.

Genotypes	leaf fresh mass (g)	Proline content (µmol g^{-1} DM)	Chlorophyll a (µmol g^{-1} DM)	Chlorophyll b (µmol g^{-1} DM)	Total Chlorophyll (µmol g^{-1} DM)	cartenoids contents (µmol g^{-1} DM)	Peroxidase activity (µmol min^{-1} g^{-1} FM)
OPRC14	8.52c	0.89b	573.17c	695.67c	1793.24b	403.88c	0.43c
OPRC18	7.94c	0.91b	735.5b	878.91b	1812.86b	513.49b	0.67b
OPRC23	12.01a	0.85b	664.84bc	851.05bc	1515.99c	493.68bc	0.78a
OPRC54	10.38b	1.58a	923.42a	1142.19a	2065.62a	647.47a	0.5c
Salinity							
*EC= 4 (control)	9.07b	0.86c	723.35ab	876.29ab	1799.51a	521.40a	0.61ab
*EC = 6	10.12a	0.93bc	782.86a	971.35a	1754.30b	567.07a	0.54b
*EC = 8	10.71a	1.15ab	769.95a	969.66a	1739.61b	555.68a	0.64a
*EC = 12	8.95b	1.28a	620.78b	750.54b	1894.30a	414.37b	0.59ab

Mean value followed by the same letters in each column are not significantly different (Duncan multiple range test).

Table. 3: Mean comparison of interaction between genotype and salinity levels on *Iris × germanica* characteristics.

Genotype × salinity	leaf length (cm)	leaf width (cm)	leaf area (cm2)	PH (cm)	leaf number	plant diameter (cm)	Relative water content (%)	Electrolyte leakage (%)
OPRC14*EC = 4	14.07f	0.94h	13.37f	14.07f	4.5g	2.86c	74.33c	9.07c
OPRC14*EC = 6	13.81f	0.74i	10.25g	13.81f	4.91g	2.60d	94.85ab	9.79bc
OPRC14*EC = 8	15.39de	0.91h	14.12f	15.39de	5.58f	2.85c	0.99a	11.37ab
OPRC14*EC = 12	15.05e	1.05g	15.97f	15.05e	5.58f	2.85c	93.79ab	9.97bc
OPRC18*EC = 4	18.75c	1.7d	32.07d	18.75c	6.25bdec	3.50b	83.38bc	9.32c
OPRC18*EC = 6	19.58b	1.76dc	34.7dc	19.58b	6.5abc	3.50b	91.89ab	9.41c
OPRC18*EC = 8	19.41bc	1.80dc	34.96dc	19.41bc	6.75ab	3.58b	84.22bc	9.94bc
OPRC18*EC = 12	20.00b	1.81dcb	36.39c	20.00b	6.66abc	3.61b	86.92abc	9.86bc
OPRC23*EC = 4	21.33a	1.52e	32.54d	21.33a	6.00fdec	3.76a	85.26abc	10.42abc
OPRC23*EC = 6	22.00a	1.87bc	41.29b	22.00a	6.58abc	3.88a	97.87ab	12.00a
OPRC23*EC = 8	22.12a	1.92ab	42.65a	22.12a	6.83ab	3.58a	87.87abc	10.21bc
OPRC23*EC = 12	21.91a	2.03a	44.82a	21.91a	7.00a	3.88a	94.49ab	11.26ab
OPRC54*EC = 4	16.16d	1.28f	20.79e	16.16d	5.75fe	2.80c	92.60ab	9.82bc
OPRC54*EC = 6	16.16d	1.32f	21.45e	16.16d	5.83fde	2.90c	94.66ab	8.83c
OPRC54*EC = 8	16.16d	1.35f	21.83e	16.16d	6.58abc	2.92c	95.62ab	9.10c
OPRC54*EC = 12	15.93d	1.35f	21.66e	15.93d	6.41abcd	2.85c	89.40ab	8.92c

Con. Table 3: Mean comparison of interaction between genotype and salinity levels on *Iris × germanica* characteristics.

Genotype × salinity	leaf fresh mass (g)	Proline content (μmol g^{-1} DM)	Chlorophyll a (μmol g^{-1} DM)	Chlorophyll b (μmol g^{-1} DM)	Total chlorophyll (μmol g^{-1} DM)	cartenoids contents (μmol g^{-1} DM)	peroxidase (μmol min^{-1} g^{-1} FM)
OPRC14*EC = 4	7.15f	0.79dc	837.4abc	1014.1abcd	1857.2dce	538.11abc	0.45fg
OPRC14*EC = 6	8.20fe	0.91dc	734.8c	837.5dc	1529.6hg	481.59bc	0.42fg
OPRC14*EC = 8	9.61dec	0.93dc	732.9abc	899.2abcd	1632.1e-h	517.54abc	0.51fe
OPRC14*EC = 12	9.12fde	0.91dc	950.00ab	1204.00ab	2154.00ab	336.28d	0.33g
OPRC18*EC = 4	7.80fe	0.80dc	669.80c	809.70dc	2273.00a	469.42c	0.62dec
OPRC18*EC = 6	7.78fe	0.76dc	710.60bc	810.80dc	1521.40h	494.81bc	0.69abcd
OPRC18*EC = 8	8.36fe	0.86dc	849.60abc	1032.90abcd	1882.40dc	593.89abc	0.73abcd
OPRC18*EC = 12	7.81fe	1.22c	712.10bc	862.30bdc	1574.4 f-h	495.85bc	0.64bdec
OPRC23*EC = 4	10.69bdc	1.12dc	577.30c	699.10c	1276.3i	468.39c	0.79ab
OPRC23*EC = 6	13.28a	1.12dc	731.60abc	1033.10abcd	1765.00d-g	565.73abc	0.74a-d
OPRC23*EC = 8	13.67ab	0.65d	626.00c	1033.10abcd	1420.6h	565.73abc	0.84a
OPRC23*EC = 12	11.41abc	0.66d	724.60abc	877.50abcd	1602.00hgf	436/05c	0.77abc
OPRC54*EC = 4	10.64bdc	1.43a	808.90abc	982.30abcd	1791.2dfe	564.68abc	0.58fde
OPRC54*EC = 6	11.23abcd	1.07dc	977.20ab	1204.00ab	2201.2ab	726.15a	0.30g
OPRC54*EC = 8	12.21ab	1.001dc	871.44abc	1151.90abc	2023.34bc	606.75abc	0.49fe
OPRC54*EC = 12	7.45fe	1.82b	1016.20a	1230.60a	2246.8ab	692.28ab	0.63bdec

Mean value followed by the same letters in each column are not significantly different (Duncan multiple range test).

and width, plant height, plant diameter, chlorophyll a, b, total chlorophyll and carotenoids contents, proline content and peroxidase enzyme activity (Table. 3).

3.2 COMPARISON OF SALINITY LEVELS AND GENOTYPES

As shown in Table 2, the highest leaf length and width, leaf area, plant height, leaf number, plant diameter, electrolyte leakage, leaf fresh and dry mass, soluble protein, and peroxidase enzyme activity were observed in OPRC23 genotype seedlings. OPRC54 genotype seedlings had the highest relative water content, proline content, chlorophyll a, chlorophyll b, total and carotenoids contents. In morphological and physiological characteristics, OPRC23 was superior to other genotypes. The lowest morphological characteristics were observed in OPRC14 genotype seedlings. OPRC54 genotype seedlings were superior to other genotypes in biochemical characteristics, especially proline content; also, the lowest biochemical characteristics were observed in OPRC14 genotype seedlings. The highest leaf length, plant height, leaf number, plant diameter, electrolyte leakage, fresh leaf mass, and peroxidase enzyme activity were obtained at 8 dS m^{-1} salinity level. At 6 dS m^{-1} salinity, the highest relative water content, chlorophyll a, b, and carotenoid content were observed. The greatest leaf width, leaf area, total chlorophyll, and proline contents corresponded to a salinity level of 12 dS m^{-1}. There was no significant difference between salinity levels with soluble protein, electrolyte leakage, and leaf dry mass between *Iris* × *germanica* genotypes**.**

3.3 INTERACTION OF SALINITY AND GENOTYPES ON *IRIS* X *GERMANICA* CHARACTERISTICS

3.3.1 Growth characteristics

Reduced growth traits are one of the earliest impacts of salt stress on plants. Plant responses to salt stress can be separated into two phases (Munns, 2005). Osmotic stress causes the first stage of growth reduction to proceed more quickly. The accumulation of salt in leaves, which results in salt toxicity in the plants, initiates the second phase, which is a much slower process. During the study period, the NaCl treatments had a considerable impact on plant development (Table 1). Based on morphological characteristics, the highest leaf length, plant height, and leaf number at all salinity levels, especially at 8 dS m^{-1}, corresponded to OPRC23 genotype seedlings (Table 3). Increasing salinity levels reduced these characteristics in the OPRC54 genotype. The highest leaf width (2.03 cm) and leaf area (44.82 cm) were observed in the OPRC23 genotype at 12 d dS m^{-1}salinity level. OPRC23 and OPRC18 genotypes showed higher morphological characteristics in comparison to others under salinity conditions. Increasing salinity levels reduced the leaf width and leaf area of the OPRC54 genotype, so that increasing salinity up to 12 dS m^{-1} decreased the leaf length. Leaf area change is an important process that controls water consumption under stress conditions. Most of the plants tolerate salinity up to a certain threshold; at higher salinity levels, plant growth decreases linearly with increasing salinity (Amir-Jani, 2010). Plants' reduced ability to absorb water as a result of osmotic stress brought on by salt is the reason of the decrease in leaf surface area (Sarvandi et al., 2020).

Chlorophyllide and sodium ion absorption has an impact on hormone synthesis and transport between roots and shoots, which lowers leaf area, plant dry biomass, and specific leaf area (SLA) In addition to lowering LA, salinity inhibits the growth of the root system, delays the development of apical buds, and results in chlorophyllorosis with subsequent necrosis on the leaf edge (Oliveira et al., 2017).

The highest plant diameter (3.88 cm) was related to the OPRC23 genotype at 6 dS m^{-1} salinity level (Table 3). The results indicated that, while increasing salinity, the diameter of the plant was reduced; in this condition, the plants of the OPRC23 genotype were more able to absorb nutrients than other genotypes.

The fresh and dry mass of leaves decreased as salinity increased. These findings were consistent with previous research on different crop species of Chinese iris (Wang et al., 2012). OPRC23 genotypes had the highest fresh and dry mass of leaf. The highest leaf dry mass was obtained in OPRC23 genotypes (1.38 g) at 8 dS m^{-1} salinity level. The leaf dry mass of OPRC18, OPRC13, and OPRC54 seedlings decreased at salinity level of 12 dS m^{-1}. The highest fresh leaf mass belonged to OPRC23 genotype seedlings (13.28 g). At 12 dS m^{-1}, the leaf fresh mass of OPRC23 and OPRC54 decreased with increasing salinity (Table 3). As a general rule, the lowest morphological characteristics (plant height, leaf length, plant diameter, leaf number, and leaf fresh mass) were observed in OPRC14 and OPRC18, respectively. Dry matter reduction under stress conditions has been reported due to decreased leaf area index, photosynthesis rate, growth of aerial organs, and the relative growth rate of the plant (Soheili-Movahed et al., 2017). Fresh and dry mass of roots and shoots in response to high salt concentrations in Poa pratensis L. declined (Esmaeili & Salehi, 2016).

Vegetative growth, including leaf area, number of leaves, number of shoots, plant height and chlorophyll content, decreased in tuberose as the concentration of sodium chlorophylloride increased. Furthermore, the decrease in root biomass caused by salinity has been attributed to either direct Na or Cl toxicity or high Na content, which results in an inability to maintain cell turgor. Root elongation may have been reduced due to an inhibition of cell expansion as cell turgor decreased under salinity stress (Dlamini et al., 2019). Salinity stress decreased length and width of leaves, relative water content, and chlorophyll content (Naseri Moghadam et al., 2020), and salinity stress was more damaging to the growth, ornamental, and physiological traits of the *N. tazetta* L. flower than drought stress (Naseri Moghadam et al., 2020). A restriction in leaf expansion followed by a reduction in leaf area is one of the first signs of plants exposed to excessive salinity (Navarro et al., 2007). Changes in the cells and a decline in leaf turgor can both be used to explain it. Reduced cell elongation and cell division cause leaves to appear more slowly and to grow to a smaller size under salt stress. Leaves become smaller and thicker as a result of a shift in cell size that reduces area more than depth (Go´mez-Bellot et al., 2013).

3.3.2 Proline content

An organic osmolyte called proline is primarily found in protoplasm. Proline increases in response to osmotic stress caused by salt. It might be connected to their excellent salinity resistance (Guan et al., 2015). The maximum proline content at 12 dS m^{-1} salinity level was found in OPRC23 (1.82 µmol gr^{-1} DM) and OPRC18 (1.22 µmol gr^{-1} DM) genotype seedlings. The results indicate that a higher proline content was observed in OPRC54 genotype seedlings at all salinity levels. The proline content increased significantly with the increase in saline stress (Table 3). Proline content increases when the water potential of the leaf decreases, which leads to the maintenance of cellular turgor and reduces the damage to the membrane in the plant; therefore, with osmotic adjustment, tolerance to water stress increases (Rahdari and Hosseini, 2012). The rate of proline synthesis depends on the development of stress, the age of the plant organ, and genetic variation (Bajji et al., 2001). In this experiment, the proline content was different among the genotypes. The changes in proline concentration at different levels of salinity showed that with increasing salinity, the proline content of genotypes increased. However, the difference in proline content between sensitive and tolerant genotypes was statistically significant. Saneoka et al. (2004) reported that proline accumulation has a positive and direct relationship with increasing salinity and drought resistance in plants. Under salt stress, proline's primary functions include osmotic adjustment, protecting membranes and enzymes, and serving as a store of energy and nitrogen that can be used (Amini et al., 2015). A well-known defence mechanism against salt stress in plants is proline accumulation. Proline accumulation has also been proposed as a selection factor for salt tolerance due to the possibility that an increase in proline content will boost salt tolerance (García-Caparrós & Lao, 2018). Proline oxidase and proline dehydrogenase have higher inhibitory rates, which helps to explain why this accumulation occurs when exposed to salt stress. However, because proline breaks down quickly when stress is removed, it is also possible to discover a decrease in proline accumulation in plants. The breakdown products provide reducing agents that aid mitochondrial oxidative phosphorylation and ATP production for stress recovery and repair (García-Caparrós & Lao, 2018). The increase in proline concentration in the roots and leaves of all species exposed to salinity could be due to a decrease in proline degradation or an increase in proline biosynthesis (García-Caparrós et al., 2016).

3.3.3 Chlorophyll content

The chlorophyll a and b content was the highest in OPRC54 genotype seedlings at a salinity level of 12 dS m^{-1}. At a salinity level of 4 dS m^{-1}, the highest content of chlorophyll a and b belonged to OPRC14. Also, at 6 and 8 dS m^{-1} salinity levels, the highest content of chlorophyll a was observed in genotype OPRC54 (Table 3). The study of salinity stress in Crocus sativus L. revealed that at a salinity level of 6 dS m^{-1}, the chlorophyll a and b content decreased. A significant decrease in total chlorophyll content in the leaves as a result of increased salinity reduces photosynthesis and exacerbates stress-induced damage (Tuna et al., 2013). Changes in the content of photosynthetic pigments, according to some reports (Noreen & Ashraf, 2009), are also affected by plants' tolerance to soil salinity, i.e., their genotype. Most studies on plant responses to salinity conditions have found a decrease in total chlorophyll content in plant leaves as a result of salt stress (Silva et al., 2010). Under stress conditions, degradation of the pigment protein complex and the oxidative complex causes damage to the chloropplast lipids, pigments, and proteins (Tambussi et al., 2005). The stimulatory effect of salinity on leaf pigment content could be attributed to an increase in the number of chloroplasts per mesophyll cell. Under salinity stress, however, both epidermal and mesophyll thickness and intercellular spaces decrease in Bruguiera parviflora (Roxb.) Wight & Arn. ex Griff. (Salachna et al., 2016). In salt-treated hyacinth, the leaf content of chlorophyll a, chlorophyll b, and carotenoids decreased as NaCl concentration increased. In

Ornithogalum saundersiae Baker, the intensity of photosynthesis and transpiration decreased as NaCl concentration increased. The chlorophyll and carotenoids content of NaCl-treated Ornithogalum saundersiae plants was significantly higher than that of control plants (Salachna et al., 2016). The leaf greenness index and proline content in Hyacinthus orientalis L. were also higher when sodium chlorophylloride was applied to the soil on the day of bulb planting, and the length of the forcing period was longer (in the second season) (Ulczycka-Walorska et al., 2020; Salachna et al., 2016) and stage Calla lilies (Zantedeschia K. Koch) (Ayad et al., 2019).

The main photosynthetic pigments that play an important role in photosynthesis are chlorophyll a, chlorophyll b, and carotenoids. The main electron donor is chlorophyll a, while the primary accessory pigment for light harvesting and energy transfer is chlorophyll b.

Plant chlorophyll content is well known to be directly related to plant health (Barry, 2009). Chlorophyll concentration decreases in response to salt stress, which can be used as a sensitive indicator of cellular metabolic state. This drop could be due to membrane deterioration. Nonetheless, under salt stress, plants may exhibit an increase in chlorophyll concentration, which may be due to an increase in the number of chloroplasts per unit leaf area in the stressed plant leaves. This decrease in photosynthetic rate in plants under salt stress could be due to a number of factors, including: a) Dehydration of cell membranes reduces their permeability to CO_2, b) salt toxicity, c) reduction of CO_2 supply due to hydroactive closure of stomata, d) increased senescence caused by salinity, and e) changes in enzyme activity caused by changes in cytoplasmic structure. Under salt stress, stomatal conductance decreases, limiting the availability of CO_2 for carboxylation reactions. Furthermore, this stomatal closure reduces water loss through transpiration, which has an impact on light-harvesting and energy-conversion systems, resulting in a change in chlorophylloroplast activity (García-Caparrós and Lao, 2018).

Plants accumulatelow-molecular-mass compounds as a compatible solutes to adjust the osmotic potential of the cytoplasm during salt stress because they do not interfere with normal biochemical reactions. Nonetheless, producing enough osmotica is metabolically costly, potentially limiting plant growth by consuming large amounts of carbon that could otherwise be used for growth. Compatible solutes include proline, sugars, glycine-betaine, and other related quaternary ammonium compounds; however, due to a lack of data on the effects of salt stress on solute accumulation in ornamental plants, we will concentrate on soluble sugars and proline (García-Caparrós and Lao, 2018).

Another study found that stabilisation of chloroplasts is a well-known mechanism for increasing stress tolerance (Veatch-Blohm et al., 2019). Calcium is required for cell division, cell wall formation, cell signalling, and the permeability of the cell membrane to ions. It may be able to mitigate some of the negative effects of salinity by reducing the impact of Na on Ca homoeostasis (Veatch-Blohm et al., 2019). Dry mass of roots is regarded as an important parameter in the response to salt stress because the greater the root growth, the greater the water and nutrient uptake that can occur, favouring the accumulation of toxic ions in roots, particularly Na^+, and thus minimising its negative effects on shoot growth. In the presence of reduced stomatal aperture, these NaCl-induced anatomical changes may facilitate CO_2 reaching the chloroplast more efficiently. These modifications appeared to be an adaptive response to protect the photosynthetic process (Acosta-Motos et al., 2015).

3.3.4 Carotenoid content

The highest carotenoid content was present in OPRC54 genotype seedlings at 6 dS m^{-1} salinity level, and the lowest carotenoid content belonged to the OPRC14 genotype seedlings at 14 dS m^{-1} salinity level. The maximum carotenoid content was observed in OPRC54 genotype seedlings at different salinity levels (Table 3). One of the plant salt resistances under salinity conditions is carotenoid content (Juon et al., 2005). Muller et al. (2010) found that salinity stress increased carotenoid content while decreasing chlorophylls a and b. This was consistent with the findings of this study on salt stress. These findings could be attributed to stressed leaves increased thickness and compacted mesophyll cells, resulting in more chloroplasts per unit area, as is often the case under stress conditions (Sarvandi et al., 2020). Controlling the salt concentration of plant aerial parts, limiting entry through the roots, and limiting transport to the shoots (by retaining these ions in the root and lower stem) is an important mechanism that allows plant survival and growth in a saline environment (Álvarez and Sánchez-Blanco, 2015).

K^+ and Na+ are important in plant growth and development, but they are also important in maintaining osmotic adjustment and cell turgor. Furthermore, plants irrigated with saline water had lower K^+/Na^+ and $K^+/Na+$ ratios, which correlated with lower biomass production (Álvarez and Sánchez-Blanco 2015). A large number of enzymes were inhibited, and several aspects of plant physiology were disrupted due to high NaCl, including photosynthesis and pigment synthesis (Munns & Tester, 2008; Cirillo et al., 2016).

3.3.5 Peroxidase activity

At 8 dS m^{-1} salinity level, OPRC23 genotype seedlings had the highest peroxidase activity (0.84 mol min^{-1}.mg $protein^{-1}$). At 6 dS m^{-1} salinity, OPRC54 genotype seedlings had the lowest peroxidase activity (0.30 mol min^{-1}.mg $protein^{-1}$). The lowest carcinoid content was observed in OPRC14 genotype seedlings at different salinity levels (Table 3). Plants tend to counteract reactive oxygen species produced by stress (Ahmad et al., 2012, 2011; Bano et al., 2013; Kaya et al., 2013). Plants subjected to salt stress exhibited up-regulation of the antioxidant defence system (Hussain et al., 2016). According to these studies, salinity increased the activity of peroxidase enzymes in salt-sensitive cultivars. Salinity inhibits cell division and also reduces cell size, resulting in a reduction in plant length. These stresses generate ROS compounds, which cause protein, lipid, carbohydrate, and nucleic acid damage. Plants use enzymatic (catalase, superoxide dismutase, and so on) and non-enzymatic (phenolic compounds and carotenoids) defence systems to scavenge and detoxify these compounds from the cell surface (Naseri Moghadam et al., 2019).

Plants commonly respond to saline conditions by accelerating the generation of reactive oxygen species (ROS), which include the cytotoxic superoxide radical (O_2), singlet oxygen ($^1O_2$), hydroxyl radical (OH^-), and hydrogen peroxide (H_2O_2). The mitochondria, chloroplasts, and peroxisomes are the primary sources of ROS generation in the cell. These reactive oxygen species participate in a variety of processes, including DNA damage, lipid peroxidation, and protein oxidation.

Plants have an antioxidative machinery composed of enzymatic and non-enzymatic components such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POX), and catalase (CAT) to mitigate the harmful effects of ROS at the cellular level (Garca-Caparrós and Lao, 2018). In contrast to what happened with CAT activity, myrtle plants experienced a sharp decline in APX activity during the experimental period. APX and CAT appear to compensate each other to some extent, as evidenced by increased CAT expression in knock-out cytAPX *Arabidopsis* plants in response to light stress. At the end of the recovery period, APX, despite showing low activity data, is partially recovered in S4 plants compared to control plants but not in S8 plants. NaCl has been shown to reduce APX activity in other plant species, including grapevine plantlets in vitro. A link has been established between CAT activity and photosynthesis because an increase in CAT reduces CO_2 photorespiratory loss (Acosta-Motos et al., 2015).

Increased CAT and SOD levels were observed in salt-stressed *Eugenia* plants at 44 and 88 mM, particularly in recovered plants. The response of CAT activity suggested that the photorespiratory pathway could be induced under salinity conditions, whereas SOD is thought to be the first line of defence against oxidative stress in plants. From the decarboxylation of glycine in the mitochondria, photorespiration can provide electron acceptors to PSI and CO_2 for the chloroplast. Furthermore, a close relationship between CAT activity and photosynthetic rate has been described. Increased CAT activity has been found to limit the H_2O_2-dependent decarboxylation of the keto-acids glyoxylate and hydroxypyruvate in the peroxisome, thereby reducing CO_2 photorespiratory loss (Acosta-Motos et al., 2015).

4 CONCLUSION

The results showed that the morphological, physiological, and biochemical characteristics of OPRC23 genotypes were superior OPRC54 genotype. The OPRC54 genotype had the highest levels of chlorophyll a, b, and proline content at 12 dS m^{-1} salinity. In conclusion, different levels of salinity can expose to different genotypes, which leads to the selection of specific salt tolerant genotypes. The genotypes examined in this experiment of *Iris × germanica* were resistant to salinity stress and this trait is of interest to landscape designers.

5 STATEMENTS & DECLARATIONS

5.1 FUNDING

There is no funding with this work. The original data were obtained from Ornamental Plants Research Center (OPRC), Horticultural Sciences Research Institute (HSRI), Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran

5.2 COMPLIANCE WITH ETHICAL STANDARDS (CONFLICT INTEREST)

The authors declare that they have no conflict of interest.

6 REFERENCES

Acosta-Motos, J.R., Diaz-Vivancos, P., & A´lvarez, S., et al. (2015). Physiological and biochemical mechanisms of the ornamental *Eugenia myrtifolia* L. plants for coping with NaCl stress and recovery. *Planta,* 1-15. https://doi.org/10.1007/s00425-015-2315-3.

Ahmad, P, Ashraf, M., Azooz, M.M., Asool, S., & Akram, N.A. (2012). Potassium starvation-induced oxidative stress and antioxidant defense responses in *Brassica juncea*. *Journalof Plant Interaction.* https://doi.org/10.1080/17429145.2012.747629.

Amir-Jani, M.R. (2010). Effect of NaCl on some physiological parameters of rice. *EJBS, 3(*1), 6-16.

Aroca, R., Ruiz-Lozano, J.M., & Zamarrenoamarreno Angel, M et al. (2013). Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. *Journal of Plant Physiology, 170,* 47–55. https://doi.org/10.1016/j.jplph.2012.08.020.

Ayad, J.Y., Othman, Y.A., & Al Antary, T.M. (2019). Irrigation water salinity and potassium enrichment influenced growth and flower quality of Asiatic lily. *Fresenius Environ Bulletin, 28*(11), 8900-8905.

Azimi M.H. (2015). An introduction of genetic inbreeding and new cultivars production of *Gladiolus grandiflorus.* Technical J Publications Organization Study. *Education, Promotion and Agriculture,* 1-22.

Azimi, M.H., Jozghasemi, S., & Barba-Gonzalez, R. (2018). Multivariate analysis of morphological characteristics in *Iris germanica* hybrids. *Euphytica 214*(161), 1-11. https://doi.org/10.1007/s10681-018-2239-7.

Azimi, M.H., Jozghasemi, S., & Edrisi, B. (2017). Diversity induction in flower color of *Iris germanica* through hybridization. *Journalof Iranian Society for Ornamental plants, 92,* 22-91.

Azimi, M.H., Sadeghyan, S.Y., Beyramizadeh, E., & Kalate-Jari, S., Tahernezhad, Z. (2010). Study of genetic variation among Iranian irises species using morphological characteristics. *Iranian Journal of Horticultural Science and Technology, 11*(1), 71-86.

Bano, S., Ashraf, M., & Akram, N.A. (2013) Salt stress regulates enzymatic and non-enzymatic antioxidative defense system in the edible part of carrot (*Daucus carota* L.). *Journal of Plant Interaction, 9*(1), 324–329. https://doi.org/10.1080/17429145.2013.832426.

Bates, L.S., Waldren, R.P., & Teare, I.D. (1973). Rapid determination of free proline water stress studies. *Plant Soil, 39,* 205-207.

Bayat, H., Alirazaie, M., Neamati, H., & Abdollahisaadabad, A. (2013). Effect of silicon on growth and ornamental traits of salt-stressed calendula (*Calendula officinalis* L.). *Journal of Ornamental Plants 3*(4), 207-214.

Chen, Q., Tao, S., Bi, X., Xu, X., Wang, L., & Li, X. (2013). Research progress in physiological and molecular biology mechanism of drought resistance in rice. *American Journal of. Molecular Biology, 3*(2), 102-107.

Cirillo, C., Rouphael, Y., & Caputo, R. et al. (2016). Effects of high salinity and the exogenous application of an osmolyte on growth, photosynthesis, and mineral composition in two ornamental shrubs. *The Journal of Horticultura Science and Biotechnology, 91*(1), 14–22. http://dx.doi.org/10.1080/14620316.2015.1110988.

Dlamini, B.B., Wahome, P.K., Masarirambi, M.T., Oseni, T.O., & Nxumalo, K.A. (2019). Effects of Salinity on the Vegetative Growth of Tuberose (*Polianthes tuberosa* L.). *Journal of Horticultural Science of Ornamental Plants, 11*(2), 144-151. https://doi.org/10.5829/idosi.jhsop.2019.144.151.

Esmaeili, S., & Salehi, H. (2016). Kentucky bluegrass (*Poa pratensis* L.) silicon-treated turfgrass tolerance to short- and long-term salinity condition. *Advances in Horticultural Science, 30*(2), 87-94.

Fatma M, Masood A, Per TS, Rasheed F, & Khan NA (2016) Interplay between nitric oxide and sulfur assimilation in salt tolerance in plants. *J Crop, 4,* 153-161.

García-Caparrós, P., & Lao, M.T. (2018). The effects of salt stress on ornamental plants and integrative cultivation Practices. *Scientia Horticulturae 240*, 430–439. http://dx.doi.org/10.1016/j.scienta.2016.01.031.

García-Caparrós, P., Llanderal, A., & Pestana M et al. (2016) Tolerance mechanisms of three potted ornamental plants grown under moderate salinity. *Scientia Horticulturae 201,* 84–91.

Go´mez-Bellot, M.J., Alvarez, S., & Castillo, M et al. (2013). Water relations, nutrient content and developmental responses of Euonymus plants irrigated with water of different degrees of salinity and quality. *Journal of Plant Research, 126,* 567–576. https://doi.org/10.1007/s10265-012-0545-z.

Guan, G., Wang, Y., Cheng, H., Jiang, Z., & Fei, J. (2015) Physiological and biochemical response to drought stress in the leaves of *Aegiceras corniculatum* and *Kandelia obovata*. *Ecotoxicology, 24*(7), 1668-1676.

Hasanuzzaman, M., Nahar, K., & Fujlta, M. (2013). Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages, In: Ahmad P, Azooz M.M, Prasad M.N.V, (Eds.), *Ecophysiology and responses of plants under salt stress*. Springer, 25-87. New York.

Hussain, S., Khan, F., Cao, W., Wu, L., & Geng, M. (2016). Seed priming alters the production and detoxification of reactive oxygen intermediates in rice seedlings grown under suboptimal temperature and nutrient supply. *Frontiers in Plant Science, 7*(439), 1-17.

Jozghasemi, S., Rrabiei, V., Soleymani, A., & Khaligi, A. (2015). Evaluation of the pigments concentration in the iris species native to Iran. J.Biodiversity. *Environmental Science, 6(1)*, 557-561.

Juon, M., Rivero, R.M., Romero, L., & Ruiz, J.M. (2005). Evalution of some nutritional and biochemical indicator selecting salt-resistant tomato cultivars. *Journal of Environment and Experimenalt Botany,* 193-201.

Kaya, C., Ashraf, M., & Sonmez, O. et al. (2009). The influence of arbuscular mycorrhizal colonisation on key growth parameters and fruit yield of pepper plants grown at high salinity.

Scientia Horticulturae, 121, 1–6. https://doi.org/10.1016/j.scienta.2009.01.001.

Kaya, C., Sonmez, O., & Aydemir, S. et al. (2013). Exogenous application of mannitol and thiourea regulates plant growth and oxidative stress responses in salt-stressed maize (*Zea mays* L.). *Journal of Plant Interaction, 8,* 234-241.

Mandhania, S., Madan, S., & Sawhney, V. (2006). Antioxidant defense mechanism under salt stress in wheat seedlings. *Journal of Plant Biology, 50,* 227-231.

Muller, T., Luttchwager, D., & Lentzsch, P. (2010). Recovery from drought stress at the shooting stage in oil seed rape (*Brassica napus* L.). *Journal of Agronomyand Crop Science, 196*(2), 81-89.

Munns, R., & Tester, M. (2008). Mechanism of salinity tolerance. *The Annual Review of Plant Biology, 59,* 651-681.

Naseri Moghadam, A., Bayat, H., Aminifard, M.H., & Moradinezhad, F. (2019). Effect of Drought and Salinity Stress on Growth, Flowering and Biochemical Characteristics of *Narsicuss tazetta* L. *Journal of Horticultural Science, 33*(3), 451-466.

Naseri Moghadam, A., Bayat, H., Aminifard, M.H., & Moradinezhad, F. (2020). Effects of drought and salinity stresses on some morphological and physiological characteristics of *Narcissus tazetta* L. flower. *Journal of Horticultural Science, 51*(1), 79-90. https://doi.org/ 10.22059/ijhs.2018.264753.1507.

Nazemi Rafi, Z., Kazemi, F., & Tehranifar, A. (2019). Morphophysiological and biochemical responses of four ornamental herbaceous species to water stress. *Acta Physiologiae Plantarum, 41*, 6-13.

Noctor, G., Mhamdi, A., & Foyer, C.H. (2014). The roles of reactive oxygen metabolism in drought: not so cut and dried. *Plant Physiology, 164,* 1636–1648. https://doi.org/10. 1104/pp.113.233478.

Noreen, Z., & Ashraf, M. (2009). Changes in antioxidant enzymes and some key metabolites in some genetically diverse cultivars of radish (*Raphanus sativus* L.). *Environmental and Experimental Botany, 67,* 395-402.

Noreen, Z., Ashraf, M., & Akram, N.A. (2010). Salt-induced regulation of some key antioxidant enzymes and physiobiochemical phenomena in five diverse cultivars of turnip (*Brassica rapa* L.). *Journaof Agronomy and Crop Science, 196,* 273-285.

Oliveira, F.I.F., JF de Medeiros, W., F. de Lacerda, C.L.R., Neves, A.R., & Oliveira, D. (2017). Saline water irrigation managements on growth of ornamental plants affecting their morphology or even reducing plant survival. *Revista Brasileira de Engenharia Agricola e Ambiental, 21*(11), 739-745. http://dx.doi.org/10.1590/1807-1929/agriambi.

Porcel, R., Aroca, R., & Ruiz-Lozano, J.M. (2012). Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. *Agronomy dor Sustainable Development, 32*, 181–200. https://doi.org/10. 1007/s13593-011-0029-x.

Rahdari, P., & Hoseini, S.M. (2012). Salinity stress: a review. *Technical Journal of Engineering. Applied Science, 1*(3), 63-66.

Rahemi, M., Karimi, S., Sedaghat, S., & Rostami, A.A. (2017). Physiological responses of olive cultivars to salinity stress. *Advances in Horticultural Science, 31*(1), 53-59.

Rostami, M., Mohammad-Parast, B., & Golfam, R. (2015). The effect of different salinity levels on some physiological characteristics of *Crocus sativus* L. *Journal of Saffron Agriculture and Technology, 3*(3), 173-179.

Ruiz-Lozano, J.M., Porcel, R., Azcon, C., & Aroca, R. (2012). Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants. New challenges in physiological and molecular studies. *Journal of Experi Botany, 63,* 33-44.

Saeed, R., Mirza, S., & Ahmad, R. (2014). Electrolyte leakage and relative water content as affected by organic mulch in okra plant (*Abelmoschus esculentus* (L.) Moench) grown under salinity. *Fuuast Journal of Biology, 4*(2), 221-227.

Saki, R. (2014). The effect of salinity on some annual ornamental plants. Master's thesis. Tabriz university of Iran.

Salachna, P., Zawadzińska, A., & Podsiadło, C. (2016). West Pomeranian University of Technology in Szczecin. 2016. Response of Ornithogalum saundersiae bak. to salinity stress. *Acta Scie Poland Hortor Cultus, 15*(1), 123-134.

Saneoka, H., Moghaieb, R.E.A., Premachandra, G.S., & Fujita, K. (2004). Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostis palustris*. *Environmental and Experimental Botany, 52,* 131-138.

Sarvandi, S., Ehtesham Nia, A., Rezaei Nejad, A., & Azimi, M.H. (2020). Morpho-Physiological Responses of Some Iris Cultivars under Drought and Salinity Stresses. *Journal of Agriculture Science andTechnology, 22*(2), 535-546.

Silva, E.N., Ribeiro, R.V., Ferreira-Silva, S.L., Viegas, R.A., & Silveira, J.A.G. (2010). Comparative effects of salinity and water stress on photosynthesis, water relations and growth of *Jatropha curcas* plants. *Journal of Arid Environmnet, 74,* 1130-1137.

Soheili-Movahed, S., Esmaili, M.A., Jabbari, F., & Fooladi, A. (2017). Evaluation of Yield and Yield Components of Some Pinto bean (*Phaseolus vulgaris* L.) Genotypes under Late Season Water Deficit Conditions. *Journal of Agroecology, 9*(2), 433-444.

Tambussi, E.A., Noques, S., & Araus, J.L. (2005). Ear of durum wheat under water stress: water relations and photosynthetic metabolism. *Planta, 221,* 446-458.

Tiwari, J.K., Munshi, A.D., Kumar, R., Pandey, R.N., Arora, A., Bhat, J.S., & Sureja, A.K. (2010). Effect of salt stress on cucumber: Na^+/K^+ ratio, osmolyte concentration, phenols and Chlorophyll content. *Acta Physiologiae Plantarum, 32,* 103-114.

Tuna, A.L., Kaya, C., Altunlu, H., & Ashraf, M. (2013). Mitigation effects of non-enzymatic antioxidants in maize (*Zea mays* L.) plants under salinity stress. *Australian Journal of Crop Science, 7*(8), 1181-1188.

Ulczycka-Walorska, M., Krzyminska, A., Bandursk, H., & Bocianowski, J. (2020). Response of Hyacinthus orientalis L. to salinity caused by increased concentrations of sodium chlorophylloride in the soil. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48*(1), 398-405. https://doi.org/10.15835/nbhOPRC548111748.

Veatch-Blohm, M.E., Roche, B.M., & Sweeney, T. (2019). The effect of bulb weight on salinity tolerance of three common Narcissus cultivars. *Scientia Horticulturae, 248*, 62–69.

Veatch-Blohm, M.E., & Morningstar, L. (2011). Calla lily growth and development under saline irrigation. *Horticultural Science, 46*(2), 222-227.

Wang, W.Y., Yan, X.F., Jian, Y., Qu, B., & Xu, Y.F. (2012) Effects of salt stress on water content and photosynthetic characteristics in *Iris acteal* var. *Chinensis* seedlings. *Middle-East Journal of Scientific Research, 12*(1), 70-74.

doi:10.14720/aas.2025.121.1.18753 Original research article / izvirni znanstveni članek

Effect of fertilisers on yield, water consumption and energy capacity of grain sorghum (*Sorghum bicolor* L.)

Vadym IVANINA [1, 2], Yevheniia IONITSOI-DOTSENKO [3], Oksana STRILETS [1], Galyna SINCHUK [1], Stanislav ORLOV [1], Olesia ZINCHENKO [1], Oleksandr HANZHENKO [1], Oleksandr KHIVRYCH [1], Galyna MAZUR[4]

Received May, 21, 2024; accepted February 05, 2025
Delo je prispelo 21. maj 2024, sprejeto 05. februar 2025

Effect of fertilisers on yield, water consumption and energy capacity of grain sorghum (*Sorghum bicolor* L.)

Abstract: This study aimed to determine how fertiliser rates affect biological yield, water use, and energy capacity of grain sorghum. Field experiment was conducted at the Uladovo-Lyulynetsk Research and Selection Station (49° 35' N, 28° 24' E) in 2017–2021. Trial was arranged using a randomized experimental design in four replications with a seeding plot area of 62 m^2, a harvesting area of 50 m^2. The investigated factors were: control, without fertiliser, winter wheat straw at the rate of 4 t ha^{-1} as a background, mineral fertilisers at rates of $N_{60}P_{60}K_{60}$, $N_{90}P_{90}K_{90}$, $N_{120}P_{120}K_{120}$ and the estimated rate of $N_{105}P_{35}K_{60}$. Grain sorghum produced high yields of biomass on black soils against the background of natural fertility with a yield of grain 5.96 t ha^{-1}, stems 30.3 t ha^{-1}. Application straw and mineral fertilisers ensured efficient moisture consumptions by plants. Estimated fertilisers rate $N_{105}P_{35}K_{60}$ against the background of 4 t ha^{-1} of straw provided similar productivity of sorghum with the maximal rate of $N_{120}P_{120}K_{120}$ and can be recommended for fertilising as more cost-effective. This fertilisation increased grain yield by 35 %, bio-ethanol output by 41 %, solid biofuel by 17 %, total energy output by 23 %.

Key words: grain sorghum, fertiliser rates, productivity, biofuel

Učinek gnojil na pridelek, porabo vode in vsebnost energije pri navadnem sirku za zrnje (*Sorghum bicolor* L.)

Izvleček: Namen raziskave je bil določiti učinek odmerkov gnojil na biološki pridelek, porabo vode in energetsko vsebnost navadnega sirka za zrnje. Poljski poskus je potekal na Uladovo-Lyulynetsk Research and Selection Station (49° 35' N, 28° 24' E) v rastnih sezonah 2017–2021. Poskus je potekal kot naključni poskus s štirimi ponovitvami s površino setve 62 m^2 in površino žetve 50 m^2. Obravnavanja so obsegala: kontrola brez gnojil, slama ozimne pšenice v odmerku 4 t ha^{-1} kot ozadje, mineralna gnojila v odmerkih $N_{60}P_{60}K_{60}$, $N_{90}P_{90}K_{90}$, $N_{120}P_{120}K_{120}$ in ocenjen odmerek $N_{105}P_{35}K_{60}$. Sirek za zrnje je imel velik pridelek biomase na črnih tleh kot ozadju naravne rodovitnosti s pridelkom zrnja 5.96 t ha^{-1} in stebel 30.3 t ha^{-1}. Uporaba slame in mineralnih gnojil je zagotovila zadostno vlago za potrebe rastlin. Ocenjen odmerek gnojila $N_{105}P_{35}K_{60}$ in uporaba slame v odmerku 4 t ha^{-1} sta dala podobno produktivnost sirka z največjo vrednostjo z odmerkom mineralnih gnojil $N_{120}P_{120}K_{120}$, kar bi lahko priporočili za cenovno učinkovito gnojenje. Takšno gnojenje je povečalo pridelek zrnja za 35 %, bioetanola za 41 %, goriva iz slame za 17 % in celokupni energetki izkopiček za 23 %.

Ključne besede: sirek za zrnje, odmerki gnojil, produktivnost, biogorivo

1 Institute of Bioenergy Crops and Sugar Beet (IBCSB), Kiev, Ukraine
2 correspondening author, e-mail: v_ivanina@ukr.net
3 National Scientific Center «Institute of agricultural economics», Kyiv, Ukraine
4 Uladovo-Lyulynetsk Research and Selection Station, Vinnitsa Region, Ukraine

1 INTRODUCTION

The problem of food and energy resources are the main challenges of the modern economy. Rapid population growth and the reduction of fossil fuels require alternative solutions capable of ensuring a delicate balance between rising living standards and the ability to satisfy the population with food and energy. A series of recent EU legislative decisions, summarized in the "European Climate Law", outlines a new map of Europe's climate neutrality, which envisages a phase-out of fossil fuels and a full transition to renewable energy sources by 2050 (Chiaramonti et al., 2021).

Ukraine lags far behind in the use of renewable energy, but large areas of land, high soil fertility, favorable climatic conditions and geographical location make it a promising player in the market of cheap energy carriers. The energy strategy of Ukraine until 2035, approved by the resolution of the Cabinet of Ministers of Ukraine dated August 18, 2017 No. 605-r, provides for increasing the share of energy obtained from renewable sources by 25 % by 2030 (Energy…, 2017).

According to Sinchenko et al. (2020), in recent years, special interest in Ukraine has focused on the production of bio-ethanol, which is planned to increase to 12 million tons by 2030.

The production of bio-ethanol requires the cultivation of highly productive and undemanding crops, among which grain sorghum deserves special attention. Grain sorghum, originating from the African continent, has genetically inherited high drought resistance, unpretentiousness to soil and climatic conditions, has high productivity, which guarantees stable biomass yields in conditions of global warming (Stamenkovic et al., 2020).

At the same time, grain sorghum, like other crops, needs sufficient supply of moisture and nutrients, particular nitrogen (Abunyewa et al., 2017). Insufficient supply of sorghum plants with water and nitrogen limits its growth, decreases yield (Obour et al., 2022), deteriorates grain quality (Modisapudi & Sebetha, 2022); the acute moisture deficit stagnates plant growth and increases nutrition requirement (Schlegel & Bond 2020). To obtain a high biological yield, grain sorghum requires the application of 70-90 kg ha^{-1} (Masebo & Menamo, 2016; Munagilwar et al., 2020; Kovalenko, 2023) to 160 kg ha^{-1} of nitrogen (Bartzialis et al., 2023; Said et al., 2023).

Organic-mineral fertilisations are much more effective in increasing the yield of grain sorghum (Kedir, 2023). The combined application of organic and mineral fertilisers has a complex effect on the soil, improves its structure and moisture supply, forms a balanced nutrition of plants in terms of macro- and micronutrients, increases the resistance of plants to drought, which is the key to obtaining stable crops in the era of global warming (Hu et al., 2018; Lu 2020, Mujdeci et al., 2020). According to Teshome et al. (2023), the application of mineral fertilisers in combination with manure in Western Ethiopia provided the highest grain sorghum yield of 5.09 t ha^{-1} with an increase compared to the control by 84.2 %.

Winter wheat straw is often used to fertilise agricultural crops. This practice is widespread in Ukraine, where there is an acute shortage of manure, while the area sown with winter wheat exceeds 6.5 million hectares with an annual straw production of over 33 million tons. For most crops, winter wheat is a good predecessor, and grain sorghum is mainly grown after it (Malyarchuk, 2019).

According to Liu et al. (2017) use of straw as a fertiliser has economic advantages over manure, it is cheaper to produce, its application does not require costs associated with transporting fertiliser to the field as is the case with manure and therefore it is more cost-effective. Application of straw in the semi-arid regions of China at the rate of 4.5-13.5 t ha^{-1} had a versatile positive effect on the soil and sorghum yield increased microbial activity in the 0-60 cm soil layer by 19.6-44.3 %, water use efficiency - by 15.7-34.6 %, grain yield by 10.6-22.8 % (Zhang et al., 2016).

According to Poliovyi et al. (2021), straw is inferior to manure in terms of filling the soil with nutrients. At a dose of straw of 5 t ha^{-1}, nitrogen enters the soil - 27 kg ha^{-1}, phosphorus - 15, potassium - 62 kg ha^{-1}. Compared to manure (30 t ha^{-1}), it is 5,6 times less for nitrogen, 5.0 times less for phosphorus and 2.9 times less for potassium, and therefore the application of mineral fertilisers is effective when applying straw into the soil.

The use of mineral fertilisers for grain sorghum against a background of straw is a little-studied issue that requires research and is relevant for obtaining high biomass yields.

This study aims to answer: (1) How fertiliser rates applied against background of straw affect biological yield, water use, and energy capacity of grain sorghum? To determine the optimal rate of fertilisers that ensure the maximum biological and energy productivity of grain sorghum.

2 MATERIALS AND METHODS

Field experiment during research years of 2017-2021 was conducted at the Uladovo-Lyulynetsk Research and Selection Station (49° 35' N, 28° 24' E). Trial was organized using a randomized experimental design in four replications with a seeding plot area of 62 m^2, a harvesting area of 50 m^2. The investigated factors were:

control, without fertiliser, winter wheat straw at the rate of 4 t ha^{-1} as a background, mineral fertilisers at rates of $N_{60}P_{60}K_{60}$, $N_{90}P_{90}K_{90}$, $N_{120}P_{120}K_{120}$ and the estimated rate of $N_{105}P_{35}K_{60}$.

Soil of the experimental site was leached chernozem, loamy composition, with agrophysical and agrochemical properties of the 0–30 cm soil layer: pH_{KCl} – 5.8-6.1, organic matter – 3.9-4.0 % (DSTU 4289:2004), easily hydrolyzed nitrogen – 140-145 mg (DSTU 7863:2015), mobile P_2O_5 – 133-137 and K_2O – 82-88 mg kg^{-1} of the soil (DSTU 4115-2002) (Soils, 2002, 2004, 2015).

Hybrid 'Dniprovskyi 39' was sown at first decade of May. An early-ripening hybrid matures in 100-110 days with a potential yield of over 8 t ha^{-1}. Predecessor of grain sorghum was winter wheat 'Bogdana'.

Sorghum was harvested at the beginning of September under grain moisture of 14 %. The content of starch was determined by a polarimeter, protein by the content of total nitrogen, determined by the Kjeldahl method (DSTU 7169-2010) with subsequent conversion to protein.

The Kalynivka meteorological station provided weather data.

To determine soil water reserves (SWR), soil samples were taken twice (sowing and harvesting) from the 0-150 cm layer.

The formula for determining the water use efficiency (WUE index) (m^3 t^{-1}):

$$WUE = (SWR_s - SWR_h + P) \times 10 / Y,$$

where SWR_s – soil water reserves at sawing, SWR_h – at harvest, mm; P – precipitation, mm; 10 – conversion coefficient, mm into m^3; Y – total yield (grain and stems), t ha^{-1} dry biomass.

The IBCSB methodology was used to determine the output of bio-ethanol, solid fuel and energy from sorghum grain (Roik et al., 2020).

The formula that defines bio-ethanol output:

$$Me = Y \times d \times S \times r \times f / 10000,$$

where Me – bio-ethanol, t ha^{-1}; Y – grain yield, t ha^{-1}; d – grain dry matter, %; S – starch content, %; r – the ratio of the molecular weight of ethanol to starch (0.5679); f – coefficient of bio-ethanol output at the factory (0.9).

The formula that defines solid biofuel output:

$$Ms = Ys \times d \times (100 + h) / 10000,$$

where Ms – solid biofuel, t ha^{-1}; Ys – stems yield, t ha^{-1}; d – dry matter, %; h – humidity coefficient of 10 %.

The heat of combustion for solid biofuel – 16 GJ t^{-1}, bioethanol – 25 GJ t^{-1} was used to calculate the energy output.

2.1 STATISTICAL ANALYSIS

All obtained data were analyzed with the technique of analysis of variance (ANOVA). Significant differences between individual means were assessed using the least significant difference test (*LSD*, $p < 0.05$). Microsoft Excel, version 2013, (USA) was used to determine correlation-regression dependencies between research data.

2.2 METEOROLOGICAL CONDITIONS

During 2017-2021, weather conditions were favorable for growing grain sorghum. 2017 was warm and uneven in precipitation distribution. The average daily temperature during the growing season exceeded the long-term average by 1.4 °C, the amount of precipitation was 37 mm less. In May, June and July, precipitation fell by 27, 36 and 52 mm more than the average multi-year norm, in August and September – by 47 and 25 mm less (Figure 1 and 2). The year 2018 was the warmest during the research period, the growing season was marked by an excess of the average long-term temperature by 2.9 °C with the amount of precipitation within the normal range (397 mm). 2019 was a warm year, with the average daily temperature exceeding the long-term norm by 1.9 °C. Precipitation corresponded to the norm with an uneven distribution during the growing season. May and June were excessively wet – the amount of precipitation exceeded the long-term norm by 52 and 61 mm, July-August were dry – 108 mm less precipitation fell in three months. 2020 was slightly dry. The average daytime temperature exceeded the norm by 1.4 °C, the amount of precipitation during the growing season was 45 mm less. A decrease in precipitation was marked in August by 20 mm and in September by 14 mm. Weather conditions the year 2021 best corresponded to the multi-year average. The amount of precipitation during the growing season was within the long-term norm – 389 mm, the temperature was higher by 0.6 °C.

3 RESULTS AND DISCUSSION

The research results showed that grain sorghum responded positively to the application of fertilisers. In the control without fertilisers and with application of 4 t ha^{-1} of straw, the yield of grain in average for research years was the lowest of 5.96 and 6.13 t ha^{-1}, respectively (Table 1). With rate of fertilisers $N_{60}P_{60}K_{60}$ over the background of 4 t ha^{-1} of straw, the yield increased compared to control without fertilisers by 0.85 t ha^{-1} or by 14.3 %, $N_{90}P_{90}K_{90}$ – by 1.78 t ha^{-1} or by 29.9 %, $N_{120}P_{120}K_{120}$ – by

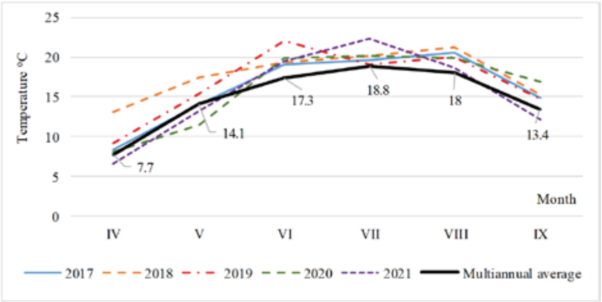


Figure 1: Average daily temperature during the growing season

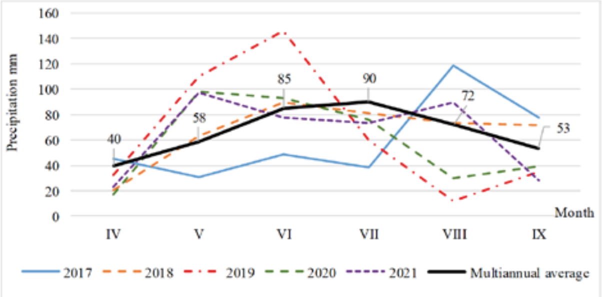


Figure 2: Amount of precipitation during the growing season

2.14 t ha^{-1} or 35.9 %, estimated rate of $N_{105}P_{35}K_{60}$ – by 2.06 t ha^{-1} or 34.6 %. All rates of mineral fertilisers statistically significantly ($p < 0.05$) increased grain yield of sorghum. The highest yield value ensured fertilisers rate of $N_{120}P_{120}K_{120}$ and estimated rate of $N_{105}P_{35}K_{60}$ applied over the background of 4 t ha^{-1}, it was amounted to 8.10 and 8.02 t ha^{-1}, respectively. Application of high rates of nitrogen fertilizers of 105 to 120 kg ha^{-1}, which is observed in these options, was decisive in obtaining a high yield of grain sorghum. In years with regular moistening during the growing season (2017-2018), mineral fertilisers showed maximum efficiency, the increase in grain yield compared to the control without fertilisers was 2.80-3.26 t ha^{-1}, in dry years (2019-2020) the effectiveness of fertilisers decreased significantly, the increase in grain yield was 0.91-1.87 t ha^{-1}, which was half as much. These results are consistent with the findings of Munagilwar et al. (2020), Bartzialis et al. (2023), Kovalenko (2023) on the effectiveness of high nitrogen doses of 70 to160 kg ha^{-1} for this crop.

The application of fertilisers substantially affected the yield of sorghum stems. An increase in the rate of fertilisers from $N_{60}P_{60}K_{60}$ to $N_{120}P_{120}K_{120}$ on the background of 4 t ha^{-1} of straw was accompanied by an increase in the yield of stems compared to the control without fertilisers by 2.8-4.7 t ha^{-1}. The maximum yield of 35.2 and 35.0 t ha^{-1} was provided by the fertilizer rate $N_{120}P_{120}K_{120}$ and the estimated rate $N_{105}P_{35}K_{60}$ against the background of 4 t ha^{-1} of straw. Fertilisers reliably increased stem yield compared to the control without fertilisers by 15.5-16.2%, which is consistent with the studies of Said et al. (2023), where the highest stem yield of 16.8 t ha^{-1} was achieved at nitrogen fertiliser rate of 150 kg ha^{-1}. Weather conditions in the years of research had a less pronounced influence on the yield of stems compared to their influence on the yield of grain. The maximum effectiveness of fertiliser application was achieved in the year 2017, which was favorable in terms of moisture, the application of high rates of fertilisers $N_{120}P_{120}K_{120}$ and $N_{105}P_{35}K_{60}$ this year increased the yield of stems compared to the control without fertilisers by 4.7-4.9 t ha^{-1}, while in the dry year of 2020 only by 2.5-2.8 t ha^{-1}.

The quality of the grain of sorghum plants depends on the accumulation of protein in it. The application of fertilisers significantly increased the protein content in sorghum grain: at rate of $N_{60}P_{60}K_{60}$ by 0.6 %, $N_{90}P_{90}K_{90}$ by 1.5 %, $N_{120}P_{120}K_{120}$ by 1.8 %, calculated rate of $N_{105}P_{35}K_{60}$ by 1.5 %, if compared to the control without fertilisers. The years of research had little effect on the quality of sorghum grain.

The starch content in sorghum grain is an indicator of its quality and a source of bio-ethanol production. The lowest starch content was recorded in the control without fertilisers – 63.2 % and against the background of straw application – 63.5 %. Fertilisers applied against the background of 4 t ha^{-1} of straw have reliably increased the accumulation of starch in sorghum grains by 2.9-4.4 %. At fertilisers rate $N_{60}P_{60}K_{60}$ starch content was 66.1 %, $N_{90}P_{90}K_{90}$ 67.0 %, $N_{120}P_{120}K_{120}$ – 67.6 %, estimated rate $N_{105}P_{35}K_{60}$ – 67.3 %. Fertilisers in the dose of $N_{120}P_{120}K_{120}$ and the estimated dose of $N_{105}P_{35}K_{60}$ maximally contributed to the increase in starch content. Weather conditions had an insignificant effect on the accumulation of starch in sorghum grains; application of fertilizers proportionally increased its content through the years of research.

The productivity of grain sorghum and the stability of obtaining crops largely depends on the accumulation of moisture in the soil and the efficiency of its use by plants during the growing season. According to Souza et al. (2021), water stress caused by water deficit in the pre-flowering stage of grain sorghum can reduce its yield by 45 %, and after the post-flowering stage by more than 48 %.

On average, for 2017-2021, the SWR in the spring during the sowing of grain sorghum was 229-244 mm in a 1.5 m soil layer (Figure 3). In the control without fertilisers, the moisture content in the soil was the lowest, and with the application of straw and mineral fertilisers, the SWR increased by 10-15 mm. The accumulation of moisture in the soil was facilitated by the application of winter wheat straw, while the application of mineral fertilisers

Table 1: Effect of fertilisers on biomass yield and grain quality of grain sorghum

Treatment	Years 2017	2018	2019	2020	2021	Mean	SD	CV
Grain yield, t ha^{-1}								
Without fertilisers (control)	6.80	6.22	5.25	5.47	6.06	5.96	0.62	10.4
4 t ha^{-1} of straw (fond)	6.96	6.50	5.44	5.68	6.08	6.13	0.61	10.0
$N_{60}P_{60}K_{60}$	8.05	7.50	5.53	6.23	6.76	6.81	1.00	14.7
$N_{90}P_{90}K_{90}$	9.39	8.69	6.04	7.02	7.55	7.74	1.33	17.2
$N_{120}P_{120}K_{120}$	10.06	9.06	6.20	7.38	7.79	8.10	1.50	18.5
estimated fertiliser rate	9.78	9.02	6.16	7.34	7.80	8.02	1.42	17.7
LSD ($p < 0.05$)	0.74	0.66	0.52	0.60	0.47	0.64		
Stems yield, t ha^{-1}								
Without fertilisers (control)	33.7	32.0	27.3	28.8	29.7	30.3	2.55	8.42
4 t ha^{-1} of straw (fond)	32.3	32.1	26.7	28.4	29.0	29.7	2.43	8.20
$N_{60}P_{60}K_{60}$	36.2	35.8	29.5	30.6	33.4	33.1	3.01	9.09
$N_{90}P_{90}K_{90}$	37.7	36.4	31.2	31.3	35.4	34.4	2.99	8.69
$N_{120}P_{120}K_{120}$	38.4	37.2	32.6	31.6	36.2	35.2	2.96	8.40
estimated fertiliser rate	38.6	36.2	32.0	31.3	36.9	35.0	3.19	9.11
LSD ($p < 0.05$)	2.44	2.52	2.06	2.12	2.33	2.14		
Content of protein, %								
Without fertilisers (control)	9.5	9.6	11.0	10.7	9.7	10.1	0.70	6.90
4 t ha^{-1} of straw (fond)	9.4	9.6	10.6	9.5	9.9	9.8	0.48	4.95
$N_{60}P_{60}K_{60}$	9.9	10.2	11.4	11.1	10.9	10.7	0.63	5.87
$N_{90}P_{90}K_{90}$	10.4	11.3	12.3	11.8	12.2	11.6	0.78	6.71
$N_{120}P_{120}K_{120}$	10.7	11.7	12.5	12.1	12.0	11.8	0.68	5.75
estimated fertiliser rate	10.4	11.5	12.5	11.9	11.7	11.6	0.77	6.62
LSD ($p < 0.05$)	0.17	0.20	0.27	0.23	0.28	0.24		
Content of starch, %								
Without fertilisers (control)	64.6	64.0	62.4	62.7	62.3	63.2	1.04	1.64
4 t ha^{-1} of straw (fond)	64.8	64.7	62.4	63.0	62.6	63.5	1.16	1.83
$N_{60}P_{60}K_{60}$	67.0	66.9	65.8	65.1	65.7	66.1	0.82	1.24
$N_{90}P_{90}K_{90}$	67.6	67.8	66.2	66.0	67.4	67.0	0.84	1.25
$N_{120}P_{120}K_{120}$	67.9	68.0	67.2	67.1	67.8	67.6	0.42	0.62
estimated fertiliser rate	67.9	67.8	66.4	67.0	67.4	67.3	0.62	0.92
LSD ($p < 0.05$)	0.43	0.37	0.40	0.34	0.42	0.40		

SD–standard deviation; CV–coefficient of variation

had an insignificant effect on its reserves. Our data are consistent with the studies of Wang et al. (2016), where the application of 15-23 t ha^{-1} of manure under corn crops formed high moisture reserves in the soil at the stage of tassel formation, which may be a consequence of improving the soil structure and increasing its ability to retain moisture.

At harvest, SWR in the 1.5 m soil layer decreased by 1.93-2.46 times. The smallest SWR was recorded for the fertiliser rate $N_{120}P_{120}K_{120}$ and the estimated rate $N_{105}P_{35}K_{60}$, it amounted to 99 and 101 mm, respectively. In these options, grain sorghum yield was the highest, which required additional moisture inputs.

During the growing season, grain sorghum used

118-145 mm of moisture from the soil, which was 24-28 % of the plant's moisture needs, the remaining 72-76 % of the needs was covered by precipitation, which averaged 378 mm over the years of research.

The yield of dry biomass (grain and stems) of grain sorghum was the lowest in the control without fertilisers and for the application of 4 t ha^{-1} of straw –12.6 and 12.7 t ha^{-1}, respectively, and the highest was for the fertilisers rate of $N_{120}P_{120}K_{120}$ and the estimated rate of $N_{105}P_{35}K_{60}$ – 15.7 and 15.4 t ha^{-1}. The use of fertilisers increased the yield of dry biomass compared to the control without fertilizers by 1.22-1.25 times (Figure 4). These results are consistent with the findings of Abunyewa et al. (2017) on a reliable increase in sorghum yield under sufficient supply of water and nutrients.

The calculation of moisture consumption for the formation of 1 ton of dry biomass (WUE index) reflects the efficiency of moisture use by plants. In the control without fertilisers, WUE was the highest – 394 m^3, and the lowest at fertilisers rate of $N_{120}P_{120}K_{120}$ and the estimated dose of $N_{105}P_{35}K_{60}$ – 333 and 337 m^3, respectively. Application of mineral fertilisers reduced moisture consumption for the formation of one ton of biomass compared to the control without fertilizers by 61 and 57 m^3. High rates of mineral fertilisers $N_{120}P_{120}K_{120}$ and $N_{105}P_{35}K_{60}$, which provided increased nitrogen nutrition, resulted in the lowest WUE. These results are consistent with the research of Bastaubayeva et al. (2022) on the effectiveness of high fertiliser rates in reducing water consumption by sugar beet.

Grain sorghum is a valuable bioenergy crop for the production of bio-ethanol. Bio-ethanol is obtained from sorghum grain, its output depends on the yield of the grain and its starch content. On average, over the years of research, the lowest output of bioethanol was recorded in the control without fertilisers – 1.66 t ha^{-1}, which was

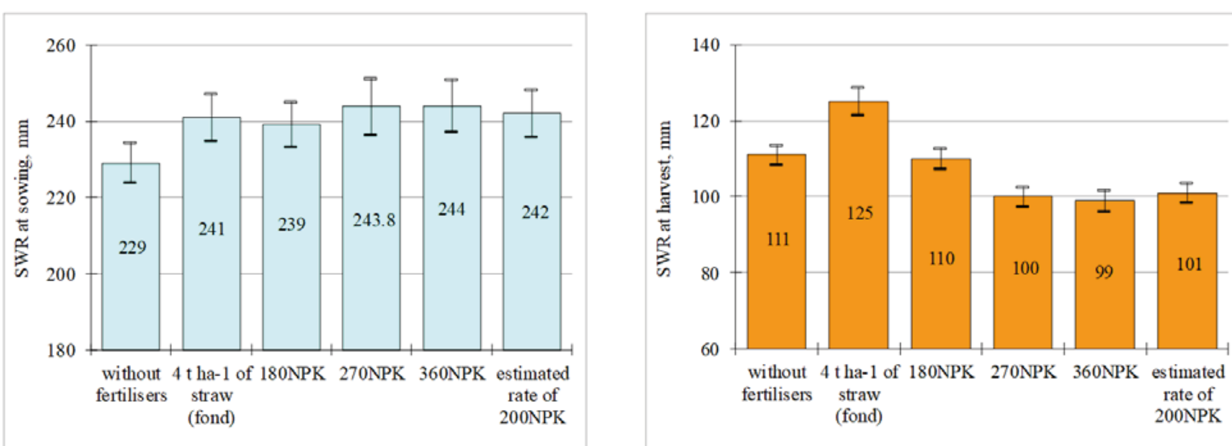


Figure 3: Effect of fertilisers on SWR in 1.5 m soil layer, mm, 2017–2021 years

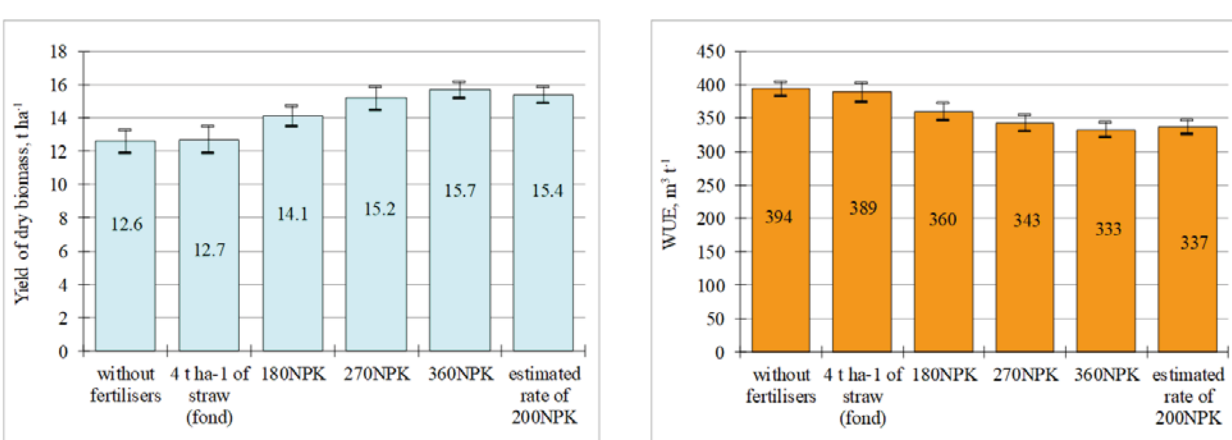


Figure 4: Effect of fertilisers on sorghum dry biomass yield and WUE index, 2017–2021 years

Table 2: Effect of fertilisers on the biofuel output and energy capacity of grain sorghum, 2017-2021 years

Treatment	Biofuel t ha-1		Energy GJ ha^{-1}		Total energy GJ ha^{-1}
	bio-ethanol	solid biofuel	bio-ethanol	solid biofuel	
Without fertilisers (control)	1.66	8.20	41.5	131	172.5
4 t ha^{-1} of straw (fond)	1.71	8.16	42.8	131	173.8
$N_{60}P_{60}K_{60}$	1.98	9.08	49.5	145	194.5
$N_{90}P_{90}K_{90}$	2.28	9.44	57.0	151	208.0
$N_{120}P_{120}K_{120}$	2.41	9.60	60.3	154	214.3
estimated fertiliser rate	2.34	9.56	58.5	153	211.5
LSD ($p < 0.05$)	0.19	0.26	4.7	5.1	6.4
Mean	2.06	9.01	51.6	144	195.8
SD	0.33	0.67	8.20	10.67	18.79
CV	15.9	7.4	15.9	7.4	9.6

SD–standard deviation; CV–coefficient of variation

28 % of the grain yield. The highest output of bio-ethanol was obtained by applying fertilisers rate of $N_{120}P_{120}K_{120}$ and the estimated rate of $N_{105}P_{35}K_{60}$ against the background of 4 t ha^{-1} of straw – 2.41 and 2.34 t ha^{-1}, respectively. The application of fertilisers increased the output of bio-ethanol compared to the control without fertilisers by 0.68-0.75 t ha^{-1} or 41-45 % (Table 2). These results are consistent with the findings of Gamayunova et al. (2022), Pravdyva et al. (2022), where application fertilisers under grain sorghum increased bio-ethanol output by 33-74 %.

When producing bio-ethanol, grain sorghum stems can be reliable source for production of solid biofuel. Research results showed that the output of solid biofuel from sorghum stems was 4.0-6.4 times higher than the output of bio-ethanol from grain. The rate of fertilisers $N_{120}P_{120}K_{120}$ and the estimated rate $N_{105}P_{35}K_{60}$ against the background of 4 t ha^{-1} of straw provided the maximum yield of solid biofuel of 9.60 and 9.56 t ha^{-1} with an increase compared to the control without fertilisers by 17 %.

Energy capacity of grain sorghum is the energy that is concentrated in bioethanol and solid biofuel and is the result of their combustion. In the current experiment, 72-76 % of the total energy was concentrated in solid biofuel, 24-28 % in bio-ethanol. Fertiliser application increased energy storage in sorghum plants compared to the unfertilised control by 22.0–41.8 GJ ha^{-1}, to the fond of 4 t ha^{-1} of straw – by 20.7-40.5 GJ ha^{-1}. The highest energy capacity of sorghum was recorded when applying fertilisers at the rate of $N_{120}P_{120}K_{120}$ and the estimated rate of $N_{105}P_{35}K_{60}$ against the background of 4 t ha^{-1} of straw, it was 214.3 and 211.5 GJ ha^{-1}, respectively.

It was established that the highest accumulation of energy per one kilogram of nutrients (NPK) of mineral fertilisers was achieved with the application of an estimated rate of $N_{105}P_{35}K_{60}$ against the background of 4 t ha^{-1}

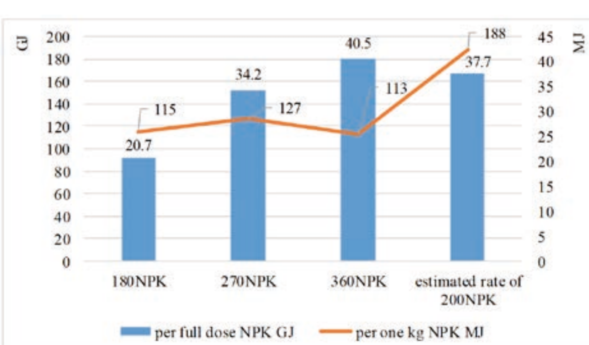


Figure 5: Total energy increase from mineral fertilisers application, 2017–2021 years

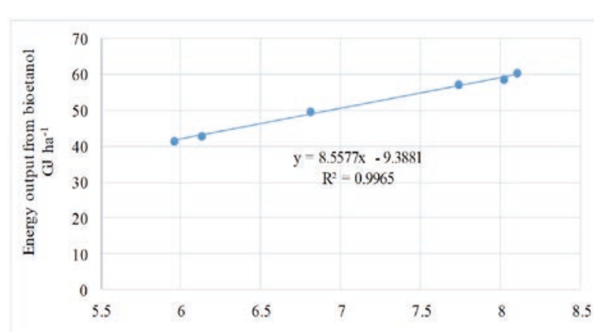


Figure 6: Correlation between grain yield and energy output from bio-ethanol, 2017–2021 years

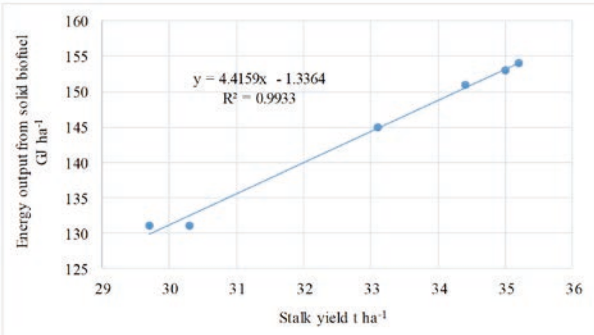


Figure 7: Correlation between stem yield and energy output from solid biofuel, 2017–2021 years

of straw, it amounted to 188 MJ (Figure 5). This rate of fertilisers was the most effective in the formation of high-energy capacity of grain sorghum.

A strong linear correlation was established between grain yield and output of energy from bioethanol, stalk yield and energy output from solid biofuel with a coefficient of determination of 0.9965 and 0.9933, respectively (Figure 6 and 7).

4 CONCLUSION

1. Grain sorghum produces high yields of biomass on black soils, which is a raw material for the production of bioethanol and solid biofuel. Against the background of natural fertility, the yield of sorghum grain was 5.96 t ha^{-1}, stems 30.3 t ha^{-1}, from which 1.66 t ha^{-1} of bio-ethanol and 8.2 t ha^{-1} of dry biofuel can be obtained, with a total energy capacity of 172 GJ ha^{-1}.

2. It is advisable to grow grain sorghum after winter wheat. The use of wheat straw for fertiliser increases the accumulation of moisture in the soil, and its application with mineral fertilisers ensures economical and efficient use of moisture by plants.

3. Estimated fertilisers rate $N_{105}P_{35}K_{60}$ against the background of 4 t ha^{-1} of straw provided almost the same productivity of sorghum with the maximal rate of $N_{120}P_{120}K_{120}$ and can be recommended for fertilising as more cost-effective. This organic-mineral fertilisation led to an increase in grain yield by 35 %, bio-ethanol output by 41 %, solid biofuel by 17 %, total energy output by 23 %.

5 REFERENCES

AAbunyewa, A. A., Ferguson, R. B., Wortmann, C. S., Mason, S. C. (2017). Grain sorghum nitrogen use as affected by planting practice and nitrogen rate. Journal of Soil Science and Plant Nutrition, 17(1), 155-166. https://doi.org/10.4067/S0718-95162017005000012

Bastaubayeva, S. O., Tabynbayeva, L. K., Yerzhebayeva, R. S., Konusbekov, K., Abekova, AM., Bekbatyrov, M. B. (2022). Climatic and agronomic impacts on sugar beet (Beta vulgaris L.) production. SABRAO Journal of Breeding and Genetics, 54(1), 141-152. https://doi.org/10.54910/sabrao2022.54.1.13

Bartzialis, D., Giannoulis, K. D., Gintsioudis, I., Danalatos, N. G. (2023). Assessing the efficiency of different nitrogen fertilization levels on sorghum yield and quality characteristics. Agriculture, 13(6), 1253. https://doi.org/10.3390/agriculture13061253

Chiaramonti, D., Talluri, G., Scarlat, N., Prussi, M. (2021). The challenge of forecasting the role of biofuel in EU transport decarbonisation at 2050: A meta-analysis review of published scenarios. Renewable & Sustainable Energy Reviews, 139, 110715. https://doi.org/10.1016/j.rser.2021.110715

Energy strategy of Ukraine for the period until 2035 "Security, energy efficiency, competitiveness". Decree of the Cabinet of Ministers of Ukraine of August 18. (2017). No. 605-r. (in Ukrainian) Available at: https://zakon.rada.gov.ua/laws/show/605-2017-%D1%80#Text

Gamayunova, V., Khonenko, L., Kovalenko, O. (2022). Bioethanol producing from sorghum crops. Ukrainian Black Sea Region Agrarian Science, 26(1), 9-18. (in Ukrainian) https://doi.org/10.56407/2313-092X/2022-26(1)-1

Hu, C., Zheng, C., Sadras, V. O., Ding, M. (2018). Effect of straw mulch and seeding rate on the harvest index, yield and water use efficiency of winter wheat. Scientific Reports, 8(1). https://doi.org/10.1038/s41598-018-26615-x

Kedir, M. (2023). Effects of organic and inorganic fertilizer on sorghum yield and growth attributes: A Review. International Journal of Current Research and Academic Review, 11(10), 10-17. doi: https://doi.org/10.20546/ijcrar.2023.1110.002

Kovalenko, M. O. (2023). The effect of mineral fertilizer on the development and productivity of grain sorghum in the conditions of the northeastern Forest-Steppe of Ukraine. Bulletin of Sumy National Agrarian University. The series Agronomy and Biology, 53(3), 16-22. (in Ukrainian) https://doi.org/10.32782/agrobio.2023.3.3

Liu, D. L., Zeleke, K. T., Wang, B., Macadam, I., Scott, F., Martin, R. J. (2017). Crop residue incorporation can mitigate negative climate change impacts on crop yield and improve water use efficiency in a semiarid environment. European Journal of Agronomy, 85, 51-68. https://doi.org/10.1016/j.eja.2017.02.004

Lu, X. (2020). A meta-analysis of the effects of crop residue return on crop yields and water use efficiency. PLoS ONE, 15(4), 0231740. https://doi.org/10.1371/journal.pone.0231740

Malyarchuk, M. P., Luzhansky, I. Yu., Markovska, O. Ye. (2019). Productivity of grain sorghum under different systems of basic soil cultivation and fertilization in crop rotation under irrigation. Tavria Scientific Bulletin. Series: Agricultural Sciences, 105, 210-217. (in Ukrainian)

Masebo, N., & Menamo, M. (2016). The Effect of application of different rate of N-P fertilisers rate on yield and yield components of sorghum (Sorghum bicolor): case of derashe woreda, SNNPR, Ethiopia. Journal of Natural Sciences Research, 6(5), 2224-3186.

Modisapudi, S. L., Sebetha, E. T. (2022). Sorghum grain quality as affected by different nitrogen fertilizer sources, cultivar and field condition. Journal of Agriculture and Crops, 8(4), 330-339. https://doi.org/10.32861/jac.84.330.339

Mujdeci, M., Demircioglu, A. C., Alaboz, P. (2020). The Effects of Farmyard Manure and Green Manure Applications on Some Soil Physical Properties. YYU Journal of Agricultural Science, 30(1), 9-17. https://doi.org/10.29133/yyutbd.628921

Munagilwar, V. A., Khurade, N. G., More, V. R., Dhotare, V. A. (2020). Response of sorghum genotypes to different fertility levels on growth and yield attributes of sorghum. International Journal of Current Microbiology and Applied Sciences, 11, 3853-3858.

Obour, A. K., Holman, J. D., Assefa, Y. (2022). Grain sorghum productivity as affected by nitrogen rates and available soil water. Crop Science, 62(3), 1360-1372. https://doi.org/10.1002/csc2.20731

Poliovyi, V. M., Yashchenko, L. A., Yuvchyk, N. O. (2021). Removing elements of nutrition with winter wheat depending on fertilizer and lime in western Polissia. Bulletin of Agricultural Science, 4(817), 5-12. (in Ukrainian) https://doi.org/10.31073/agrovisnyk202104-01

Pravdyva, L. A., Doronin, V. A., Dryha, V. V., Khakhula, V. S., Vakhniy, S. P., Mykolaiko, I. I. (2022). Yield capacity and energy value of sorghum grain depending on the application of mineral fertilisers. Zemdirbyste-Agriculture, 109(2), 115-122. https://doi.org/10.13080/-a.2022.109.015

Roik, M. V., Pravdyva, L. A., Hanzhenko, O. M., Doronin, V. A., Sinchenko, V. M., Kurylo V. L., Fuchylo, Ya. D., Kvak, V. M., Khivrych, O. B., Zykov, P. Yu., Honcharuk, H. S., Smirnykh, V. M., Ivanova, O. H., Dubovyi, Yu. P., Atamaniuk, O. M., Yalanskyi, O. V. (2020). Technology of grain sorghum cultivation as raw material for food industry and biofuel production, 21. (in Ukrainian)

Said, M., Ahmed, I. A., Osman, M. N., Muqtar, J. A. (2023). Impact of varied nitrogen fertilizer rates on growth and yield of local sorghum (Sorghum Bicolor L.) variety in Somalia. IOSR Journal of Agriculture and Veterinary Science, 16(8), 36-39. doi: 10.9790/2380-1608013639

Schlegel, A., & Bond, H. D. (2020). Long-term nitrogen, phosphorus, and potassium fertilisation of irrigated grain sorghum. Kansas Agricultural Experiment Station Research Reports, 6(8). https://doi.org/10.4148/2378-5977.7960

Sinchenko, V. M., Bondar, V. S., Gumentyk, M. Ya., Pastukh, Yu. A. (2020). Ecological bioenergy materials in Ukraine – current state and prospects of production development. Ukrainian Journal of Ecology, 10(1), 85-89. (in Ukrainian) https://doi.org/10.15421/ 2020_13

Soils (2002). Determination of mobile phosphorus and potassium compounds by the modified Chirykov method: DSTU 4115: 2002. [Valid from 2003–01–01]. Kyiv: Derzhspozhyvstandart of Ukraine, 2003. 9 p. (National Standard of Ukraine). (in Ukrainian)

Soil quality (2004). Methods for determining organic matter: DSTU 4289:2004. [Valid from 2005–07–01]. Kyiv: Derzhspozhyvstandart Ukrainy, 2005. 14 p. (National Standard of Ukraine). (in Ukrainian)

Soil quality (2015). Determination of easily hydrolyzable nitrogen by the Kornfield method: DSTU 7863:2015. [Valid from 2016–07–01]. Kyiv: Derzhspozhyvstandart Ukrainy, 2016. 13 p. (National Standard of Ukraine). (in Ukrainian)

Souza, A. A., Carvalho, A. J., Bastos, E. A., Cardoso, M. J., Mingote, M. P., Batista, P. S., Julio, B. H., Campolina, C. V., Portugal, A. F., Menezes, C. B., Oliveira, S. M. (2021). Grain sorghum under pre- and post-flowering drought stress in a semiarid environment. Australian Journal of Crop Science, 15(08), 1139-1145. doi: 10.21475/ajcs.21.15.08.p3162

Stamenković, O. S., Siliveru, K., Veljković, V. B., Banković-Ilić, I. B., Tasić, M. B., Ciampitt, I. A., Dalović, I. G., Mitrović, P. M., Sikora, V. S., Prasad, P. V. V. (2020). Production of biofuels from sorghum. Renewable and Sustainable Energy Reviews, 124. https://doi. org/10.1016/j.rser.2020.109769

Teshome, T., Alemayehu, Y., Regasa, A. (2023). Integrated use of farmyard manure and NPS influenced soil chemical properties, yield and yield attributes of sorghum (Sorghum bicolor L.) in Assosa district, western Ethiopia. East African Journal of Sciences, 17(2), 185-198.

Wang, X., Jia, Z., Liang, L., Yang, B., Ding, R., Nie, J., Wang, J. (2016). Impacts of manure application on soil environment, rainfall use efficiency and crop biomass under dry land farming. Scientific Reports, 6, 20994. https://doi.org/10.1038/srep20994

Zhang, P., Chen, X., Wei, T., Yang, Z., Jia, Z., Yang, B., Han, Q., Ren, X. (2016). Effects of straw incorporation on the soil nutrient contents, enzyme activities, and crop yield in a semiarid region of China. Soil and Tillage Research, 160, 65-72. https://doi.org/10. 1016/j.still.2016.02.006

doi:10.14720/aas.2025.121.1.19183 Original research article / izvirni znanstveni članek

Effect of chemical thinning on the fruit organoleptic parameters of 'Majhoul' date palm during the ripening stage

Mohamed ARBA [1, 2], Atmane ELLADI [3] and Hicham OUACHOUO [4]

Received July 01, 2024, accepted February 13, 2025
Delo je prispelo 1. julij 2024, sprejeto 13. februar 2025

Effect of chemical thinning on the fruit organoleptic parameters of 'Majhoul' date palm during the ripening stage

Abstract: The aim of our research work was to study the effect of chemical thinning on the fruit organoleptic parameters of 'Majhoul' date palm during ripening. Trials were carried out in the Tinejdad region. Four thinning treatments, including two NAA (naphthalene acetic acid) treatments were used on the fruits of three flowering phases. Obtained results showed that thinning treatments and flowering phases did not affect ($p < 0.05$) the titratable acidity of the fruit juice, while the two factors affected ($p \leq 0.05$) the pH of the fruit juice (5.16 in not thinned early flowering and 6.75 in the late flowering treated with T2 NAA). The content of sugar in the fruit was higher in the control and manual thinning (17.62-19.58 °Brix) than in NAA treatments (13.89-15.05 °Brix). It was also higher in the early flowering phase (18.35 °Brix) than in the other phases (15.50-15.76 °Brix). At the ripening stage, thinning treatments and flowering phases affected significantly ($p \leq 0.001$) the rate of ripening, but there was no interaction between the two factors. Thinning treatments and flowering phases affected some parameters of the fruit (pH, sugar content), but did not affect some other parameters (titratable acidity).

Key words: 'Majhoul' date palm, chemical thinning, NAA, flowering phase, fruit ripening, fruit organoleptic parameters.

Učinek kemičnega redčenja v obdobju zorenja na organoleptične lastnosti plodov dateljeve palme 'Majhoul'

Izvleček: Namen raziskave je bil preučiti učinek kemičnega redčenja v obdobju zorenja plodov na organoleptične lastnosti dateljeve palme 'Majhoul'. Poskusi so bili izvedeni na območju Tinejdad. Obravnavanja so obsegala štiri načine redčenja v treh fazah cvetenja, od katerih sta dva vsebovala naftalen ocetno kislino. Rezultati so pokazali, da redčenja in faze cvetenja niso vplivale ($p < 0,05$) na titrabilno kislost soka plodov medtem, ko sta oba dejavnika vplivala ($p \leq 0,05$) na pH soka plodov (5,16 pri plodovih zgodnje faze cvetenja brez redčenja in 6,75 pri plodovih pozne faze cvetenja in obravnavanju T2). Vsebnost sladkorjev v plodovih je bila večja pri kontroli in ročnem redčenju (17,62-19,58°Brix) kot pri obravnavanjih z naftalen ocetno kislino (13,89-15,05 °Brix). Vsebnost sladkorja je bila tudi večja v plodovih zgodnje faze cvetenja (18,35 °Brix) kot v plodovih ostalih faz (15,50-15,76 °Brix). Faze cvetenja in obravnavanja z redčenjem so značilno vplivala ($p \leq 0,001$) na stopnjo zrelosti plodov, vendar med obema dejavnikoma ni bilo povezave. Zaključimo lahko, da so redčenja in faze cvetenja vplivale na nekatere organoleptične lastnosti plodov (pH in vsebnost sladkorja) a ne na nekatere druge parametre (titratibilna kislost).

Ključne besede: 'Majhoul' dateljeva palma, kemično redčenje, naftalen ocetna kislina, faza cvetenja, zrelost plodov, organoleptične lastnosti plodov

1 Former Professor at Hassan II Institute of Agronomy and Veterinary Medicine, Horticultural Complex of Agadir, BP 121, Ait Melloul 15086, Morocco.
2 corresponding author: arbamohamed@yahoo.fr
3 National Agency for the Development of the Oasis and Argan Zones (ANDZOA), Department of oasis zones. Route Rissani, Erfoud PK 7 RASZ, Morocco
4 Agricultural Materials Company (AGRIMATCO), Department of horticultural products. 27 Boulevard Zerktouni, 20100 Casablanca, Morocco.

1 INTRODUCTION

To obtain good quality dates with market value, farmers are required to perform the thinning practice, which is an important management practice in the production system of date palm. Because of the expensive cost of manual thinning due to its performance by skilled labor, the need for a thinning practice, which is not expensive, such as chemical thinning, is necessary. The chemical thinning may be an alternative to manual thinning. Preliminary trials have shown that the use of some chemical regulators, such as NAA (naphthalene acetic acid), improved date yield and quality. This synthetic auxin has been shown effective in the chemical thinning of date palm (Ali Dinar et al., 2002; Al-Juburi & Al-Masry, 2003; Abd El-Kader et al., 2008). As reported by several authors, the chemical NAA product is also used to improve dates quality and to control their ripening (Al-Juburi et al., 2000 and 2001; Aboutalebi & Beharoznam, 2006). Abd El-Kader et al. (2008) indicated that to improve dates yield and quality much progress is needed in using the growth regulators such as NAA product.

The elimination of 15, 30, or 45 % of fruits per cluster improved fruit quality, including fruit and pulp mass and the content of sugar in the fruit (Samouni et al., 2016). Applications of 150 and 200 ppm NAA on 'Barhe' and 'Shahl' date palm 10 weeks after fruit set decreased the content of sugar in the fruit compared to the control not treated (Harhash & Al Obeed, 2007). Applying 100 and 200 ppm NAA on 'Majhoul' date palm 20 to 38 days after pollination also decreased the content of sugar in the fruit. The application of 100 ppm NAA decreased the content of dry matter in the fruit and that of 200 ppm did not affect this content of dry matter. While, the pH of the fruit juice was almost similar for all treatments (Arba & Oumou, 2024). However, Taim (2010) reported that applying 100 ppm NAA on 'Barhe' date palm 10 days and 7 weeks after pollination increased the titratable acidity and the content of sugar in the fruit. While, the same application decreased the pH of the fruit juice and the content of dry matter in the fruit. Other authors indicated that application of 80 and 100 ppm NAA on 'Barhee' and 'Succary' date palm fifteen weeks after pollination led to an increase in the acidity and the content of sugar in the fruit (Al Obeed et al., 2003; Al Qurashi & Awad, 2011).

Our research work aimed to study the effectiveness of chemical thinning as an alternative to manual thinning, because it's less expensive for the farmetrs and easy to use. Moreover, the objective of our research work was to study the effect of chemical thinning by using NAA on the organoleptic parameters of the fruits. These organoleptic parameters are amang the physico chemical constituants that are important in the determination of the date quality. They are focusing on the pH and titratable acidity of the fruit, and the content of sugar and dry matter in the fruit. Applications of NAA are used 30 days and 60 days after pollination on different flowering phases of 'Majhoul' date palm: the early flowering phase, the seasonal phase, and the late flowering phase.

2 MATERIALS AND METHODS

2.1 THE SITE OF TRIALS

Trials are carried out on fifteen-year-old plantations in the Tinejdad region, Tafilalet area: latitude 31° 32' north, longitude 4° 52' west, 1062 m altitude. The site of trials is characterized by an arid climate with warm temperatures in summer (mean temperature is 42 °C) and cold temperatures in winter (mean low temperature is -0.5 °C), and low rainfall (less than 100 mm per year) which is poorly distributed in the year. The site of trials is also characterized by warm and dry winds from the east, which can reach more than 57 km per hour during the summer period (May to August) (DGCL, 2015). The soil of the site of trials included 46 % fine sand, 30 % silt, 12 % clay, 7 % limestone, and 0.21 % organic matter.

The irrigation system used in the farm of trials was drip irrigation with two drip rails per row of palm trees and two drips per palm tree (one drip per drip rail). Organic manure was brought once a year, in May, and the amount provided was 150 kg per palm tree and mineral manure was brought twice a year, in February and June. Watering and fertilizing programs used in the cultivation of 'Majhoul' date palm in the farm of trials are presented in Table 1.

2.2 THE EXPERIMENTAL DESIGN

The experimental design adopted was a split-plot with 3 blocks including 4 palm trees each one, and on each palm tree, the three flowering phases were randomly chosen. The spathe-opening and pollination periods of the three flowering phases are presented in Table 2. Studied palm trees were 2-3 m high, the number of palms per palm tree was 72 to 80 and the number of clusters kept per palm tree was more than one per 10 palms because the clusters were not loaded. The pollination was carried out manually by putting three spikelets of male inflorescence inside the female inflorescence.

Table 1: Watering and fertilizing programs used in the cultivation of 'Majhoul' date palm in the farm of trials in the Tinejdad region, Tafilalet area.

Fertilizing program				Watering program	
Fertilizers used	Principal constituants	Amount brought per palm tree on February	Amount brought per palm tree on June	Watering dose per palm tree and frequency of the apports	Periods of the apports
Composed fertilizers	14 % N, 7 % P_2O_5, 21 % K2O, 3 % MgO	2 kg (0.28 kg N, 0.14 kg P_2O_5, 0.42 kg K_2O and 0.06 kg MgO)	1 kg (0.14 kg N, 0.07 kg P_2O_5, 0.21 kg K_2O and 0.03 kg MgO)		
Silica-based fertilizers	60 % SiO2, 3.7 % MgO, 3.4 % Fe_2O_3, 3 % CaO	1 Kg (0.6 kg SiO_2, 0.037 kg MgO, 0.034 kg Fe_2O_3, 0.03 kg CaO)	1 Kg (0.6 kg SiO_2, 0.037 kg MgO, 0.034 kg Fe_2O_3, 0.03 kg CaO)	400 to 600 liters per palm tree per 4 days	December to April
Ammonium nitrate	33,5% N	2 kg (0,67 kg N)	1 kg (0,335 kg N)		
Potash nitrate	13 % N, 46 % K2O	-	1 kg (0.13 kg N, 0.46 kg K2O)	400 to 600 liters per palm tree per 2 days	May to November
DAP Diammonium-triphosphate	18 % N, 46 % $P2O_5$	1 kg (0.18 kg N, 0.46 kg $P2O_5$)	-		

Table 2: Spathe-opening and pollination periods of the three flowering phases, thinning treatments used, and application dates of NAA treatments on 'Majhoul' date palm in the Tinejdad region, Tafilalet area.

		Flowering phase		
		Early flowering	Seasonal flowering	Late flowering
Spathe-opening period		March 15 to 25 2017	March 26 to April 5 2017	After April 5 2017
Pollination period		March 30 to April 6 2017	April 7 to 14 2017	April 15 to 21 2017
Thinning treatments used	T0	Control treated with liquefied water		
	T1	150 ppm NAA 30 days after pollination and 300 ppm 60 days after pollination		
	T2	250 ppm NAA 30 days after pollination and 500 ppm 60 days after pollination		
	T3	Manual thinning used by the farmer: retaining 8 to 10 fruits per spikelet and removing the rest		
Application dates of NAA	1st application	30 days after pollination of the early flowering phase (May 08, 2017)		
	2nd application	60 days after pollination of the early flowering phase (June 10, 2017)		

2.3 THINNING TREATMENTS USED IN THE STUDY

Thinning treatments used in the study and their applying dates are presented in table 2. They were applied to the fruits of the early, seasonal, and late flowering phases of 'Majhoul' date palm to study their effect on the fruit organoleptic parameters. The fruit morphological parameters as affected by thinning treatments, including NAA treatments, during fruit development are published in another journal (Arba et al., 2023b). Manual thinning began on May 15, 2017, 45 days after pollination and the fruit diameter at this stage was 12-13 mm. It was used to assess the effectiveness of chemical thinning using NAA on 'Majhoul' date palm. The diameter of the fruits during the first treatment of NAA was 8 mm for the early flowering phase, 6.5 mm for the seasonal flowering, and 5 mm for the late flowering phase, and their diameter during the second treatment was 22 mm for the early flowering, 20 mm for the seasonal flowering and 18 mm for the late flowering. Each palm of the trial received a thinning treatment, which was repeated three times per flowering phase. Precautions were taken during NAA treatments because the NAA product is sensitive to climatic conditions. The mean temperature should be 18-24 °C, the relative humidity between 5-17 %, and the wind speed should not exceed 5 km per hour. The NAA product used was a powder with 99 % purity and the amounts used were measured using a precision balance with an error of 0.01 mg.

2.4 CHEMICAL ANALYSIS OF THE FRUITS

For chemical analysis of pH and titratable acidity, a sample of 10 fruits per flowering phase or thinning treatment was washed with tap water, cleared of their seeds, and finely ground in mixture with a double their volume in distilled water. The grind was centrifuged for 40 minutes, the recovered supernatant was filtered and the filtrate was adjusted with distilled water up to 200 ml to constitute the raw solution to be analyzed. The pH of juice was measured with a pH meter and the titratable acidity was determined by titration of 10 g juice diluted with 10 ml distilled water using a 0.1 N NaOH solution until a pH of 8.1 is obtained with a pH meter. The juice of 10 g fruit per flowering phase or thinning treatment, which was crushed using a garlic press was used to determine the content of sugar or the Brix using a refractometer. The content of dry matter in the fruit was determined according to Shamim et al. (2013) by measuring the fresh mass of 3 fruits per combination of thinning treatment-flowering phase and their dry mass after cutting them into small pieces and drying them in an oven at 70 °C for 48 hours. Fruit ripening monitoring was performed on a sample of 10 spikelets on the two clusters (5 per cluster) of each combination of flowering phase-thinning treatment.

2.5 STATISTCAL ANALYSIS OF DATA

Data processing of studied parameters, as well as the determination of the means and the design of the graphs, were carried out using Microsoft Excel 16. The analysis of variance (ANOVA) amang the means values of repeated mesures and the multiple comparison of means according to the Tukey test, with a significance level of $p \leq 0.05$, were carried out using Minitab 16 statistical software.

3 RESULTS AND DISCUSSION

3.1 THE ORGANOLEPTIC PARAMETERS OF THE FRUITS

3.1.1 Titratable acidity and the content of sugar in the fruit

Obtained results showed that thinning treatments and flowering phases and the interaction of the two factors did not affect significantly ($p > 0.05$) the titratable acidity of the fruit juice. The titrable acidity of the fruit juice was 0.16 to 0.21 % citric acid for all thinning treatments and flowering phases (Table 3). Our results are different from those of several authors who reported that applying 80 to 100 ppm NAA on 'Barhe' and 'Succary' date palm fifteen weeks after pollination led to an increase in the titratable acidity of the dates (Al Obeed et al., 2003; Al Qurashi & Awad, 2011). Taaim (2010) also reported that applying 100 ppm NAA on 'Barhe' date palm 10 days to 7 weeks after pollination increased the titratable acidity of the dates. However, Harhash & Al Obeed (2007) indicated that an application of 150 and 200 ppm NAA on 'Barhe' and 'Shahl' date palm ten weeks after fruit set led to a decrease in the titratable acidity of the dates. The difference between their outcome and our results is probably due to the difference in studied date palm varieties and the ripening stage where chemical analysis of the fruits is carried out. In our case the chemical analysis of the fruits is carried out at the end of the 'Khalal' stage and in their case the chemical analysis is realized during the 'Tamar' stage, which is the late ripening stage in date palm.

However, thinning treatments and the flowering phases affected significantly ($p \leq 0.05$) the content of sugar in the fruit, but there was no interaction between the two factors for this chemical parameter. The content of sugar in the fruit was higher in the control and manual thinning treatment (17.62-19.58 °Brix) than in T1 and T2 NAA treatments (13.89-15.05 °Brix) (Table 3). Our results are consistent with those of several authors who reported that applying 150 and 200 ppm NAA on 'Succary' date palm seven weeks after pollination (Harhash & Al Obeed, 2005) or on 'Barhe' and 'Shahl' date palm ten weeks after fruit set (Harbash & Al Obeed, 2007) led to a decrease in the content of sugar in the fruit. Arba & Oumou (2024) also indicated that applying 100 and 200 ppm NAA on 'Majhoul' date palm 20 to 38 days after pollination resulted in a decrease in the content of sugar in the fruit of the NAA treatments (9.60-9.67 °Brix) compared to the fruits of the manual thinning treatment (12.88 °Brix). Some other authors reported that manual

Table 3: Titratable acidity and the content of sugar in the fruit according to thinning treatments used (T0, T1, T2, and T3) and flowering phases (early, seasonal, and late flowering) of 'Majhoul' date palm in the Tinejdad region, Tafilalet area.

Thinning treatments used	Titratable acidity of the fruit juice (% citric acid)		Sugar content in the fruit (°Brix)	
T0	0.20 ± 0.04		19.58 ± 1.02	
T1	0.16 ± 0.03	ns	15.05 ± 1.12	*
T2	0.23 ± 0.07		13.89 ± 1.11	
T3	0.21 ± 0.03		17.62 ± 1.36	
Flowering phases				
Early flowering	0.21 ± 0.04		18.35 ± 1.01	
Seasonal flowering	0.21 ± 0.05	ns	15.76 ± 0.74	*
Late flowering	0.18 ± 0.04		15.50 ± 1.02	

T0: Control treated with liquefied water
T1: 150 ppm NAA 30 days after pollination and 300 ppm 60 days after pollination
T2: 250 ppm NAA 30 days after pollination and 500 ppm 60 days after pollination
T3: Manual thinning used by the farmer: retaining 8 to 10 fruits per spikelet and removing the rest
*: significant difference at $p \leq 0.05$
ns: No significant difference (p > 0.05)
The numbers after the ± sign indicate the error deviation

thinning in 'Samany', 'Barhe', 'Seewy', 'Saqei', and 'Succary' date palm increased most of physical (fruit mass and length) and chemical properties (total solid solubles) compared to not thinned palm trees (Dawoud & El-Rauof, 2021; Abdelhalim Ahmed, 2022; Ghazzawy et al., 2023; Sallam, 2023; Zakaria et al., 2023). However, our results are different from those of some other authors who reported that applying 80 and 100 ppm NAA on 'Barhe' and 'Succary' date palm fifteen weeks after pollination (Al Obeed et al., 2003; Al Qurashi & Awad, 2011) or 100 ppm NAA on 'Barhe' date palm ten days and seven weeks after pollination (Taaim, 2010) led to an increase in the content of sugar in the fruit. Arba et al. (2023a) also reported that an application of NAA at the 'Hababouk' stage (20-30 days after pollination) improves fruit quality of retained fruits in the clusters. Applying 75 ppm Cytophex on 'Zaghloul' and 'Samany' date palm four weeks after pollination also increased the content of sugar in the fruit (El Kosary, 2009). Jalali et al. (2024) showed that the interaction of manual and chemical thinning improved fruit quality and the content of sugar in the fruit of 'Khadrawi' date palm at the 'Tamar' stage. The chemical thinning affected significantly the titratable acidity of the fruit juice and the manual thinning affected the total phenols and non-reducing sugars in the fruit. Their results also showed that there was an increase trend in the amount of sugars, pH, and total acidity from the 'Kimri' to 'Tamar' stage, but a decrease trend in the amount of total phenols and titratable acidity of the fruit from the first stage to the second one.

The differences in the results of cited authors may be due to the ripening stage of studied varieties and management practices used in each case. The two main factors are the water and mineral supply of plants, and the environmental conditions prevailing in the growing site of date palm in each case of study. Regarding the flowering phases, the content of suger in the fruit was also higher in the fruits of the early flowering phase (18.35 °Brix) than in those of the other flowering phases (15.50-15.76 °Brix) (Table 3) probably because of the long period of the fruit development period and the ripening stage where the fruits of the early flowering phase have accumulated more sugar than those of the other flowering phases due to the shorter period of their fruit development period and ripening stage. However, Arba & Oumou (2024) indicated that at the end of the 'Khalal' stage, the flowering phases did not affect significantly ($p > 0.05$) the content of sugar in the fruit. Probably because of the fruit load of the palm trees, which is less loaded in their study due to the low number of clusters kept per palm tree (a mean of 7 clusters per palm tree).

3.1.2 The pH of the fruit juice

Characterized by high sugar content, the organoleptic quality of the dates depends on several factors, mainly the balance between their content of sugar and acidity. Our results on the pH of the fruit juice showed also that the flowering phases and thinning treatments affected significantly ($p \leq 0.05$) this chemical parameter and that

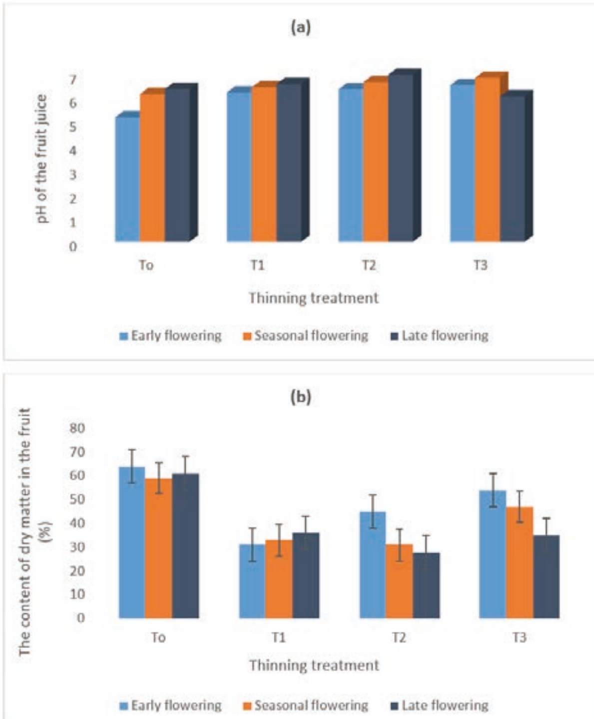


Figure 1: Variation in the pH of the fruit juice (a) and in the content of dry matter in the fruit (b) according to the thinning treatments T0, T1, T2, and T3, and the flowering phases (early, seasonal, and late flowering) of 'Majhoul' date palm in the Tinejdad region, Tafilalet area.

T0: Control treated with liquefied water
T1: 150 ppm NAA 30 days after pollination and 300 ppm 60 days after pollination
T2: 250 ppm NAA 30 days after pollination and 500 ppm 60 days after pollination
T3: Manual thinning used by the farmer: retaining 8-10 fruits per spikelet and removing the rest

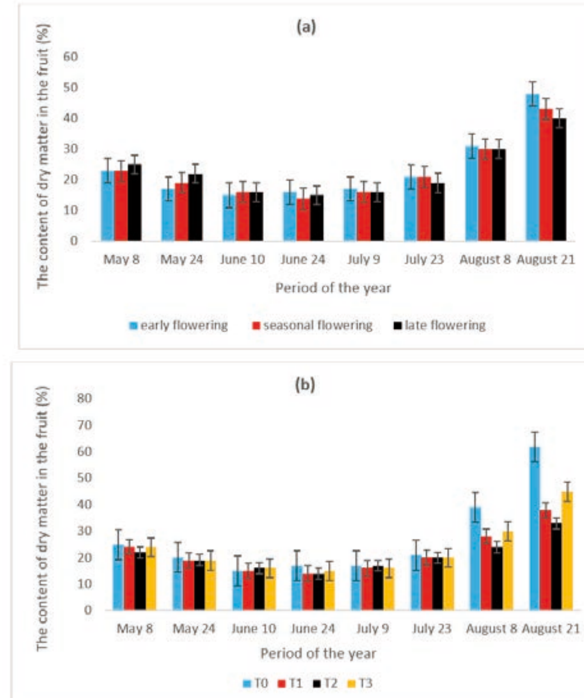


Figure 2: Evolution of the content of dry matter in the fruit of 'Majhoul' date palm according to flowering phases (early, seasonal and late flowering) (a), and thinning treatments used (T0, T1, T2 and T3) (b) in the Tinejdad region, Tafilalet area.

T0: Control treated with liquefied water
T1: 150 ppm NAA 30 days after pollination and 300 ppm 60 days after pollination
T2: 250 ppm NAA 30 days after pollination and 500 ppm 60 days after pollination
T3: Manual thinning used by the farmer: retaining 8-10 fruits per spikelet and removing the rest

there is an interaction between the two factors (Figure 1a). The lowest pH of the fruits (5.16) was obtained in the early flowering phase without thinning treatment (Figure 1a). The fruit load of the clusters, which was higher in this flowering phase and may influence the air circulation in the clusters, might explain this lowest pH in the fruits of the early flowering phase. According to Chafi et al. (2015), this pH value may be the lowest value for 'Majhoul' date palm, and according to Acourene et al. (2001), this pH level may be a bad chemical character for the preservation of dates. The pH of the other thinning treatment-flowering phase combinations is above 5.8 and this may be a good chemical character for the preservation of dates. Our results are different from those of Arba & Oumou (2024) who reported that the flowering phases and thinning treatments used in their study (control without application of NAA, 100 and 200 ppm NAA) did not affect significantly ($p > 0.05$) the pH of the fruit juice. Probably because of the ripening stage and NAA treatments used in their study and which were different from the ours.

3.1.3 Evolution of the content of dry matter in the fruit during fruit development

The evolution of the content of dry matter in the fruit during fruit development is presented in Figure 2. It shows that except for May 24 and August 21 2017 where the flowering phases affect significantly ($p \leq 0.05$) the content of dry matter in the fruit, these flowering phases do not affect significantly ($p > 0.05$) this content of dry matter during the rest of the fruit development period (Figure 2). During the first days of application of NAA treatments, the content of dry matter in the fruit is almost similar for all the thinning treatments. However, during May 22-July 24 2017, a slight decrease in the content of dry matter in the fruit is observed in all the thinning treatments. This may be due to the coincidence of this period with the fruit enlargement stage during which the demand for fruit for water is important. At the end of the fruit enlargement stage (July 24, 2017), there is a resumption in the increase of the content of dry matter in the fruit which is a sign of the beginning of the ripening stage.

The results also showed that there is an interaction between the flowering phases and thinning treatments (Figure 1b). Thus, for the flowering phases without thinning treatment, the content of dry matter in the fruit was higher and reached 64 % for the early flowering phase, 57 % for the seasonal phase, and 61 % for the late phase. Arba & Oumou (2024) also reported that in 'Majhoul' date palm, the content of dry matter in the fruit of the early flowering phase was higher than in the other phases and the late flowering phase was the latest. In the case of the manual thinning and the control without thinning treatment, the content of dry matter in the fruit was similar for all the flowering phases. While the content of dry matter in the fruit of the NAA treatments was the lowest, mainly in the higher NAA treatment in the late flowering phase. These results confirm the difference between thinning treatments and flowering phases in terms of the ripening rate as the increase in the content of dry matter in the fruit is a sign of the beginning of the ripening stage. This difference may be due to the fruit load which is higher in the clusters of the early flowering phase and which contributed to early ripening. This early ripening is due to the high content of dry matter in the fruit, because the higher the fruit load of the cluster is, the faster the fruit reaches its final diameter and its ripening begins. Our results are consistent with those of Harshash & Al Obeed (2007) who reported that applying 50, 100, 150, and 200 ppm NAA on 'Barhe' and 'Shahl' date palm ten weeks after fruit set resulted in a decrease in the content of dry matter in the dates. Arba & Oumou (2024) also indicated that applying 100-ppm NAA on 'Majhoul' date palm 20-38 days after pollination leads to a decrease in the content of dry matter in the fruit, while applying 200 ppm NAA does not affect this content of dry matter in the fruit. This may be due to the studied date palm variety and to the application period of NAA treatments, which coincided with an advanced stage of fruit development.

3.2 FRUIT RIPENING MONITORING

Change in the fruit color may be a criterion for the beginning of the ripening stage of dates. It was observed from July 23, 2017, on the clusters of the early flowering phase without NAA treatment. Fruit ripening monitoring for the period July 23-August 16, 2017 showed that flowering phases and thinning treatments affect significantly ($p \leq 0.001$) the ripening of 'Majhoul' date palm. While there is no interaction between the two factors. The evolution of the ripening rate according to thinning treatments and flowering phases is presented in Figure 3. It shows that the ripening of NAA treatments (T1 and T2) is six days later (July 23-29) compared to the control T0 and manual thinning treatment T3. At the beginning of the ripening stage, all thinning treatments have a ripening rate that is more or less negligible, and one week after there is a slight increase in the ripening rate of the control (6 %) and manual thinning treatment (4 %), while NAA treatments (T1 and T2) are still at a negligible level (Figure 3a). On August 4, 2017, twelve days after the

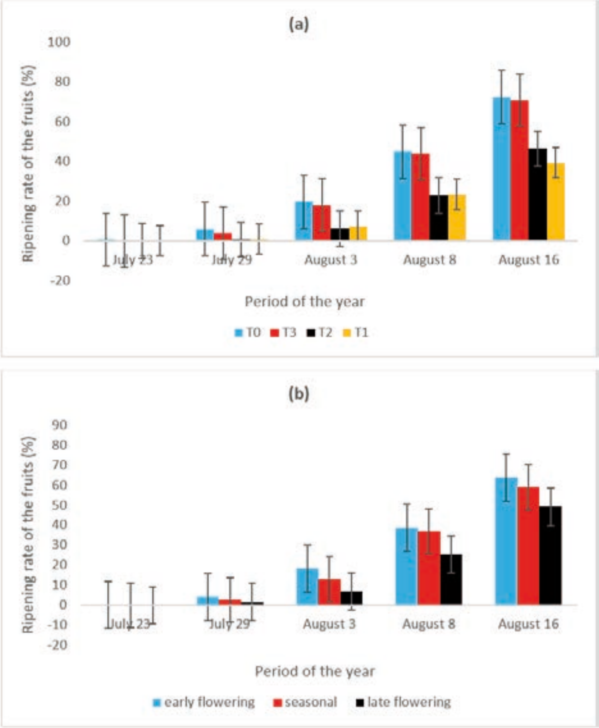


Figure 3: Evolution of the ripening rate of 'Majhoul' date palm according to thinning treatments T0, T1, T2 and T3 (a), and flowering phases (early, seasonal and late flowering) (b) in the Tinejdad region, Tafilalet area.

T0: Control treated with liquefied water
T1: 150 ppm NAA 30 days after pollination and 300 ppm 60 days after pollination
T2: 250 ppm NAA 30 days after pollination and 500 ppm 60 days after pollination
T3: Manual thinning used by the farmer: retaining 8-10 fruits per spikelet and removing the rest

beginning of the change in the fruit color, the ripening rate increased to 20 % for T0, 18 % for T3, 6 % for T1 and 4 % for T2. Following a heat wave at the site of study from the end of July, an increase in temperatures ranging from 30 to 37 °C for the mean temperature and 39 to 44 °C for the maximum is observed increasing the ripening rate of all thinning treatments (Figure 3a). Babahani & Eddoud (2012) also reported that these levels of temperatures might trigger the ripening of dates. At the end of the period of observations on the ripening of dates (August 16, 2017), thinning treatments affect significantly ($p \leq 0.001$) the ripening rate. It is almost similar for T0 and T3 (72 and 71 % respectively), while it is only 46 and 40 % respectively for T1 and T2 NAA treatments.

Regarding the flowering phases, it is obvious that the change in fruit color and the ripening began in the clusters of the early flowering phase, followed by the clusters of the seasonal flowering and the clusters of the late phase are the latest. From the end of July 2017, the ripening rate of the early flowering phase is the highest compared to the other flowering phases, it is followed by that of the seasonal phase and the ripening rate of the late flowering is the latest (Figure 3b). On August 16, 2017 (the end of the period of observations), the ripening rate was 64 % for the early flowering phase, 59 % for the seasonal flowering, and 49 % for the late phase.

4 CONCLUSION

Our study has shown that chemical thinning with NAA affected the organoleptic parameters of the date by improving some biochemical parameters of the dates. Obtained level of pH in the fruits of chemical and all thinning treatments was a good biochemical criterion for the preservation of 'Majhoul' dates. Fruit yield was lower in the chemical thinning treatments than in the control treated with liquefied water and the manual thinning treatment, but chemical thinning has improved some biochemical parameters of the dates. The ripening period was delayed by the chemical thinning what involves a grouped harvesting of dates in a short period and a significant saving in the cost of labor for harvesting dates.

Following these results, we suggest repeating the experiments by using the same concentrations of NAA after a short period of pollination (approximately one month after pollination) because the results we obtained on the chemical thinning of 'Majhoul' date palm using NAA have shown that the application of this product approximately one month after pollination gave the best results (Arba et al., 2023a and b; Arba & Oumou, 2024). We also recommend the use of other products such as Ethephon, Cytophex and gibberellins, with the aim of studying the effect of these products on the chemical thinning of 'Majhoul' date palm and to compare their effect with that of NAA.

Acknowledgements: We want to thank so much the farmer Mr Zahni ho has made his date palm farm to our disposal for the trials. Many thanks also to the Agricultural Research Center (CRA) of Errachidia and Hassan II Institute of Agronomy and Veterinary Medicine in Agadir for their support

5 REFERENCES

Abdelhamid Ahmed, A. (2022). Response of 'Samany' date palm to different methods and times of fruit thinning under Assiut conditions. *Egyptian Journal of Horticulture*, *49*(1), 87-93. 10.21608/ejoh.2022.120989.1190.

Abd El-Kader, A. M., El-Makhtoun, F. B., Aly, H. S. H. & El-Roby, K. A. (2008). Effect of naphthalene acetic acid (NAA) spray on yield and fruit characteristics of 'Zaghloul' date palm. *Alexandra Science Exchange Journal*, *29*(4), 252-256. https://doi.org/10.3923/ijar.2013.77.86.

Aboutalebi, A. & Beharoznam, B. (2006). Study on the effects of plant growth regulators on date fruit characteristics. *International Conference on Date palm Production and Processing Technology*. Muscat, 9-11 May, Oman.

Acourene, S., Buelguedj, M., Tama M. & Taleb, B. (2001). Caractérisation, évaluation de la qualité de la datte et identification des cultivars rares de palmier dattier de la région des Ziban. *Recherche Agronomique, 5*(8), 19-39.

Ali Dinar, H. M., Alkhateeb, A. A., Al Abdulhadi, I., Alkhateeb, A., Abugulia, K. A. & Abdulla, G. R. (2002). Bunch thinning improves yield and fruit quality of date palm (*Phoenix dactylifera* L.). *Egyptian Journal of Applied Sciences*, *17*(11), 228-238.

Al-Juburi, H. J. and H. H. Al-Masry (2003). The Effects of plant growth regulators application on production and fruit characteristics of date palm trees (*Phoenix dactylifera* L.) *The International Conference on Date Palm and Joint Events*. King Saudi University Al-Quassem, 16-19 September, Saudi Arabia.

Al-Juburi, H. J., Al-Masry, H. H. & Al-Muhanna, S. A. (2001). Effect of some growth regulators on some fruit characteristics and productivity of the 'Barhee' date palm tree cultivar (*Phoenix dactylifera* L.). *Fruits*, *56*, 325-332. 10.1051/fruits:2001133.

Al-Juburi, H. J., Al-Masry, H. & Al-Muhanna S. A. (2000). Fruit characteristics and productivity of date palm trees (*Phoenix dactylifera* L.) as affected by some growth regulators. *HortScience*, *35*, 476-477.
https://doi.org/10.21273/HORTSCI.35.3.476E.

Al Obeed, R.S., Harhash, M. & Fayez, N. S. (2003). Effect of chemical thinning on yield and fruit quality of 'Succary' date palm cultivar grown in Riyadh region. In *Proceedings of the International Conference on Date Palm*, EL-Kassim, Saudi Arabia, p. 725-738.

Al-Qurashi, A. D. & Awad, M. A. (2011). Naphatalene acetic acid increase bunch weight and improve fruit quality of 'Barhe' date palm cultivar under hot arid climate. *American Eurasian Journal of Agricultural and Environmental Sciences*, *10*(4), 569-573.

Arba, M. & Oumou, L. (2024). Effect of chemical thinning on fruit growth and fruit quality of 'Majhoul' date palm at the end of the 'Khalal' stage. *World Journal of Advanced Pharmaceutical and Life Sciences*, *7*(1), 23–33. https://doi.org/10.53346/wjapls.2024.7.1.0036.

Arba, M., Oumou, L. & Sabri, A. (2023a). Fruit set in 'Majhoul' date palm and fruit drop by chemical thinning. *World Journal of Advanced Science and Technology*, *4*(1), 1-9.
https://doi.org/10.53346/wjast.2023.4.1.0068.

Arba, M, Elladi, O., Ouachouo, H. & Sabri, A. (2023b). Effect of chemical thinning on the fruit parameters of 'Majhoul' date palm during fruit development. *World Journal of Biological and Pharmaceutical Research*, *5*(2), 1-11. https://doi.org/10.53346/wjbpr.2023.5.2.0069.

Babahani, S. & Eddoud A. G. (2012). Effet de la température sur l'évolution des fruits chez quelques variétés du palmier dattier (*Phoenix dactylifera* L.). *Algerian Journal of Arid Environment*, *2*(1), 36-71.

Chafi, A., Benabbes, R., Bouakka, M., Hakkou, A., Kouddane, N. & Berrichi A. (2015). Pomological study of dates of some date palm varieties cultivated in Figuig oasis, *Journal of Materials and Environmental Science*. *6*(5), 1266-1275.

Dawoud, H. D. & El-Rauof, F. A. (2021). Improving of fruit quality and yield of 'Barhi' date palm cultivar (*Phoenix dactylifera* L.) by thinning practices. *Egyptian International Journal of Palms*, *1*(2), 43-50.
10.21608/ESJP.2021.247991.

DGCL (2015). *Monographie générale de la région de Drâa-Tafilalet*. Rabat: Direction générale des collectivités locales (DGCL), Ministère de l'intérieur.

El-Kosary, S. (2009). Effect of GA3, NAA and Cytophex spraying on 'Samany' and 'Zaghloul' date palm yield, fruit retained and characteristics. *Journal of Plant Production*, 1(4), 49-59. 10.21608/jpp.2009.1171.

Ghazzawy, H. S. Alqahtani, N., Munir, M., Alghanim, N. S. & Maged, M. (2023). Combined impact of irrigation, potassium fertilizer, and thinning treatments on yield, skin separation, and physicochemical properties of date palm fruits. *Plants*, 12(5), 1003. 10.3390/plants12051003.

Harbash, M. M. & Al-Obeed, R. S. (2007). Effect of naphthalene acetic acid on yield and fruit quality of 'Barhee' and 'Shahl' date palm cultivars. *Assiut Journal of Agricultural Sciences,* 38(1), 63-73. 10.21608/ajas.2007.271099.

Harhash M. A., Al Obeed, R. S. & Fayez, N. S. (2005). Effect of bunch thinning on yield and fruit quality of 'Succary' date palm cultivar grown in the Riyadh region. *Journal of King Saud University-Agricultural Sciences*, 17(2), 235-249.

Jalali, M., Moallemi, N., Khaleghi, E., Zivdar, S & Rahmati-Joneidabad, M. (2024). Effect of chemical and hand thinning on the fruit biochemical properties of 'Khadrawi' date palm (*Phoenix dactylifera* L.) during fruit development. *Journal of Horticultural Science*, *38*(4), 655-673. https://doi.org/10.22067/jhs.2024.87570.1337.

Samouni, M. T. M., El-Salhy, A. M., Ibtesam, F. M. & Badawy, E. F. A. (2016). Effect of pollination and thinning methods on yield and fruit quality of 'Saidy' date palms. *Assiut Journal of Agricultural Sciences*, *47*(3), 92-103. 10.21608/AJAS.2016.907.

Sallam, A. A. M. (2023). Thinning methods effect on yield and fruit quality of date palm cv Saqei. *Journal of Plant Production*, *14*(2), 45-49. 10.21608/jpp.2023.188923.1208.

Shamim, F., Johnson, G. N., Saqlan S. M. & Waheed, A. (2013). Higher antioxidant capacity protects photosynthetic activities as revealed by chl a fluorescence in drought tolerant tomato genotypes. *Pakistan Journal of Botany*, *45*(5), 1631-1642.

Taaim, D. A. (2010). Effect of NAA on physiology of growth and ripening of date palm fruits (*Phoenix dactylifera* L) cv.Barhe. *Journal of Karbalaa University of Science, 8*(1), 156-175.

Zakaria, B. A., Abdelaal, A. H., Khodair, O. A. & Diab, Y. M. (2023). Effect of thinning treatments on yield and fruit quality of 'Seewy' date palm under New Valley conditions. *International Journal of Chemical and Biochemical Sciences*, *24*(12), 789-795.

doi:10.14720/aas.2025.121.1.19520 Original research article / izvirni znanstveni članek

Experimental study of the effect of a glyphosate-based herbicide on a species of earthworm *Aporrectodea caliginosa* (Savigny, 1826)

Ghenima LANDRI-AIT BOUDRARE [1], Lynda OULTAF [1], Samira ALI AHMED [1], Monia BAHA [2], Djamila SADOUDI-ALI AHMED [1]

Received August 24, 2024; accepted November 09, 2024
Delo je prispelo 24. avgust 2024, sprejeto 09. november 2024

Experimental study of the effect of a glyphosate-based herbicide on a species of earthworm *Aporrectodea caliginosa* (Savigny, 1826)

Abstract: This work aims to demonstrate the effect of a glyphosate-based herbicide on a species of earthworm, *Aporrectodea caliginosa* (Savigny, 1826) to provide scientific justification for the species rarity in cultivated environments where this phytosanitary product is widely used. During the fifty-two days of contamination in the laboratory, the life traits monitored and evaluated were growth and mortality. Herbicide concentrations ranging from the lowest (C30) of around 240 l kg^{-1} to the highest (C210), equivalent to 1840 l kg^{-1} were tested. The study found that the herbicide is toxic to the A*porrectodea caliginosa* species, causing growth to slow and mortality to rise as concentrations rise. We report a delayed negative effect at low concentrations, which appears after several weeks of product exposure, depending on the exposure time. However, at the highest concentrations of the herbicide studied, the early negative effect is visible after the second week of exposure.

Key words: earthworm, *Aporrectodea caliginosa*, formulation, glyphosate, dose, toxicity

Raziskava učikov herbicidov na osnovi glifosata na vrsto deževnika *Aporrectodea caliginosa* (Savigny, 1826)

Izvleček: Namen raziskave je bil prikazati učinek herbicidov na osnovi glifosata na vrsto deževnika, *Aporrectodea caliginosa* za znanstveno potrditev ogroženosti te vrste v urbanem okolju, kjer se ta herbicid veliko uporablja. Med dvainpetdesetimi dnevi zastrupljanja v laboratoriju sta bili spremljani in ovrednoteni rast in mortaliteta. Preiskušene koncentracije herbicida so bile v območju od najmanjših, C30, okrog 240 l kg^{-1}, do največjih, C210, ki so ustrezale 1840 l kg^{-1}. Izsledki raziskave so pokazali, da je herbicid strupen za to vrsto in povzroča upočasnjevanje rasti in veča njeno mortaliteto s povečevanjem koncentracije. Opaženi so bili tudi negativni učinki manjših koncentracij, ki so se pojavili več tednov po izpostavitvi in so bili odvisni od časa izpostavitve. Pri večjih koncentracijah herbicida so bili negativni učinki vidni že po dveh tednih izpostavitve.

Ključne besede: deževnik, *Aporrectodea caliginosa,* odmerki herbicida, glifosat, doza, strupenost

1 PSEMRVC Laboratory, Faculty of Biological Sciences and Agronomic Sciences, Mouloud Mammeri University, Tizi-Ouzou, Algeria
2 Laboratory of Eco-Biology Animals (L.E.B.A.), École Normale Supérieure de Kouba Bachir El Ibrahimi, B.P. 92, 6050, Algiers, Algeria

1 INTRODUCTION

Earthworms play an important role in many soils. They are commonly called "ecosystem engineers" due to their importance in pedogenesis and soil profile development. Their actions have an impact on the physical, chemical, and microbiological properties of the soil, as well as the activity of other soil organisms (Bartlett, 2010). Herbicides negatively impact them through the water that soaks into the soil (Mamy et al., 2011). Despite the efficacy of glyphosate-based herbicides (GBH) in controlling weeds in agricultural and non-agricultural soils, their residues in the soil system have long been a concern (Owagboriaye et al., 2020). After spraying, glyphosate is distributed between the plant, the soil, and the atmosphere (Beckert et al., 2011). It attacks specific rhizosphere functions, which can have environmental consequences due to its immobilization via cation chelation; it is thus highly stable and not easily degradable (Huber et al., 2005). Earthworms exposed to glyphosate show a decrease in growth rate and a tendency to avoid treated areas (Casabé et al., 2007; Gill et al., 2018).

Previous research has shown that certain earthworm species, such as *Octolasion tyrtaeum* (Savigny, 1826) and *Lumbricus terrestris* L., 1857, are sensitive to glyphosate. Other species, such as *Esisenia fetida* (Savigny, 1826) and *Aporrectodea caliginosa* (savigny, 1826), were less sensitive to glyphosate or not at all (Correia, Moreira, 2010; Domnguez et al., 2016; Garca-Torres et al., 2014; Gaupp-Berghausen et al., 2015). The species *Aporrectodea caliginosa* is declining in our country's cultivated land (vegetable and cereal crops), leading us to believe that it is sensitive to pesticides, particularly glyphosate-based pesticides, which are the most widely used in Algeria (Oultaf et al., 2022). To provide scientific answers to the species' scarcity in cultivated environments due to glyphosate-based pesticides, laboratory work was carried out in which the species' life history (mortality and growth) was studied. The current study focuses on two important points: demonstrating the sensitivity of *Aporrectodea caliginosa* to the herbicide glyphosate and determining the species' toxic concentration range.

2 METHODS

2.1 COLLECTION OF EARTHWORMS

The earthworms used in the experiment were *Aporrectodea caliginosa*, collected in sufficient quantity (to avoid the laboratory rearing stage) on a lawn far from any source of pollution (healthy soil) in Beni Douala, Kabylia. We extracted the earthworms using Bouché's (1972) physical method. The adult worms (clitellum clearly visible) were collected after the species *Aporrectodea caliginosa* was identified, primarily using morphological criteria that vary greatly between species. Two hundred and ten healthy earthworms were sent to the laboratory in preparation for herbicide exposure. At the laboratory, the individuals were introduced into a large plastic tray containing soil from the same sampling station, moistened and without any additions. For a two-day acclimatization period, the tray was kept in the dark at room temperature (22-23 °C)

2.2 SOIL SAMPLING AND PREPARATION

The soil used in the experiment is from the same worm sampling station, and we chose to use soil from the same station without any additions to get as close to natural conditions as possible. According to Chevillot (2017), using real soil in toxicological tests appears to be the most relevant choice for getting as close to a system representative of the environment as possible. After freeze-drying, the sampled soil was transported to the laboratory in plastic boxes and sieved to 2 mm to ensure an easy-to-handle texture and a suitable fraction for physicochemical analysis. According to the preliminary analysis, the soil was clayey in texture, with a slightly acidic pH (6.06) and 4.85 % organic matter.

2.3 PREPARATION OF TEST CONCENTRATIONS

Glyfozell 36SL®, a liquid formulation (soluble concentrate) containing glyphosate as the active ingredient, was used as the herbicide. The glyphosate content of one liter of commercial product (360 g l^{-1}) and the maximum authorized agronomic dose (6 l ha^{-1}) was used to calculate the amount of product to be mixed with the soil. Eight concentrations (C0, C30, C60, C90, C120, C150, C180, C210) were considered, each representing a quantity of product in l.

2.4 SOIL CONTAMINATION

The pre-prepared soil (40 kg) was distributed over forty-one-kilogram boxes of soil; into each box, a precise quantity of the product was added by spraying to ensure good distribution of the product in the soil, which was then well stirred by hand for better homogenization, and left to stabilize overnight.

2.5 EXPERIMENTAL DESIGN

The earthworms (200 individuals) are rinsed with distilled water, weighed, and distributed at a rate of five individuals per box and five replicates per concentration; to keep the soil moist, the soil surface is moistened each time, if necessary, with spring water to approximate natural conditions as closely as possible. The experiment was carried out in the dark and at room temperature. To avoid altering the worms' behavior or the concentrations tested, no additional food was added during the test (Fig. 1).

2.6 TOXICITY ASSESSMENT

Earthworms were removed from each box once a week for 52 days, counted, weighed, and returned to the box. Mortality and growth mass were also assessed.

2.7 GROWTH RATE (MASS)

The average mass was recorded at each exposure time interval allowed us to calculate the relative growth rate, which we calculated using the Martin (1986) equation:

$$Relative\ growth\ rate = ln\ \frac{P_m}{P_0} * 100$$

(1)

P_0 is the average mass of earthworms before exposure to a concentration, and P_m is the average mass of earthworms after exposure to glyphosate at each concentration.

2.8 MORTALITY

The number of dead earthworms in each box was used to calculate mortality. Earthworms were considered dead when an advanced body alteration was observed, and the individual did not respond to a stimulus. Throughout the experiment, abnormal behaviors such as restlessness and coiling were assessed.

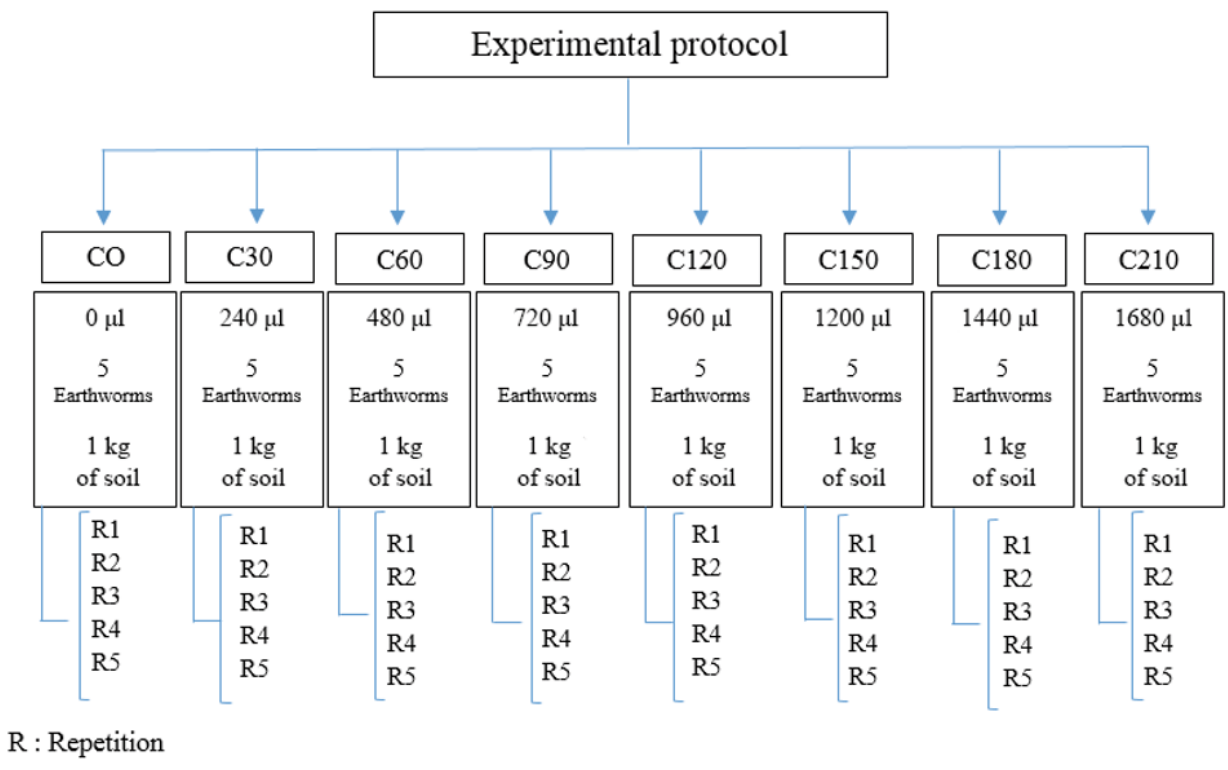


Figure 1: Summary diagram of the experimental protocol

2.9 DATA ANALYSIS

Multiple regressions were calculated using R software version 3.3.4 for both growth and mortality as functions of glyphosate concentration and exposure duration of earthworms.

3 RESULTS

3.1 RELATIVE GROWTH

The results of the multiple regression analysis conducted to explain the evolution of growth rate as a function of exposure duration and herbicide concentration are provided by the equation:

Growth = −0.0093 (Time) −0.00073 (Concentration) + 0.79

The low probability value (*p*-value: 1.351e-10) indicates that the regression model is significant. The coefficient of determination (0.5097) showed that the quality of the fit was good. Therefore, growth decreases as concentration increases and exposure is prolonged.

The results show that after the eighth day of pollutant exposure, there is difference in the growth of control worms. These differences were observed in comparison to the first day of the experiment and all other periods from day 16 to day 40. Indeed, these periods are distinguished by a mass loss from day eight to day forty-three. After 52 days of experimentation, a resumption of growth is observed for the control, as evidenced by an increase in mass (Fig. 2).

The results of earthworms exposed to various glyphosate concentrations show mass gain during the first week of exposure for all concentrations ranging from C30 to C210. These findings suggest that *Aporrectodea caliginosa* species can tolerate glyphosate concentrations equivalent to 1680 µl kg^{-1} soil for a short period (one week). Overall, a relative and gradual decrease in mass was observed beginning on the eighth day of exposure for all concentrations, except for concentrations C60, C150, and C210, where the decrease in mass began on the sixteenth day of exposure. Glyphosate has a negative effect on growth starting on the sixteenth day of exposure for concentrations C120 and C180 and on the twenty-fourth day of exposure for concentrations C150 and C210. After several weeks of exposure, the negative effect of contamination on growth is demonstrated for concentrations below C180, particularly the lowest C30 (240 µl kg^{-1}). However, high concentrations have a negative impact on earthworm growth after only a short period of exposure.

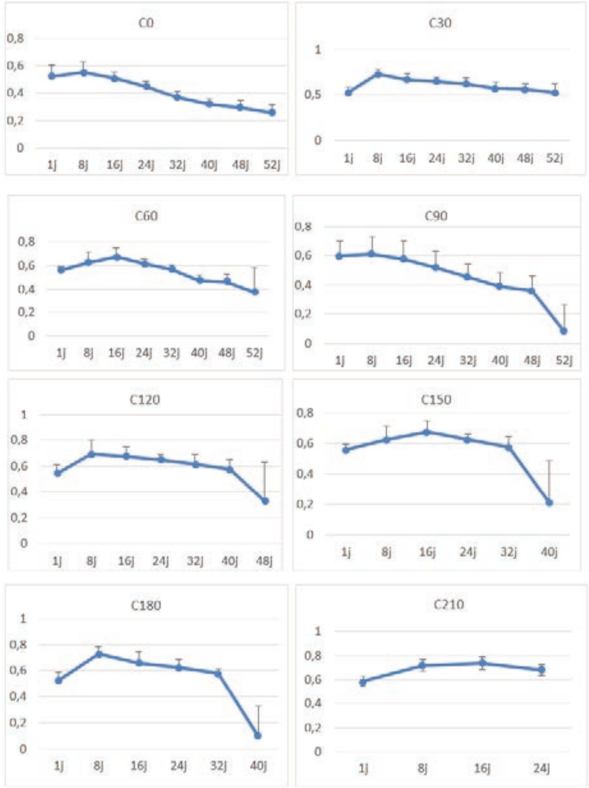


Figure 2: Variations in relative growth rate (%) as a function of time (j = day) and concentration

The multiple regression calculated to explain the evolution of mortality rate as a function of exposure duration and herbicide concentration is given by the equation:

Mortality = 0.078 (Time) + 0.0126 (Concentration) −2.17

The low probability value (7.321e-15) indicates that this regression model is significant. The coefficient of determination (0.6447) showed that the quality of the fit was good. Therefore, mortality increases as concentration increases and exposure is prolonged.

The results show that no mortality was observed for the control (C0 = 0 µl) for the entire duration of the experiment (52 days) (Fig. 3). For all concentrations tested, there was a very marked effect of time and degree of contamination on the mortality rate of *Aporrectodea caliginosa* earthworms. The lowest concentrations (240 µl kg^{-1}, 480 µl kg^{-1} and 720 µl kg^{-1}) had a delayed lethal effect, starting only on the forty-eighth day of exposure to the pesticide, with mortality rates of 1 ± 0.707, 2.2 ± 0.836 and 3 ± 0 respectively. This lag time decreases with increasing concentrations. In fact, at the highest concen-

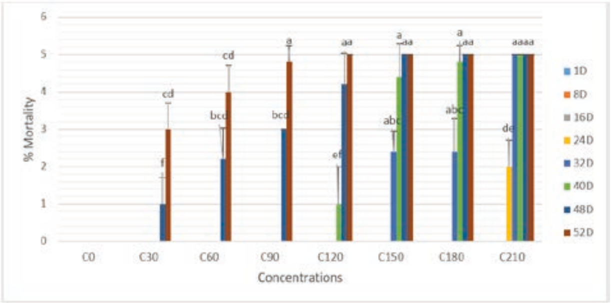


Figure 3: Mortality rate (number of individuals) of *Aporrectodea caliginosa* earthworms as a function of time and glyphosate concentration.

trations (1200 µl, 1440 µl, 1680 µl), the lag time is much reduced, resulting in 100 % mortality as early as day 32 of glyphosate exposure. According to the findings, mortality increases with time and concentration.

4 DISCUSSION

This ecotoxicity study aims to determine the toxicity of a glyphosate-based herbicide on *Aporrectodea caliginosa* earthworms. This species is uncommon in agricultural plots where the herbicide of choice is widely used. The biological parameters considered were mortality and growth. Throughout the experiment, no mortality was recorded for the control worms, indicating that the experimental conditions, aside from glyphosate contamination, did not contribute to earthworm mortality. Over the forty-eight days of experimentation, control earthworms showed a slight decrease in initial biomass. Conversely, the controls showed a significant mass recovery (0.266 ± 0.044 to 0.357 ± 0.181) at the end of the experiment.

The findings show that glyphosate is toxic to *Aporrectodea caliginosa* even at low soil concentrations. This toxicity is observed at low concentrations of around 240 µl kg^{-1} soil (C30). Toxicity levels vary according to exposure time and pollutant dose. Mass loss in contaminated individuals is significantly greater than in the control group. As a result, the sensitivity of *Aporrectodea caliginosa* to glyphosate was confirmed. Observing at least one death for low concentrations (C30) after forty-eight days of experimentation indicates that each concentration was potentially lethal for *Aporrectodea caliginosa* in this study. The worms' inability to resist the herbicide's lethal effects, particularly at high doses, may explain why mortality increased proportion to herbicide concentration. This finding is consistent with the findings of Casabé et al. (2007), who demonstrated that glyphosate is highly toxic to earthworms at recommended concentrations. Furthermore, for all doses above 960 mg kg^{-1}, this result could explain the total death of adults (100 % mortality) on the thirty-second day of contamination.

Thus, living in glyphosate-contaminated soil causes the species *Aporrectodea caliginosa* earthworms to lose mass, beginning as early as the first week of exposure to the pollutant. The researchers hypothesize that worms living in uncontaminated soil have more metabolic resources than worms living in contaminated soil; worms in uncontaminated soil are thus unaffected by contamination stress and can thus invest in mass gain (Pochron et al., 2017; Pochron et al., 2021). Body mass and survival time are affected by the glyphosate-based formula. Taken together, the data show that increasing glyphosate doses result in a significant decrease in survival time and a significant decrease in body mass when compared to the control. Toxicity studies on *Eisenia fetida* with the same contaminant show a steady decline in body mass in the organisms tested (Yasmin, D'Souza, 2007; Correia, Moreira, 2010). Tolerance to high concentrations of glyphosate was recently discovered in the same species, indicating its use in bioremediation processes (Lescano et al., 2020).

Growth, as measured by body mass, is a common assessment criterion in toxicological studies. However, body mass does not always decrease in response to contamination (Pochron et al., 2018; Pochron et al., 2019). When organisms are exposed to various contaminants, their body mass may increase. Recent research shows that earthworms living in nutrient-rich soil can gain mass even while metabolically processing contaminants (Pochron et al., 2020). However, our findings show that *Aporrectodea caliginosa* does not use this strategy in response to glyphosate. Herbicide concentrations of C30, C60, C90, C120, C150, C180, and C210 were tested on earthworms. Worms exposed to the lowest dose (240 µl kg^{-1}) for 48 days died at a low rate. At concentrations greater than 720 µl kg^{-1}, there was 100 % mortality at the end of the experiment. These findings are consistent with those obtained on the *Octolasion tyrtaeum* (Savigny, 1826): after 32 days of treatment, 100 % mortality was observed at the highest glyphosate concentration (50,000 mg kg^{-1}).

However, most studies on the acute effects of glyphosate at various concentrations on *Eisenia fetida* show that no product dose results in mortality. The effect of glyphosate-based herbicides on other earthworm species, *Lumbricus terrestris* and *Aporrectodea caliginosa* reveals that *L. terrestris* activity decreased three weeks after herbicide application. However, no change in activity was observed for *A. caliginosa* species (Gaupp-Berghausen et al., 2015), confirming that not all earthworm species respond similarly to soil contamination by glyphosate-based formulations and that the species *Aporrectodea caliginosa* exhibits a clear sensitivity to this herbicide. In addition to mass loss and mortality, individuals exposed to glyphosate showed behavioral abnormalities such as coiling and excitation, even at low concentrations.

Previous research on the toxicity of glyphosate indicates that while it does not directly cause earthworm mortality, it can have serious long-term consequences (Verrell, Van Buskirk, 2004). Sublethal effects on the population dynamics of the *Eisenia fetida* species exposed to glyphosate were observed, as evidenced by decreased cocoon fertility. This resulted in the extinction of the soil's earthworm population (Santadino et al., 2014). Anatomical changes were also observed after 30 days of experimentation. According to Correia, Moreira (2010), morphological abnormalities such as body elevation and curling were observed in all specimens exposed to high concentrations of glyphosate in soil. As a result, our findings support previous findings that different agrochemicals affect different earthworm body parameters (Van Gestel et al., 1992; Zaller et al., 2021).

Previous studies observed that earthworm biodiversity is reduced in intensively farmed fields (Smith et al., 2008), and laboratory studies on the species *Aporrectodea* and *Allolobophora* sp. conclude that pre-exposure to pesticides in the field enhances earthworm physiological responses (Givaudan, 2014).

Glyphosate is the most widely used herbicide in the world (Müller, 2021). Few experimental studies evaluating its impact on earthworms under field conditions have been carried out. Overall, these studies conclude that there is a high risk of chronic toxicity of these substances on earthworms if recommended doses are not respected (Pelosi et al., 2021). However, studies evaluating the impact of glyphosate-based pesticides under laboratory and controlled conditions are numerous, but their results remain controversial, due to the fact that these studies, do not reflect real field conditions and do not take into account potential variations in abiotic and biotic factors such as temperature and organic matter (Schmidt et al., 2024). In general, recent laboratory results still highlight the negative effect of glyphosate on earthworms, and they recommend further research on glyphosate in the laboratory and in the field, taking all variants into account, to prevent future threats to soil biodiversity from glyphosate (De Lima E Silva & Pélosi, 2024).

The present study will thus contribute to a broader understanding of the impact of glyphosate on soil fauna in general and earthworms in particular.

5 CONCLUSION

A study of the effect of the herbicide glyphosate on the earthworm *Aporrectodea caliginosa* revealed the species' sensitivity to this plant protection product. This sensitivity manifests itself in reduced growth and increased mortality in the species. Glyphosate has a negative effect that is dose and time-dependent. The effect appears after several weeks of exposure to glyphosate at the low concentration C30, equivalent to 240 µl kg^{-1} of product. At high concentrations (C210 equivalent to 1680 µl kg^{-1} product), however, the effect is visible after the second week of glyphosate exposure. According to our hypothesis, the use of glyphosate-based pesticides, in particular, significantly impacts the growth and mortality of the *Aporrectodea caliginosa* species, explaining its low abundance in cultivated land where the product is widely used. We believe this species could be a good candidate for bioindication of soil pollution caused by glyphosate-based pesticides. Its application in soil quality biomonitoring is also suggested.

6 CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

7 DATA AVAILABILITY STATEMENT

Data available on request from the corresponding author.

8 REFERENCES

Bartlett, M. D., Briones, M. J., Neilson, R., Schmidt, O., Spurgeon, D., & Creamer, R. E. (2010). A critical review of current methods in earthworm ecology: from individuals to populations. *European Journal of Soil Biology, 46*(2), 67-73. https://doi.org/10.1016/j.ejsobi.2009.11.006

Beckert, M., Dessaux, Y., Charlier, C., Darmency, H., Richard, C., Savini, I., & Tibi, A. (2011). Les variétés végétales tolérantes aux herbicides. Effets agronomiques, environnementaux, socio-économiques. Expertise scientifique collective, rapport, CNRS-INRA. Retrieved from http://inra.dam.front.pad.brainsonic.com/ressources/afile/223295-8f9d9-resource-expertise-vth-rapport-complet.html.

Bouché, M.B. (1972). *Lombriciens de France. Ecologie et systématique*. Paris: INRA Editions.

Casabé, N., Piola, L., Fuchs, J., Oneto, M. L., Pamparato, L., Basack, S., & Kesten, E. (2007). Ecotoxicological assessment of the effects of glyphosate and chlorpyrifos in an Argentine soya field. *Journal of Soils and Sediments*, *7*, 232-239. https://doi.org/10.1065/jss2007.04.224

Chevillot, F. (2017). *Etude de la bioaccumulation et autres effets sublétaux de contaminants organiques sur le vers de terre Eisenia andrei exposé à des concentrations environnementales*. Doctoral dissertation, Sherbrooke, Québec, Canada.

Correia, F. V., & Moreira, J. C. (2010). Effects of glyphosate and 2, 4-D on earthworms (*Eisenia foetida*) in laboratory tests. *Bulletin of Environmental Contamination and Toxicology, 85*(3), 264-268. https://doi.org/10.1007/s00128-010-0089-7

De Lima E Silva, C., & Pelosi, C. (2024). Effects of glyphosate on earthworms: From fears to facts. *Integrated Environmental Assessment and Management*, *20*(5), 1330-1336. https://doi.org/10.1002/ieam.4873

Domínguez, A., Brown, G. G., Sautter, K. D., Ribas de Oliveira, C. M., de Vasconcelos, E. C., Niva, C. C., & Bedano, J. C. (2016). Toxicity of AMPA to the earthworm *Eisenia andrei* Bouché, 1972 in tropical artificial soil. *Scientific Reports, 6*(1), 19731. https://doi.org/10.1038/srep19731

García-Torres, T., Giuffré, L., Romaniuk, R., Ríos, R. P., & Pagano, E. A. (2014). Exposure assessment to glyphosate of two species of annelids. *Bulletin of Environmental Contamination and Toxicology, 93*, 209-214. https://doi.org/10.1007/s00128-014-1312-8

Gaupp-Berghausen, M., Hofer, M., Rewald, B., & Zaller, J. G. (2015). Glyphosate-based herbicides reduce the activity and reproduction of earthworms and lead to increased soil nutrient concentrations. *Scientific Reports, 5*(1), 12886. https://doi.org/10.1038/srep12886

Gill, J. P. K., Sethi, N., Mohan, A., Datta, S., & Girdhar, M. (2018). Glyphosate toxicity for animals. *Environmental Chemistry Letters,16*, 401-426. https://doi.org/10.1007/s10311-017-0689-0

Givaudan, N. (2014). *Stratégies d'adaptation de la biodiversité des sols (vers de terre) aux pesticides: mécanismes en jeu et* évaluation *des coûts* écosystémiques. Doctoral dissertation, Rennes University, Syddansk Universitet, Rennes, France.

Lescano, M. R., Masin, C. E., Rodríguez, A. R., Godoy, J. L., & Zalazar, C. S. (2020). Earthworms to improve glyphosate degradation in biobeds. *Environmental Science and Pollution Research, 27*, 27023–27031. https://doi.org/10.1007/s11356-020-09002-w

Mamy, L., Barriuso, E., & Gabrielle, B. (2011). Impacts sur l'environnement des herbicides utilisés dans les cultures génétiquement modifiées. *Le Courrier de L'environnement de l'INRA*, *60*, 15-24.

Martin, N. A. (1986). Toxicity of pesticides to *Allobophora caliginosa* (Oligochaeta: Lumbricidae). *New Zealand Journal of Agricultural Research, 29*(4), 699-706. https://doi.org/10.1080/00288233.1986.10430466

Müller, B. (2021). Glyphosate, une histoire d'amour. *Monde Commun*, *5*(2), 48-64. https://doi.org/10.3917/moco.005.0048

Oultaf, L., Metna Ali Ahmed, F., & Sadoudi Ali Ahmed, D. (2022). Environmental and health risks of pesticide use practices by farmers in the region of Tizi-Ouzou (northern Algeria). *International Journal of Environmental Studies,* 1-11. https://doi.org/10.1080/00207233.2022.2044693

Owagboriaye, F., Dedeke, G., Bamidele, J., Bankole, A., Aladesida, A., Feyisola, R., & Adekunle, O. (2020). Wormcasts produced by three earthworm species (*Alma millsoni, Eudrilus eugeniae* and *Libyodrilus violaceus*) exposed to a glyphosate-based herbicide reduce growth, fruit yield and quality of tomato (*Lycopersicon esculentum*). *Chemosphere*, *250*, 126-270. https://doi.org/10.1016/j.chemosphere.2020.126270

Pelosi, C., Bertrand, C., Daniele, G., Coeurdassier, M., Benoit, P., Nélieu, S., & Fritsch, C. (2021). Résidus de pesticides actuellement utilisés dans les sols et les vers de terre: une menace silencieuse ?. *Agriculture, Ecosystems & Environment, 305*, 107-167.

Pochron, S., Choudhury, M., Gomez, R., Hussaini, S., Illuzzi, K., Mann, M., & Tucker, C. (2019). Temperature and body mass drive earthworm (*Eisenia fetida*) sensitivity to a popular glyphosate-based herbicide. *Applied Soil Ecology, 139*, 32-39. https://doi.org/10.1016/j.apsoil.2019.03.015

Pochron, S., Nikakis, J., Illuzzi, K., Baatz, A., Demirciyan, L., Dhillon, A., & Moawad M. (2018). Exposure to aged crumb rubber reduces survival time during a stress test in earthworms (*Eisenia fetida*). *Environmental Science and Pollution Research, 25*(12), 11376–11383. https://doi.org/10.1007/s11356-018-1433-4

Pochron, S.T., Fiorenza, A., Sperl, C., Ledda, B., Patterson, C.L., Tucker, C.C., … & Panico N. (2017). The response of earthworms (*Eisenia fetida*) and soil microbes to the crumb rubber material used in artificial turf fields. *Chemosphere, 173*, 557–562. https://doi.org/10.1016/j.chemosphere.2017.01.091

Pochron S.T., Mirza A., Mezic M., Chung E., Ezedum Z., Geraci G., ... & Smith R., (2021). Earthworms *Eisenia fetida* recover from Roundup exposure. *Applied Soil Ecology, 158*, 103793. https://doi.org/10.1016/j.apsoil.2020.103793

Santadino, M., Coviella, C., & Momo, F. (2014). Glyphosate sublethal effects on the population dynamics of the earthworm *Eisenia fetida* (Savigny, 1826). *Water, Air, & Soil Pollution, 225*, 1-8. https://doi.org/10.1007/s11270-014-2207-3

Sekhara-Baha, M. (2008). *Etude bioécologique des oligochètes du nord de l'Algérie*. Doctoral dissertation, INA, Algiers, Algeria.

Schmidt, R., Spangl, B., Gruber, E., Takács, E., Mörtl, M., Klátyik, S., ... & Zaller, JG. (2022). Glyphosate Effects on Earthworms: Active Ingredients vs. Commercial Herbicides at Different Temperature and Soil Organic Matter Levels. *Agrochemicals*, *2*(1), 1-16.

Smith, R. G., McSwiney, C. P., Grandy, A. S., Suwanwaree, P., Snider, R. M., & Robertson, G. P. (2008). Diversity and abundance of earthworms across an agricultural land-use intensity gradient. *Soil and Tillage Research, 100*(1-2), 83-88.

Valle, A. L., Mello, F. C. C., Alves-Balvedi, R. P., Rodrigues, L. P., & Goulart, L. R. (2019). Glyphosate detection: methods, needs and challenges. *Environmental Chemistry Letters, 17*, 291-317. https://doi.org/10.1007/s10311-018-0789-5

Van Gestel, C. A. M., Dirven-Van Breemen, E. M., Baerselman, R., Emans, H. J. B., Janssen, J. A. M., Postuma, R., & Van Vliet, P. J. M. (1992). Comparison of sublethal and lethal criteria for nine different chemicals in standardized toxicity tests using the earthworm *Eisenia andrei*. *Ecotoxicology and Environmental Safety, 23*(2), 206-220. https://doi.org/10.1016/0147-6513(92)90059-C

Van Gestel, C. A., & Loureiro, S. (2018). Terrestrial isopods as model organisms in soil ecotoxicology: a review. *ZooKeys*, (801), 127. https://doi.org/10.3897/zookeys.801.21970

Verrell, P. A. V. B., & Van Buskirk, E. (2004). As the worm turns: *Eisenia fetida* avoids soil contaminated by a glyphosate-based herbicide. *Bulletin of Environmental Contamination and Toxicology, 72*(2), 219-224. https://doi.org/10.1007/s00128-003-9134-0

Yasmin, S., & D'Souza, D. (2007). Effect of pesticides on the reproductive output of *Eisenia fetida*. *Bulletin of Environmental Contamination and Toxicology, 79*, 529-532. https://doi.org/10.1007/s00128-007-9269-5

Zaller, J.G., Weber, M., Maderthaner, M., Gruber, E., Takács, E., Mörtl, M., … & Leisch F. (2021). Effects of glyphosate-based herbicides and their active ingredients on earthworms, water infiltration and glyphosate leaching are influenced by soil properties. *Environmental Sciences Europe, 33*(1), 1–16. https://doi.org/10.1186/s12302-021-00492-0

doi:10.14720/aas.2025.121.1.19698 Original research article / izvirni znanstveni članek

Health risk assessment of heavy metals in basil (*Ocimum basilicum* L.) grown in artificially contaminated substrates

Ajla SMAJLOVIĆ [1], Adnan HADŽIĆ [1], Bekir TANKOSIĆ [1], Mirza VALJEVAC [1], Amina ŠERBO [1], Adna HADŽIĆ [1], Nermina HASIČEVIĆ [1], Senad MURTIĆ [1,2]

Received September 02, 2024; accepted March 23, 2025
Delo je prispelo 2. september 2024, sprejeto 23. marec 2025

Health risk assessment of heavy metals in basil (*Ocimum basilicum* L.) grown in artificially contaminated substrates

Abstract: This study aimed to determine the levels of Cd, Cr, and Pb in basil (*Ocimum basilicum* L.) cultivated on artificially contaminated substrates and to assess their potential harmful effects on human health via the calculation of the target hazard quotients (THQ). A pot experiment was performed in a completely randomized design for each tested heavy metal. It included four contamination treatments (0, 20, 50, and 100 mg kg^{-1} for Cd, and 0, 100, 250, and 500 mg kg^{-1} for Pb and Cr). Concentrations of Cd, Cr, and Pb in plant samples were determined by atomic absorption spectroscopy. The results of this study showed that the concentrations of Cd, Cr, and Pb were several times higher in the roots than in the aboveground parts of basil regardless of contamination levels. These are desirable results because only aboveground parts of basil are used for medicinal purposes or consumption. The THQ values for Cd, Cr, and Pb observed in this study were lower than 1 regardless of contamination levels, indicating that the consumption of basil from the study site (up to 10 g per day) does not pose a risk to human health from the point of view of heavy metal investigated.

Key words: cadmium, chromium, lead, pollution

Ocena zdravstvenega tveganja s težkimi kovinami pri navadni baziliki (*Ocimum basilicum* L.) rastoči v umetno kontaminiranih substratih

Izvleček: Namen raziskave je bil določiti vsebnosti Cd, Cr, in Pb v navadni baziliki (*Ocimum basilicum* L.) rastoči na namensko onesnaženih tleh in in oceniti njihove potencialno škodljive učinke na zdravje ljudi preko izračuna potencialnega koeficienta tveganja (THQ). Za testiranje posameznih kovin je bil izveden lončni poskus s popolno naključno zasnovo. Poskus je obsegal štiri obravavanja kontaminacije (0, 20, 50, in 100 mg kg^{-1} za Cd, in 0, 100, 250, in 500 mg kg^{-1} za Pb in Cr). Koncentracije Cd, Cr in Pb v rastlinah so bile določene z atomsko obsorpcijsko spektroskopijo. Rezultati raziskave so pokazali, da so bile koncentracije Cd, Cr, in Pb večje v koreninah kot v nadzemnih delih bazilike, ne glede na stopnjo kontaminacije. Ti izsledki so zaželjeni, kajti za medicinske in prehrambene namene se uporabljajo le nadzemni deli bazilike. Vrednosti indeksa toksičnosti (THQ) za Cd, Cr, in Pb, pridobljeni v tej raziskavi, so bile manjše od 1, ne glede na stopnjo kontaminacijer, kar nakazuje, da je uživanje bazilike v dnevnem odmerku do 10 g na dan ne predstavlja rizika za zdravje ljudi glede na preučevane težke kovine.

Ključne besede: kadmij, krom, svinec, onesnaženje

1 University of Sarajevo, Faculty of Agriculture and Food Sciences, Department for Plant Production, Sarajevo, Bosnia and Herzegovina
2 corresponding author: murticsenad@hotmail.com

1 INTRODUCTION

Medicinal plants represent a special class of plants that have been used for therapeutic and medical purposes since ancient times. The use of medicinal plants is more easily available and accessible to the population compared to conventional medicine, and therefore, the demand for herbal remedies is growing worldwide and is expected to increase continuously (Ekor, 2014). However, due to the continuous increase of environmental pollution caused mainly by the urbanization, extensive use of chemical fertilizers and pesticides in plant production and rapid development of highly polluting industries, consumer concerns regarding the safety of their use have been highlighted (Łuszczki et al., 2019).

Inorganic pollutants, such as heavy metals, are of great concern to public health because of their persistence and high toxicity (Jadaa & Mohammed, 2023). Cd, Cr and Pb are among the most dangerous heavy metals because they can cause adverse effects on human health even in small quantities (Isinkaralar et al., 2024). As a result, the abovementioned heavy metals represent a category of pollutants of particular interest for health risk studies. There are numerous scientific studies which have provided evidence that an excessive amount of Cd in the human body can damage the kidneys, liver, bones and heart, and in severe cases can even cause death (Fatima et al., 2019; Charkiewicz et al., 2023). Adverse health effects associated with Cr exposure include respiratory, gastrointestinal and cardiovascular damage (Shin et al., 2023), while exposure to Pb has been found to be associated with a high risk of brain and kidney damage and cardiovascular diseases (Bhasin et al., 2023). Considering these aspects, monitoring toxic heavy metals, particularly Cr, Cd and Pb, in plants intended for consumption or medicinal use has become imperative.

Basil (*Ocimum basilicum* L., Lamiaceae) is a well-known culinary and medicinal plant originating from South Asia, from where it is spread all over the world, especially around the Mediterranean Sea (Azizah et al., 2023). As a traditional herbal remedy, basil leaves have been used since ancient times to treat a wide range of respiratory and gastrointestinal ailments. In addition, basil is easy to grow and has a high harvest index, making it very popular among farmers and crop producers. On the one hand, this has resulted in increased production and consumption of basil on the global scene (Polyakova et al., 2015), but on the other hand, this has led to increased consumer concerns regarding the quality and safety of its use (Nadeem et al., 2022).

Given the fact that the information on the safety of basil is relatively limited, especially from the heavy metal contamination point of view, this study aimed to determine the levels of Cd, Cr, and Pb in different parts of basil plants grown on artificially contaminated substrates and to assess their potential harmful effects on human health *via* the calculation of the target hazard quotients (THQ).

Our hypotheses were: (1) levels of Cd, Cr and Pb in basil plants tend to increase with increasing their content in growing substrates, and (2) basil plants grown on artificially contaminated substrates would pose a risk to human health from the point of view of Cd, Cr and Pb contamination.

2 MATERIALS AND METHODS

2.1 EXPERIMENTAL SITE AND GREENHOUSE EXPERIMENTAL DESIGN

This study was carried out from mid-May to end of June 2024 in a naturally ventilated greenhouse at the experimental station of the Faculty of Agriculture and Food Sciences (Sarajevo, Bosnia and Herzegovina). On May 18, 2024, one-month-old basil plants (*Ocimum basilicum* 'Genovese Gigante', purchased from a local nursery), which exhibited minimal variation in size and appearance, were transplanted into plastic pots (12 cm diameter × 20 cm height, one plant per plot) previously filled with a commercial growing substrate artificially contaminated with heavy metals (Cd, Cr and Pb). The selected substrate composition was a mixture of black and white peat, coconut fibre, composted plant material and other organic

Table 1: Chemical properties of the growing substrate before adding the contaminants

Parameter	Measure unit	Value
pH H_2O	pH range	6.3
pH KCl	pH range	6.0
organic matter	%	56.5
available forms of potassium (K_2O)	mg 100 g^{-1}	37.6
available forms of phosphorus (P_2O_5)	mg 100 g^{-1}	43.2
Cd content	mg kg^{-1}	0.1
Cr content	mg kg^{-1}	4.7
Pb content	mg kg^{-1}	1.1
Cu content	mg kg^{-1}	13.4
Zn content	mg kg^{-1}	28.9
Mn content	mg kg^{-1}	125.9
Fe content	mg kg^{-1}	631.7
Ni content	mg kg^{-1}	2.8

material (wood fibre and compost) with 1 g fertilizer N-P-K 15-15-15 per litre of the substrate. Information on both substrate composition and physical-chemical properties was provided by the manufacturer. The chemical properties of the growing substrate are listed in Table 1.

From the point of view of chemical properties, the growing medium used in this study is suitable for basil growth and development. All of the tested heavy metals in the growing medium were found to be below the permissible limits recommended by the Food and Agriculture Organization of the United Nations (FAO). According to FAO, the permissible limits for Cd, Cu, Zn, Mn, Ni and Cr in agricultural soils are 3, 100, 300, 2000, 50 and 100 mg kg^{-1} dry mass, respectively (FAO, 1985).

Before starting the experiment, the substrate was artificially contaminated by adding a solution containing heavy metals (Cd, Cr and Pb) and then mixed thoroughly to ensure a uniform distribution of the heavy metal solution. Cd, Cr and Pb were applied as $CdCl_2$, $K_2Cr_2O_7$ and $Pb(NO_3)_2$, respectively.

This study included four contamination treatments within each heavy metal tested i.e. 0, 20, 50, and 100 mg kg^{-1} for Cd, and 0, 100, 250, and 500 mg kg^{-1} for Pb and Cr, with three replications per treatment. Each contamination treatment consisted of five pots/plants, resulting in 180 pots in this study. Air temperature and relative humidity inside the greenhouse varied from 19 ± 3 °C to 29 ± 3 °C and from 60 % to 90 %, respectively. Air circulation was achieved by opening the roof vents and main door during the day. A green shade cloth was used to reduce heat build-up during hot days. Each plant/pot was regularly watered every other day. There was no fertilizers application during the investigation.

After two months of plant growth under these conditions, the experiment was terminated. The plants, i.e., leaves and roots of control and heavy metal-stressed basil plants, were harvested and then separated into roots and aboveground parts of plants. The fresh mass was recorded right after harvesting, whereas the dry mass was determined after being oven-dried at 60 °C until a stable weight was reached. Plant height was measured with a ruler, and the leaf area was computed based on the method outlined by Pandey and Singh (2011). The plant material for heavy metal analysis was dried in an oven at 80 °C to a constant mass, ground into powder using an electric blender, and kept dry in paper bags in a desiccator until analysis.

2.2 HEAVY METAL ANALYSIS

Wet digestion method was used to extract the heavy metals of the plant samples (Lisjak et al., 2009). In short, 1g of dry plant sample was placed in an Erlenmeyer flask and then 10 ml of HNO_3 and 4 ml of H_2SO_4 were added. The mixture was left overnight at room temperature and then heated on a hot plate for 2 h at 60 °C and then at 100 °C until the formation of brown fumes stopped (approximately 1 h). After cooling to room temperature, the mixture was filtered to the mark through quantitative filter paper (Whatman, No. 42) in a volumetric flask (25 ml) and diluted with deionized water.

Heavy metal concentrations (Cd, Cr, Pb) in plant samples were determined by atomic absorption spectrophotometry using the Shimadzu AA-7000 device (Shimadzu Instruments, Tokio, Japan). The standard and working solutions of investigated heavy metals were prepared on a daily basis by diluting the certified stock solutions (Merck, Darmstadt, Germany) with deionized water as necessary.

2.3 HEALTH RISK ANALYSIS

The potential human health risk of Cd, Cr and Pb through consumption of basil plants was assessed using target hazard quotient (THQ), which was described in detail by the USEPA (United States Environmental Protection Agency). In short, the THQ value describes the non-carcinogenic health risk posed by exposure to the respective toxic element. The following equation was used for THQ calculation (USEPA, 20111).

$$THO = \frac{c\ x\ IR\ x\ EF\ x\ ED}{ET\ x\ BW\ x\ RfD}$$

where is:

c - concentration of contaminant in analysed plant sample (mg kg^{-1})

IR – food (basil) dietary intake (0.01 kg/person/day)

EF – exposure frequency (365 days per year)

ED – exposure duration for adult (70 years)

ET – averaged exposure time (25550 days (EF x ED))

BW – body mass (for adults 70 kg, for children 32 kg)

RfD – the oral reference dose i.e. the highest level of contaminant at which no adverse health effects are expected (according to USEPA (2011) the RfD values for Cd, Cr and Pb are 0.001, 0.004 and 0.003 mg/kg/day, respectively.

Table 2: Growth characteristics of basil plants cultivated on contaminated substrates

Treatments	Plant height (cm)	Leaf area (cm^2)	Fresh aboveground mass (g)	Dry aboveground mass (g)
Control	31.3 ± 6.2	8.5 ± 5.9	26.7 ± 2.7	3.7 ± 0.6
100 mg kg^{-1} of Cr	30.5 ± 6.3	8.6 ± 5.2	26.3 ± 4.1	3.5 ± 0.9
250 mg kg^{-1} of Cr	29.7 ± 6.2	8.4 ± 5.1	26.1 ± 3.7	3.2 ± 0.7
500 mg kg^{-1} of Cr	28.2 ± 7.6	7.7 ± 6.9	25.5 ± 2.1	2.9 ± 0.9
100 mg kg^{-1} of Pb	31.1 ± 5.9	8.2 ± 6.2	25.7 ± 6.3	3.1 ± 1.3
250 mg kg^{-1} of Pb	28.2 ± 7.9	7.9 ± 5.3	25.8 ± 3.9	3.1 ± 0.9
500 mg kg^{-1} of Pb	26.5 ± 7.2	7.3 ± 4.2	25.5 ± 2.5	3.0 ± 1.2
20 mg kg^{-1} of Cd	29.9 ± 5.9	8.5 ± 5.3	25.9 ± 7.2	3.4 ± 1.1
50 mg kg^{-1} of Cd	28.1 ± 6.3	8.2 ± 5.6	25.5 ± 6.5	3.3 ± 0.9
100 mg kg^{-1} of Cd	28.6 ± 5.2	7.7 ± 7.2	25.8 ± 4.2	3.4 ± 1.2
$LSD_{0.05}$	-	-	-	-

Any THQ value lower than the threshold value of 1 indicates that there is no risk of non-carcinogenic diseases through the consumption of the tested food.

2.4 STATISTICAL ANALYSIS

All data were analysed using Microsoft Excel software. The least significant difference (LSD) test at a 5 % probability level was performed to establish significant differences between treatment's means.

3 RESULTS

The basic growth characteristics of basil plants grown in substrates contaminated with Cr, Pb, and Cd are given in Table 2.

The basil plants grown in the substrates artificially contaminated with Cd, Cr and Pb, presented similar phenotypes comparing with the plants from the non-contaminated growing medium. No considerable difference in plant height, leaf area and fresh and dry aboveground mass among basil plants was observed during the exposure period, regardless of the level of substrate contamination. Furthermore, no signs of heavy metal toxicity were observed in basil plants.

Heavy metal concentrations (Cd, Cr, Pb) in roots and leaves of basil plants grown on contaminated substrates are shown in Table 3, 4 and 5. All results are expressed on a dry mass basis in mg kg^{-1}.

As expected, the concentrations of Cd, Cr and Pb were significantly higher in basil plants cultivated on contaminated substrates compared to control plants. The results also showed that the uptake of Cd, Cr and Pb by basil plants corresponded to the increasing level of soil contamination.

In this study, the concentrations of Cd, Cr, and Pb were several times higher in the roots than in the aboveground parts of basil plants, regardless of the level of

Table 3: Heavy metal levels in basil plants grown on Cd-contaminated substrates

Treatments	Roots	Leaves
0 mg kg^{-1} of Cd	0.1 ± 0.1^{d*}	0.3 ± 0.1^{c}
20 mg kg^{-1} of Cd	58.7 ± 10.1^{c}	17.9 ± 5.7^{b}
50 mg kg^{-1} of Cd	145.3 ± 9.7^{b}	18.8 ± 7.9^{b}
100 mg kg^{-1} of Cd	223.7 ± 23.1^{a}	30.1 ± 5.8^{a}
$LSD_{0.05}$	13.1	6.5

*Averages denoted by the same letter in the same column indicate no significant difference ($p < 0.05$)

Table 4: Heavy metal levels in basil plants grown on Cr-contaminated substrates

Treatments	Roots	Leaves
0 mg kg^{-1} of Cr	2.9 ± 1.4^{d*}	1.9 ± 1.8^{c}
100 mg kg^{-1} of Cr	73.7 ± 10.1^{c}	12.1 ± 1.9^{b}
250 mg kg^{-1} of Cr	394.2 ± 22.5^{b}	12.3 ± 7.7^{b}
500 mg kg^{-1} of Cr	625.9 ± 20.1^{a}	25.3 ± 17.2^{a}
$LSD_{0.05}$	15.9	9.4

*Averages denoted by the same letter in the same column indicate no significant difference ($p < 0.05$)

substrate contamination. These results strongly indicate that basil plants have the ability to significantly reduce the transfer of Cd, Cr and Pb from roots to aboveground parts. From the public health point of view, these results are extremely desirable because only aboveground parts of basil are used for medicinal purposes or consumption.

The target health quotient (THQ) was estimated to assess the human health risks posed by the intake of those potentially toxic elements (Cr, Cd, and Pb) from the consumption of the basil plants. The THQ values calculated for each tested heavy metal found in basil leaves are shown in Table 6.

Table 5: Heavy metal levels in basil plants grown on Pb-contaminated substrates

Treatments	Roots	Leaves
0 mg kg^{-1} of Pb	1.4 ± 0.3^{d*}	0.4 ± 0.2^{c}
100 mg kg^{-1} of Pb	90.9 ± 7.7^{c}	12.3 ± 2.1^{b}
250 mg kg^{-1} of Pb	369.4 ± 38.3^{b}	53.2 ± 46.1^{a}
500 mg kg^{-1} of Pb	1041.7 ± 111.1^{a}	75.3 ± 43.8^{a}
$LSD_{0.05}$	59.8	31.4

*Averages denoted by the same letter in the same column indicate no significant difference ($p < 0.05$)

Table 6: THQ values for Cd, Cr and Pb due to consumption of basil leaves

Treatment	Adult	Child	Treatment	Adult	Child	Treatment	Adult	Child
0 mg kg^{-1} of Cd	0.004	0.008	0 mg kg^{-1} of Cr	0.01	0.02	0 mg kg^{-1} of Pb	0.002	0.004
20 mg kg^{-1} of Cd	0.26	0.57	100 mg kg^{-1} of Cr	0.06	0.13	100 mg kg^{-1} of Pb	0.05	0.06
50 mg kg^{-1} of Cd	0.27	0.59	250 mg kg^{-1} of Cr	0.06	0.13	250 mg kg^{-1} of Pb	0.22	0.48
100 mg kg^{-1} of Cd	0.42	0.92	500 mg kg^{-1} of Cr	0.12	0.26	500 mg kg^{-1} of Pb	0.31	0.52

The THQ values of Cd, Cr and Pb for adults and children in all tested basil leaves were much less than 1, indicating no significant health risks associated with basil leaves consumption.

4 DISCUSSION

The basil plants that were grown in the substrates artificially contaminated with Cd, Cr and Pb showed similar phenotypic traits to those grown in uncontaminated media. Additionally, no indications of heavy metal toxicity were detected in the basil plants. On the basis of these findings, basil can be considered as plant species that have a high ability to grow successfully in a Cd, Cr and Pb rich growing medium. Similar finding was also reported by Dinu et al. (2020) and Hlihor et al. (2022).

Adaptation of basil or any other plant species to heavy metal stress conditions is a complex process and depends not only on the specific metals present, their speciation and amounts in the growing medium, but also on the plant genotype and substrate/soil physical and chemical properties (Rashid et al., 2023). In this regard, it is important to note that there is no adaptation to heavy metal stress in general, suggesting that tolerance to heavy metal stress is mainly related to the individual heavy metal. However, there is a growing body of studies that show that some plants exhibit combined tolerance to different heavy metals (Mehes-Smith et al., 2013; Viehweger, 2014). The results of the current study point to the conclusion that basil can be considered one of those plants.

In general, plant tolerance to heavy metal stress can be achieved by 'avoidance mechanisms' through which plants can restrict the heavy metal uptake into the body and/or by 'tolerance mechanisms' based on the hyperaccumulation of heavy metal ions within the plant body without negative effects on its growth and development (Yan et al., 2020).

Given the fact that the Cd, Cr, and Pb concentrations in this study were several times higher in the roots than in the above-ground parts of basil plants, regardless of the level of substrate contamination, it can be assumed that basil plants possess very effective mechanisms to absorb Cd, Cr and Pb from growing medium and then accumulate them in roots through various mechanisms including metal binding to cell walls and sequestration and compartmentalization of heavy metal ions in root cells, mainly in vacuoles. This finding was in line with findings in earlier studies where it was found that basil accumulates more Cd, Cr and Pb in its roots than in other plant parts (Adamczyk-Szabela et al., 2017; Lycas et al., 2022; Ur Rahman et al., 2024). However, these results are opposite to those obtained in study of Jena and Gupta (2012). In their study, the leaves of basil plants were found to contain higher concentrations of heavy metals, including Cr, than roots. According to the authors, the reason for this is the atmospheric deposition of pollutants by both dry and wet deposition.

In this study, the levels of Cd, Cr and Pb in basil plants grown on contaminated substrates increased with increasing the level of substrate contamination and ranged from 17.9 to 30.1 mg kg^{-1}, from 12.1 to 25.3 mg kg^{-1} and from 12.3 to 75.3 mg kg^{-1}, respectively. These values were much higher than the acceptable levels established by World Health Organization (WHO). According to WHO, the maximum acceptable levels for Cd, Pb and Cr in foodstuffs are 0.2 mg kg^{-1}, 0.3 mg kg^{-1} and 2.3 mg kg^{-1} dry mass, respectively (WHO/FAO, 2007). From this point of view, consumption of basil leaves from the studied site cannot be considered safe for human health.

However, in the case of THQ, the results showed the opposite; the mean THQ values of Cd, Cr, and Pb for adults and children were lower than 1, suggesting no significant human health risks through consumption basil from studied site. The disparity in the results can be explained by the fact that assessing the potential health risks associated with heavy metal in foodstuffs strongly depends on the average daily dietary intake (Rai et al., 2019). Given that basil and spices are consumed in amounts several times smaller than other food ingredients, the THQ values obtained in this study seem reasonable and logical. In this regard, it is important to note that the health risks of consuming heavy metals through spices increase sharply with increasing dietary spice intake.

However, the quantification of spice intake at the individual level is a difficult task and represents a great challenge because the frequency and quantity of spice intake depend considerable on the type of spice, form in which it is used, and consumers' consumption preferences (Siruguri & Bhat, 2015). In this study, the average daily intake of spices per person was estimated to be 10.0 g/person/day, since this value is mainly used in scientific studies to calculate THQ (Tefera & Teklewold, 2021; Ezez et al., 2024; Oladeji et al., 2024). If this data is relevant, it seems there is no human health risks associated with consumption of basil plants from the study site (up to 10 g per day). However, consumption of these plants in an amount higher than 10 g per day could potentially have a negative impact on human health, from a toxicological point of view. These findings strongly suggest that the knowledge of the consumption pattern regarding spices has a remarkable relevance in human health risk assessment.

5 CONCLUSIONS

Cd, Cr, and Pb concentrations were several times higher in the roots than in the above-ground parts of basil regardless of contamination levels, suggesting that basil possesses very effective mechanisms to prevent their transport from roots to other parts of plants. These are desirable results because only aboveground parts of basil are used for medicinal purposes or consumption. The THQ values for Cd, Cr, and Pb observed in this study were lower than 1 regardless of contamination levels, indicating that the consumption of basil from the study site (up to 10 g per day) does not pose a risk to human health from the point of view of heavy metal investigated.

6 REFERENCES

Adamczyk-Szabela, D., Romanowska-Duda, Z., Lisowska, K., & Wolf, W. M. (2017). Heavy metal uptake by herbs. V. Metal accumulation and physiological effects induced by thiuram in *Ocimum basilicum* L. *Water, Air, & Soil Pollution, 228*(9), 334. https://doi.org/10.1007/s11270-017-3508-0

Azizah, N. S., Irawan, B., Kusmoro, J., Safriansyah, W., Farabi, K., Oktavia, D., Doni, F., & Miranti, M. (2023). Sweet basil (*Ocimum basilicum* L.) – A review of its botany, phytochemistry, pharmacological activities, and biotechnological development. *Plants, 12*(24), 4148. https://doi.org/10.3390/plants12244148

Bhasin, T., Lamture, Y., Kumar, M., & Dhamecha, R. (2023). Unveiling the health ramifications of lead poisoning: A narrative review. *Cureus, 15*(10), e46727. https://doi.org/10.7759/cureus.46727

Charkiewicz, A. E., Omeljaniuk, W. J., Nowak, K., Garley, M., & Nikliński, J. (2023). Cadmium toxicity and health effects – A brief summary. *Molecules, 28*(18), 6620. https://doi.org/10.3390/molecules28186620

Dinu, C., Vasile, G. G., Buleandra, M., Popa, D. E., Gheorghe, S., & Ungureanu, E-M. (2020). Translocation and accumulation of heavy metals in *Ocimum basilicum* L. plants grown in a mining-contaminated soil. *Journal of Soils and Sediments, 20*, 2141–2154. https://doi.org/10.1007/s11368-019-02550-w

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology, 4*, 177. https://doi.org/10.3389/fphar.2013.00177

Ezez, D., Birhanu, H., Shamena, S., & Engidaw, S. (2024). Bioaccumulation of heavy metals, assessment of carcinogenic and non-carcinogenic health risk in various spices. *Journal of Hazardous Materials Advances, 15*, 100441. https://doi.org/10.1016/j.hazadv.2024.100441

FAO. (1985). Guidelines: land evaluation for irrigated agriculture. *Soils Bulletin 55*. Food and Agriculture Organization of the United Nations, Rome, Italy. Retrieved from https://www.fao.org/4/x5648e/x5648e00.htm

Fatima, G., Raza, A. M., Hadi, N., Nigam, N., & Mahdi, A. A. (2019). Cadmium in human diseases: It's more than just a mere metal. *Indian Journal of Clinical Biochemistry, 34*(4), 371–378. https://doi.org/10.1007/s12291-019-00839-8

Hlihor, R. M., Roșca, M., Hagiu-Zaleschi, L., Simion, I. M., Daraban, G. M., & Stoleru, V. (2022). Medicinal plant growth in heavy metals contaminated soils: Responses to metal stress and induced risks to human health. *Toxics, 10*(9), 499. https://doi.org/10.3390/toxics10090499

Isinkaralar, O., Isinkaralar, K., & Nguyen, T. N. T. (2024). Spatial distribution, pollution level and human health risk assessment of heavy metals in urban street dust at neighbourhood scale. *International Journal of Biometeorology*, 2024. https://doi.org/10.1007/s00484-024-02729-y

Jadaa, W., & Mohammed, H. K. (2023). Heavy metals – definition, natural and anthropogenic sources of releasing into ecosystems, toxicity, and removal methods – An overview sStudy. *Journal of Ecological Engineering, 24*(6), 249–271. https://doi.org/10.12911/22998993/162955

Jena, V., & Gupta, S. (2012). Study of heavy metal distribution in medicinal plant basil. *Journal of Environmental & Analytical Toxicology, 2*, 8. https://doi.org/10.4172/2161-0525.1000161

Lisjak, M., Špoljarević, M., Agić, D., & Andrić, L. 2009. *Practicum-Plant Physiology*. Osijek: Faculty of Agriculture in Osijek.

Łuszczki, J. J., Gustaw-Rothenberg, K., Chmielewski, J., & Florek-Łuszczki, M. (2019). Prospects for the use of herbal medicines in relation to progressing environmental pollution. *Medycyna Środowiskowa, 22*(1-2), 5–8. https://doi.org/10.26444/ms/117884

Lycas, C., Zografou, M., & Kazi, M. (2022). Cadmium, nickel, chromium, and lead accumulation in roots, shoots, and leaves of basil plants (*Ocinum basilicum* L.). *International Journal of Agriculture and Environmental Science, 9(*2), 1–14. https://doi.org/10.14445/23942568/IJAES-V9I2P101

Mehes-Smith, M., Nkongolo, K., & Cholewa, E. (2013). Coping mechanisms of plants to metal contaminated soil. In S. Silvern & S. Yang (Eds.), *Environmental Change and Sustainability*. London: InTechOpen. https://doi.org/10.5772/55124

Nadeem, H. R., Akhtar, S., Sestili, P., Ismail, T., Neugart, S., Qamar, M., & Esatbeyoglu, T. (2022). Toxicity, antioxidant activity, and phytochemicals of basil (*Ocimum basilicum* L.) leaves cultivated in southern Punjab, Pakistan. *Foods, 11*(9), 1239. https://doi.org/10.3390/foods11091239

Oladeji, O. M., Kopaopa, B. G., Mugivhisa, L. L, & Olowoyo, J. O. (2024). Investigation of heavy metal analysis on medicinal plants used for the treatment of skin cancer by traditional practitioners in Pretoria. *Biological Trace Element Research, 202*(2), 778–786. https://doi.org/10.1007/s12011-023-03701-4

Pandey, S. K., & Singh, H. (2011). A simple, cost-effective method for leaf area estimation. *Journal of Botany, 2011*, 658240. https://doi.org/10.1155/2011/658240

Polyakova, M. N., Martirosyan Y. T., Dilovarova, T. A., & Kosobryukhov, A. A. (2015). Photosynthesis and productivity of basil plants (*Ocimum basilicum* L.) under different irradiation. *Sel'skokhozyaistvennaya Biologiya, 50*(1), 124–130. https://doi.org/10.15389/agrobiology.2015.1.124eng

Rai, P. K., Lee, S. S., Zhang, M., Tsang, Y. F, & Kim, K. H. (2019). Heavy metals in food crops: Health risks, fate, mechanisms, and management. *Environment International, 125,* 365–385. https://doi.org/10.1016/j.envint.2019.01.067

Rashid, A., Schutte, B. J., Ulery, A., Deyholos, M. K., Sanogo, S., Lehnhoff, E. A., & Beck, L. (2023). Heavy metal contamination in agricultural soil: Environmental pollutants affecting crop health. *Agronomy, 13*(6), 1521. https://doi.org/10.3390/agronomy13061521

Shin, D. Y., Lee, S. M., Jang, Y., Lee, J., Lee, C. M., Cho, E-M., & Seo, Y. R. (2023). Adverse human health effects of chromium by exposure route: A comprehensive review based on toxicogenomic approach. *International Journal of Molecular Sciences, 24*(4), 3410. https://doi.org/10.3390/ijms24043410

Siruguri, V., & Bhat, R. V. (2015). Assessing intake of spices by pattern of spice use, frequency of consumption and portion size of spices consumed from routinely prepared dishes in southern India. *Nutrition Journal, 14*, 7. https://doi.org/10.1186/1475-2891-14-7

Tefera, M., & Teklewold, A. (2021). Health risk assessment of heavy metals in selected Ethiopian spices. *Heliyon, 7*(5), e07048. https://doi.org/10.1016/j.heliyon.2021.e07048

Ur Rahman, S., Qin, A., Zain, M., Mushtaq, Z., Mehmood, F., Riaz, L., . . . Shehzad, M. (2024). Pb uptake, accumulation, and translocation in plants: Plant physiological, biochemical, and molecular response: A review. *Heliyon, 10*(6), e27724. https://doi.org/10.1016/j.heliyon.2024.e27724

USEPA. (2011). United States Environmental Protection Agency – Regional Screening Level (RSL) Summary Table: November 2011. Retrieved from http://www.epa.gov/regshwmd/risk/human/Index.htm

Viehweger, K. (2014). How plants cope with heavy metals. *Botanical Studies, 55*, 35. https://doi.org/10.1186/1999-3110-55-35

WHO/FAO. (2007). Joint FAO/WHO Food Standard Codex Alimentarius Commission 13th Session. *Report of the Thirty Eight Session of the Codex Committee on Food Hygiene. Houston, United States of America, 4 – 9 December, 2006.*

Yan, A., Wang, Y., Tan, S. N., Mohd Yusof, M. L., Ghosh, S. & Chen, Z. (2020). Phytoremediation: A promising approach for revegetation of heavy metal-polluted land. *Frontiers in Plant Science, 11*, 359. https://doi.org/10.3389/fpls.2020.00359

doi:10.14720/aas.2025.121.1.19926 Original research article / izvirni znanstveni članek

Beneficial effect of organic and inorganic forms of selenium on yield and nutritional characteristics of beetroot

Nadezhda GOLUBKINA [1, 2], Vladimir ZAYACHKOVSKY [1], Pavel POLUBOYARINOV [3], Zarema AMAGOVA [4], Agnieszka SĘKARA [5], Otilia Cristina MURARIU [6], Alessio Vincenzo TALLARITA [7], Gianluca CARUSO [7]

Received October 06, 2024; accepted December 18, 2024
Delo je prispelo 6. oktober 2024, sprejeto 18. december 2024

Beneficial effect of organic and inorganic forms of selenium on yield and nutritional characteristics of beetroot

Abstract: Beetroot (garden beet) is an essential component of the human diet and a source of biologically active compounds, valuable for the pharmaceutical and food industry. To increase the content of essential nutrients and develop a functional food product with enhanced antioxidants, the effect of foliar sodium selenate and selenocystine supply on yield and biochemical characteristics of two table beet cultivars (Marusia and Nezhnost) was assessed. Compared to the untreated control, foliar application of 26.4 mM sodium selenate increased root yield by 1.20-1.25 times, monosaccharide content by 1.49-2.25 times, betalain pigments by 1.56-2.17 times and total antioxidant activity (AOA) by 1.38-1.79 times, whereas the selenocystine supply increased the same parameters by 1.44-1.85, 1.64-3.4, 1.28-1.50 and 1.31-1.33 times, respectively. Compared to pulp, root peel demonstrated 2-2.6 times higher levels of betalain pigments, 1.9-2.4 times higher levels of polyphenols (TP), and 1.5-2.2 times higher antioxidant activity. Significant varietal differences in biochemical characteristic changes due to organic and inorganic Se supply were recorded. Taking into account the relatively low Se biofortification levels of roots (3-3.5 in pulp and 7-12 in peel), the results of the present research prove the importance of Se application mostly to improve beetroot yield, antioxidant content, including betalain pigments, and root peel utilization as a significant source of pharmaceuticals.

Key words: *Beta vulgaris* L. ssp. *vulgaris* var. *vulgaris*; antioxidant status; betalain pigments; selenate; selenocystine; yield

Ugodni učinki organskih in anorganskih oblik selena na pridelek in prehranske lastnosti rdeče pese

Izvleček: Rdeča pesa je bistvena sestavina človeške prehrane in vir biološko aktivnih snovi, ki so pomembne za farmacevtsko in prehrambeno industrijo. Za povečanje vsebnosti esencialnih hranil in razvoj funkcionalnih prehrambenih produktov s povečano vsebnostjo antioksidantov je bil ocenjen vpliv foliarnega dodajanja natrijevega selenata in selenocistina na pridelek in biokemične lastnosti dveh sort rdeče pese (Marusia in Nezhnost). V primerjavi s kontrolo je foliarno dodajanje 26,4 mM natrijevega selenata povečalo pridelek rdeče pese za 1,20-1,25 krat, vsebnost monosaharidov za 1,49 do 2,25 krat, vsebnost betalaina za 1,56-2,17 krat in celokupno antioksidacijsko aktivnost (AOA) za 1,38-1,79 krat. Dodajanje selenocistina je iste paramatre povečalo za 1,44-1,85, 1,64-3,4, 1,28-1,50 in 1,31-1,33 krat. V primerjavi s pulpo je olupek rdeče pese izkazal 2-2,6 krat večje vsebnost betalaina, 1,9-2,4 krat večje vsebnosti polifenolov (TP) in 1,5-2,2 krat večjo antioksidacijsko aktivnost. Pri dodatkih organskega in anorganskega Se so bile ugotovljene tudi značilne razlike v biokemičnih lastnostih med sortama rdeče pese. Glede na relativno majhno kopičenje Se v koreninskem gomolju rdeče pese (3-3,5 krat v pulpi in 7-12 krat v olupu) rezultati raziskave dokazujejo pomen dodajanja selena, predvsem za povečanje pridelka, vsebnosti antioksidantov vključno z betalainom in uporabo olupkov kot pomemben vir farmacevtsko zanimivih snovi.

Ključne besede: *Beta vulgaris* L. ssp. *vulgaris* var. *vulgaris*; stanje antioksidantov; betalaini; selenate; selenocistin; pridelek

1 Federal Scientific Vegetable Center, Moscow region 143072, Russia
2 Corresponding author: segolubkina45@gmail.com; tel.+7-903-118-50-30 (Nadezhda Golubkina)
3 Medical Faculty, Department of General and Clinical Pharmacology, Penza State University, 440026 Penza, Russia
4 Chechen Scientific Institute of Agriculture, 366021Gikalo, Grozny, Chechen republic, Russia
5 Department of Horticulture, Faculty of Biotechnology and Horticulture, University of Agriculture, 31-120 Krakow, Poland
6 Department of Food Technology, 'Ion Ionescu de la Brad' Iasi University of Life Sciences, 700490 Iasi, Romania
7 Department of Agricultural Sciences, University of Naples Federico II, 80055 Naples, Italy

1 INTRODUCTION

Beetroot (*Beta vulgaris* L. ssp. *vulgaris* var. *vulgaris*) is one of the most important root vegetables, an outstanding source of biologically active compounds and nutrients, such as carbohydrates, betalain pigments (Da Silva et al., 2019), polyphenols and vitamins (Wruss et al., 2015). Among sugar beet, fodder beet, mangold (leaf beet) and beetroot, the latter plays a special role in human nutrition, health maintenance and pharmacology (Zhang et al., 2016).

Betalains are known to be the main antioxidants in beetroot (Czapski et al., 2009), and have important effects such as cardio-protective and analgesic effects (Lundberg & Weitzberg, 2005; Sadowska-Bartosz & Bartosz, 2021), as well as antimicrobial, anticancer, antilipidemic activity, hepato- and neuroprotective, antidiabetic, anti-inflammatory and immuno-modulatory, reduce systolic and diastolic blood pressure, and normalize blood glucose level (Fu et al., 2020). Furthermore, betalain pigments of beetroot are highly valued as food coloring pigments (Chikara et al., 2019), thus contributing to quality improvement. Different factors affect beetroot yield and quality, such as genetic peculiarities, environmental conditions, light intensity, soil characteristics, fertilization technique including microbial supply, water availability, etc. (Agic et al., 2018), phytohormones and growth stimulators, humic and amino acids (El-Gamal et al., 2016), soil bacteria (Rašovský et al., 2022).

In recent years, much attention has been paid to growth stimulation and antioxidant properties of Se compounds (Hegedűsová et al., 2021; Khan et al., 2023). At low concentrations, Se may promote photosynthesis, enhance protein synthesis, protect plants against biotic and abiotic stresses, elicit monosaccharide increase, and activate the formation of phytohormones (Liu et al., 2023).

Biofortification of beetroot with Se has not been a common technique so far. Only Sentkowska & Pyrzynska (2023) described the accumulation of SeMet in beetroot juice, though the Se biofortification conditions have not been indicated. Besides, in previous experiments, low efficiency of foliar Se biofortification of root vegetables, such as carrots, was recorded (De Oliveira et al., 2018). According to the Periodic Table of Elements, Se mimics the chemical properties of S, forming appropriate salts (selenates Se^{+6}, and selenites Se^{+4}) and Se-amino acids (Se^{-2}): selenomethionine (SeMet) and selenocysteine (SeCys). Among them, selenates are the most mobile, selenites the most toxic and Se-amino acids are characterized by the highest bioavailability to plants (Dinh et al., 2019). Up to date, selenates have been used most frequently for plant biofortification (Malagoli et al., 2015), whereas Se-amino acid application was restricted because of their high cost. In 2022, an effective non expensive synthesis of selenocystine ($SeCys_2$) was developed (Poluboyarinov et al., 2022), providing the opportunity to investigate this compound's efficiency for plant biofortification more intensively.

The present work aimed to evaluate the efficiency of sodium selenate and selenocystine ($SeCys_2$) foliar supply on yield and nutritional quality of beetroot.

2 MATERIAL AND METHODS

2.1 EXPERIMENTAL DESIGN

The research was conducted on beetroot (*Beta vulgaris* L. ssp. *vulgaris* var. *vulgaris*) in 2022-2023 at the experimental fields of Federal Scientific Vegetable Center, Russia (55°39.510 N, 37°12.230 E). The mean values of monthly temperature and humidity during the crop cycles are presented in Table 1.

Plants were grown in a loam sod-podzolic soil with the following characteristics: pH 6.2; 2.12 % organic matter; 1.32 mg-eq 100 g^{-1} hydrolytic acidity; 18.5 mg kg^{-1} mineral nitrogen; 21.3 mg kg^{-1} ammonium nitrogen; sum of the absorbed bases as much as 93.6 %; 402 mg kg^{-1} mobile P; 198 mg kg^{-1} exchangeable K; 1 mg kg^{-1} S; 10.95 mg kg^{-1} Ca; 2.05 mg kg^{-1} Zn; 0.86 mg kg^{-1} B; and 220 µg kg^{-1} Se. The soil quality was assessed using the certified methods described in the agrochemical workshop (Carter & Gregorich, 2008), and its mineral composition was determined by an AAS Shimadzu GFA-7000 spectrophotometer (Shimadzu, Kyoto, Japan).

Seeds were sown on 8-10 May with a density of 2.9 plants per m^2 (50 × 70 cm). The experimental protocol was based on the factorial combination between two red beetroot cultivars selected at the Federal Scientific Vegetable Center (Nezhnost and Marusia) and two Se treatments (50 mg l^{-1} of sodium selenate solution, 26.4 mM;

Table 1: Mean values of monthly temperature and precipitation in 2022 and 2023

	Temperature (°C)		Precipitation (mm)	
Month	2022	2023	2022	2023
May	10.7	12.7	61	35
June	18.9	16.8	42	71
July	20.7	18.5	91	151
August	21.9	19.7	4	63
September	10.1	15.0	75	6
October	7.2	5.5	58	114

87 mg l^{-1} of $SeCys_2$ solution, 26.4 mM) plus an untreated control (water foliar spray). The plants were sprayed with the mentioned solutions twice: at the stage of beetroot formation (10-13 July) and 14 days later (1-4 August). A split plot design was used for the treatment distribution in the field, with three replicates, and each experimental unit covered a 9.8 m^2 surface area. Before sowing, the soil was accurately ploughed at 40 cm depth, and during the growing season, hoeing and manual weeding were carried out according to the needs determined through constant monitoring. The fertilization was performed twice during the crop cycles (30 June and 30 July) using 30 kg ha^{-1} of $N_{15}P_{15}K_{15}$. The irrigation was activated when the soil humidity dropped to 80 % of the available water capacity at 20 cm depth. Plants were harvested on 4-8 October.

2.2 SAMPLE PREPARATION

After harvesting and removing soil particles, the roots were separated from the aerial parts of 10 plants, washed with distilled water, dried with filter paper, and 0.5 mm peel was removed using a special knife. Both root pulp and peel were homogenized and used to determine betalain pigments, nitrates, and total dissolved solids (TDS). The remainder of the beetroot fractions was dried at 70 ˚C to constant mass and homogenized, and the resulting powders were used to determine the total antioxidant activity (AOA), total polyphenols (TP), and carbohydrate content.

2.3 DRY MATTER

The dry matter content was determined gravimetrically by drying beetroot samples at 70 °C for 72 hours. The results were expressed as a mass/mass percentage of dry matter (% m/m).

2.4 NITRATES

Nitrates in beetroot pulp samples were assessed using ion-selective electrode with an ionomer Expert-001 (Econix Inc., Moscow, Russia).

2.5 TOTAL DISSOLVED SOLIDS (TDS)

Total dissolved solids were analyzed on water extracts of beetroot pulp using a portable conductometer HM Digital TDS-3 (South Korea, Seoul). The results were expressed in mg kg^{-1} d. m.

2.6 SELENIUM

The selenium content was measured using the micro-fluorimetric method based on the acidic digestion of dried homogenized samples with a mixture of nitric and perchloric acids, subsequent conversion of selenate (Se^{+6}) to selenite (Se^{+4}) using a solution of 6 N HCl and fluorescence value determination of piazoselenol, formed as a result of a condensation between Se^{+4} and 2,3-diaminonaphtalene (Alfthan, 1984). The analysis was performed in hexane at λ emission 519 nm and λ excitation – 376 nm. As an external standard, Se-fortified mitsuba stem powder with a Se content of 1865 µg kg^{-1} (Federal Scientific Vegetable Center) was used. The results were expressed in µg kg^{-1} d m, as mean of three replications.

2.7 TOTAL POLYPHENOLS (TP)

The total polyphenols (TP) were determined in 70 % ethanol extracts of dried peel/pulp samples using the Folin–Ciocâlteu colorimetric method with some modifications (Golubkina et al., 2020). The extraction of samples was performed at 80 °C (1 h.) using a 70 % ethanol/water solution while the condensation with Folin–Ciocâlteu reagent was managed in the presence of saturated Na_2CO_3 solution at room temperature. The polyphenol concentration was calculated based on the absorption value of the resulting mixture at 730 nm by a spectrophotometer (Unico 2804 UV, Suite E Dayton, NJ, USA) using the external standard solution of 0.02 % gallic acid. The results were expressed as mg of gallic acid equivalent per g of dry mass (mg GAE g^{-1} d. m).

2.8 ANTIOXIDANT ACTIVITY (AOA)

The antioxidant activity of beet roots and leaves was assessed on 70 % ethanolic extracts of dry samples using a redox titration method (Golubkina et al., 2020). The values were expressed in mg gallic acid equivalents (mg GAE g^{-1} d. m.).

2.9 BETALAIN PIGMENTS

The betalain pigment analysis was carried out spectrophotometrically on water extracts of homogenized beetroot pulp and peel using the absorption values at 535 nm (betacyanins, extinction 60,000) and 485 nm (betaxantins, extinction 48,000) according to Bucur et al. (2016). The results were expressed in mg g^{-1} f. m.

2.10 SUGARS

The monosaccharides were determined using the ferricyanide colorimetric method, based on the reaction of monosaccharides with potassium ferricyanide (Swamy, 2008). Total sugars were analogically determined after acidic hydrolysis of water extracts with 20 % hydrochloric acid. Fructose was used as an external standard. The results were expressed in % per dry mass.

2.11 STATISTICAL ANALYSIS

The data were statistically processed using the analysis of variance (ANOVA), and the mean separations were performed through the Duncan's test at $p < 0.05$ probability level, using the SPSS software version 29 (IBM, Armonk, NY, USA).

3 RESULTS AND DISCUSSION

3.1 YIELD AND BIOMETRICAL PARAMETERS

Our previous investigation regarding Savoy cabbage biofortification with Se demonstrated a significantly higher growth stimulation effect of selenocystine than sodium selenate (Antoshkina et al., 2023). The present results were in accordance with the mentioned observation indicating high prospects of selenocystine supply in beetroot production (Table 2, Figure 1). Furthermore, the possibility of increasing beetroot yield by 1.44-1.66 times using selenocystine and by 1.20-1.26 times *via* foliar application of sodium selenate was recorded under low Se concentration not exceeding 26.4 mM. In this respect, similar beneficial effect of inorganic forms of Se was recorded on the two cultivars studied and significant varietal differences in the case of selenocystine application (Figure 1). The data presented in Table 2 also indicate the increase of root marketability due to Se supply with higher efficiency of organic Se compared to sodium selenate. No significant differences in the dry matter content between control and Se-treated plants were recorded.

Table 2: Yield, biometrical and growth parameters of beet roots

Parameter	Treatment	'Nezhnost'	'Marusia'
Mass (g)	Control	157 ± 15 c	165 ± 16 b
	Se^{+6}	197 ± 19 b	198 ± 19 a
	$SeCys_2$	290 ± 28 a	237 ± 25 a
Yield (t ha $^{-1}$)	Control	46.9 ± 4.7 c	49.5 ± 4.8 c
	Se^{+6}	59.1 ± 6.0 b	59.4 ± 5.8 b
	$SeCys_2$	78.0 ± 7.7 a	71.2 ± 7.0 a
Marketability level (%)	Control	91.0 ± 0.9 e	94.0 ± 0.9 cd
	Se^{+6}	93.5 ± 0.9 d	96.2 ± 0.9 ab
	$SeCys_2$	95.6 ± 0.9 bc	98.2 ± 0.9 a
Length (cm)	Control	12.2 ± 1.0 a	5.1 ± 0.5 a
	Se^{+6}	12.6 ± 1.0 a	5.5 ± 0.5 a
	$SeCys_2$	12.2 ± 1.0 a	5.5 ± 0.5 a
Diameter (cm)	Control	5.0 ± 0.5 b	5.1 ± 0.5 b
	Se^{+6}	5.2 ± 0.5 a	5.4 ± 0.5 b
	$SeCys_2$	5.9 ± 0.5 a	6.0 ± 0.6 a
Dry mass (%)	Control	18.8 ± 1.7 a	18.8 ± 1.8 a
	Se^{+6}	18.4 ± 1.6 a	19.0 ± 1.8 a
	$SeCys_2$	17.5 ± 1.6 a	18.5 ± 1.8 a

For each parameter, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$.

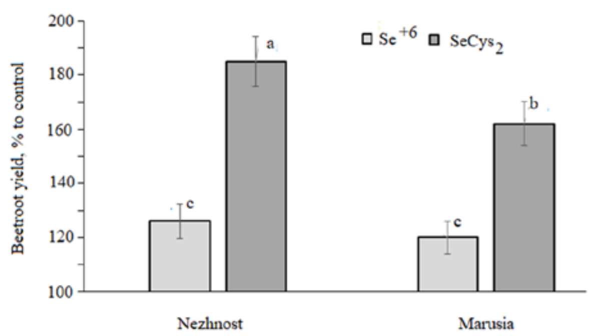


Figure 1: Changes in beetroot yield due to Se application. Values with the same letters do not differ statistically according to Duncan test at $p < 0.05$

3.2 CARBOHYDRATES, NITRATES AND TOTAL DISSOLVED SOLIDS (TDS)

The accumulation of carbohydrates is one of the most important characteristics of beetroot. In the present study, Se treatment did not change the total sugar content in roots but significantly increased the concentration of minor monosaccharides (Table 3).

The results were in accordance with the corresponding phenomenon of monosaccharide increase for environmental stress alleviation, improving photosynthesis, osmotic homeostasis, protein synthesis and membrane stabilization (Sami et al., 2016). De-

Table 3: Content of carbohydrate, nitrate and total dissolved solids in beetroot roots

Parameter	Treatment	cv. Nezhnost	cv. Marusia
Monosaccharides (% d. m.)	Control	3.76 ± 0.33 c	2.58 ± 0.22 b
	Se^{+6}	8.15 ± 0.80 b	3.85 ± 0.34 a
	$SeCys_2$	12.79 ± 1.12 a	4.22 ± 0.40 a
Total sugar (% d. m.)	Control	72.50 ± 7.01 a	73.10 ± 7.00 a
	Se^{+6}	68.00 ± 6.62 a	67.80 ± 6.55 a
	$SeCys_2$	66.80 ± 6.45 a	66.20 ± 6.51 a
Nitrates (mg kg^{-1} d. m.)	Control	1055 ± 110 a	1282 ± 120 a
	Se^{+6}	1204 ± 110 a	1442 ± 140 a
	$SeCys_2$	1159 ± 110 a	1429 ± 140 a
TDS (%)	Control	3.97 ± 0.40 a	3.80 ± 0.35 a
	Se^{+6}	4.28 ± 0.40 a	4.14 ± 0.40 a
	$SeCys_2$	3.76 ± 0.38 a	4.37 ± 0.42 a

For each parameter, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$.

spite the significant varietal differences in Se effect on monosaccharide accumulation, $SeCys_2$ showed higher efficiency than selenate, increasing the monosaccharide levels by 1.8-3.4 times compared to 1.5-2.2 times recorded under sodium selenate application. Nevertheless, considering the low monosaccharide accumulation levels in beetroot, the mentioned phenomenon did not have a significant effect on disaccharides whose concentration was over 10 times higher than the levels of monosaccharides.

Beetroot belongs to a group of vegetables capable of accumulating high concentrations of nitrates, thus being highly valuable in supporting human health, preventing hypertension and protecting against cardiovascular diseases (Brzezinska-Rojek et al., 2023; Dos S.Baião et al., 2020) via regulation of gene expressions of proteins and enzymes involved in the nitric oxide synthesis. Selenium is known to affect nitrogen metabolism, usually decreasing the nitrate content in plants (Golubkina et al., 2018; Pilon-Smits & Quinn, 2010). In the present investigation, we did not record significant differences between beetroot plants supplied with Se and control plants regarding nitrate accumulation.

The lack of significant differences in total dissolved solids (TDS) between control and Se treated plants corresponds to the same situation referring to total sugars in roots.

3.3 BETALAIN PIGMENTS

Among beetroot natural antioxidants, betalain pigments are considered the most valuable (Fu et al., 2020; Sadowzka-Nartosz & Nartosz, 2021). The results of the present investigation revealed that sodium selenate supply increased the total betalain content by 2.16 times ('Marusia') and 1.55 times ('Nezhnost'), while selenocystine produced a lower effect, with 1.5 and 1.28 times increase, respectively (Table 4, Figure 2). Contrary, though the initial levels of betalain pigments were much higher in peel than in pulp, the pigment content changes due to Se application were lower in 'Nezhnost' (1.18-1.19 for both forms of Se) and higher in 'Marusia' (1.39 ($SeCys_2$)-1.64 (Se^{+6}) times). Betalain peel/pulp ratio in control and $SeCys_2$ treated plants of both cultivars was equal to 2.54, whereas a slightly lower value was recorded for selenate supplied plants (2.27).

Furthermore, the varietal differences between the two cultivars tested revealed that the magnitude of betalain pigment increase upon Se supply may be due to either differences in the intensity of pulp or peel pigment accumulation (Figure 2).

According to literature data, red pigments betacyanins account for approximately 75–95 % of beetroot pigments, the remaining 5–25 % being yellow betaxanthins (Delgado-Vargas et al., 2000; Ninfali & Angelino, 2013). Betaxanthin content in roots of investigated cultivars reached 67-68 % and showed a slight tendency to the increase up to 70-71 % under

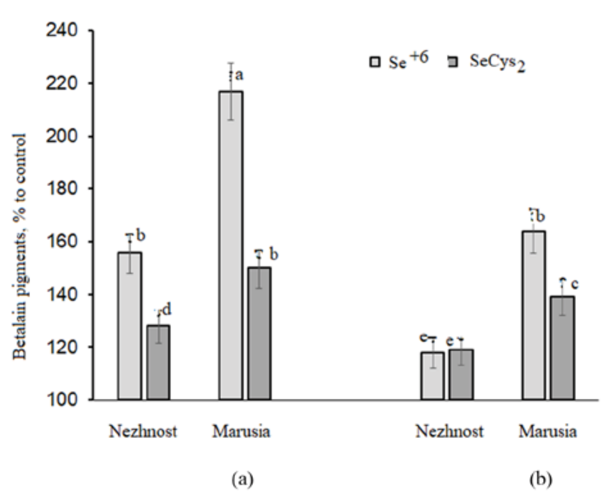


Figure 2: Effect of selenium biofortification on betalain pigment content in pulp (a) and peel (b) of beetroot. Values with the same letters do not differ statistically according to Duncan test at $p < 0.05$

Se supply (Table 4). The betacyanin/betaxantin ratios were close to 1.9:1 in peel and 2.2:1 in pulp and were not significantly affected by Se supply.

The predominant accumulation of betalain pigments in beet root peel is well documented (Kujala et al., 2002; Slatnar et al., 2015), indicating great prospects of peel utilization as a valuable source of natural pigments.

The significance of high betalain pigment accumulation in peel and pulp of beetroot relates to the nutritional value increase due to Se supply and the possibility of betalain utilization as food colorant (Calva-Estrada et al., 2022). Indeed, betalains are widely used as food colorants due to their prominent and consistent colours at pH 3–7. The development of packaging films incorporated with betalains is used as the colorimetric indicators and smart packaging films capable of improving the functional properties of packaging films, including higher water resistance, tensile strength, elongation at break, and antioxidant and antimicrobial activities (Abedi-Firoozjah et al., 2023).

3.4 TOTAL ANTIOXIDANT ACTIVITY (AOA) AND POLYPHENOLS (TP)

According to literature data, beet roots contain a significant amount of catechins and polyphenolic acids, including ferulic, protocatechuic, vanillic, caffeic and others, providing increased antioxidant activity of root ethanolic extracts (Kavalcova et al., 2015; Platosz et al., 2020). The main beet root flavonoids are rutin, kaempherol, rhamnetin, rhamnecitrin and astragalin (Sentkowska & Pyrzynska, 2020).

The analysis of the total antioxidant activity (AOA) using 70 % ethanolic extracts of beet roots revealed significant increase in the parameter due to Se supplementation (Table 4, Figure 3).

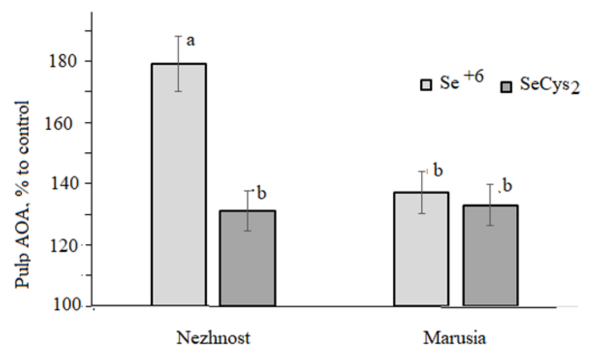
Contrary, TP levels did not differ significantly between control and Se treated plants, reaching 10.9-12.8 mg GAE g^{-1} d. m. in pulp and 23.7- 26.2 mg GAE g^{-1} d. m. in peel. The beneficial effect of Se on beetroot antioxidant activity was greater in 'Nezhnost' than 'Marusia' (Figure 3), contrary to water soluble betalain pigments whose level was higher in cultivar Marusia roots, compared to 'Nezhnost' (Figure 3 a,b).

Furthermore, 'Marusia' did not demonstrate differences in the AOA between organic and inorganic Se supply, while 'Nezhnost' showed more intensive AOA increase under selenate application. The latter phenomenon may be connected with varietal differences in polyphenol content of roots. However, there is no data about the mechanism of Se effect on polyphenol accumulation

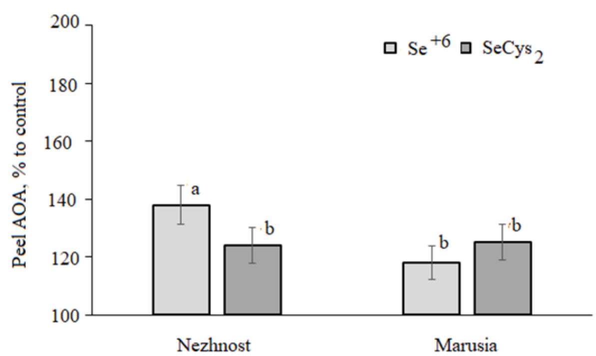
Table 4: Antioxidant levels in beetroot as affected by organic and inorganic forms of Se

		'Nezhnost'		'Marusia'	
Parameter	Treatment	Pulp	Peel	Pulp	Peel
Betacyanin	Control	1.34 ± 0.15 b	3.40 ± 0.31 a	1.20 ± 0.11 c	3.07 ± 0.29 b
(mg g^{-1})	Se^{+6}	2.13 ± 0.20 a	3.98 ± 0.36 a	2.73 ± 0.24 a	5.10 ± 0.49 a
	$SeCys_2$	1.81 ± 0.17 a	4.16 ± 0.40 a	1.89 ± 0.16 b	4.34 ± 0.41 a
Betaxantin	Control	0.64 ± 0.06 b	1.79 ± 0.16 a	0.60 ± 0.05 c	1.67 ± 0.15 b
(mg g^{-1})	Se^{+6}	0.94 ± 0.09 a	2.14 ± 0.20 a	1.18 ± 0.10 a	2.69 ± 0.24 a
	$SeCys_2$	0.72 ± 0.07 b	2.02 ± 0.19 a	0.81 ± 0.08 b	2.27 ± 0.20 a
Total betalain pigments	Control	1.97 ± 0.16 b	5.19 ± 0.50 b	1.80 ± 0.17 c	4.74 ± 0.44 b
(mg g^{-1})	Se^{+6}	3.07 ± 0.29 a	6.12 ± 0.60 a	3.91 ± 0.36 a	7.79 ± 0.75 a
	$SeCys_2$	2.53 ± 0.23 a	6.18 ± 0.60 a	2.70 ± 0.25 b	6.61 ± 0.62 a
AOA	Control	20.6 ± 2.0 c	40.5 ± 3.8 b	19.4 ± 1.5 b	41.9 ± 4.0 b
(mg GAE g^{-1} d. m.)	Se^{+6}	36.8 ± 3.3 a	55.9 ± 5.2 a	26.8 ± 2.4 a	49.4 ± 4.0 a
	$SeCys_2$	27.0 ± 2.5 b	52.3 ± 5.0 a	25.8 ± 2.3 a	52.4 ± 5.0 a
TP	Control	11.1 ± 1.0 a	25.8 ± 2.2 a	11.4 ± 1.0 a	25.2 ± 2.3 a
(mg GAE g^{-1} d. m.)	Se^{+6}	11.3 ± 1.0 a	22.7 ± 2.0 a	13.1 ± 1.0 a	24.8 ± 2.3 a
	$SeCys_2$	10.4 ± 0.9 a	22.7 ± 2.0 a	13.8 ± 1.0 a	28.6 ± 2.4 a

For each parameter, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$.



(a)



(b)

Figure 3: Effect of organic and inorganic forms of Se on antioxidant activity of beet root pulp (a) and peel (b). Values with the same letters do not differ significantly according to Duncan test at $p < 0.05$

and further studies are needed to reveal the mechanism of these changes.

3.5 SE ACCUMULATION

Our previous investigations on Se biofortification of shallot (Golubkina et al., 2019) and Savoy cabbage (Antoshkina et al., 2023) demonstrated higher biofortification levels under $SeCys_2$ supply, compared to the values recorded in selenate treated plants. The present results indicate that the mentioned effect greatly depends on plant part and varietal differences in beetroot. Indeed, beet leaves were the most sensitive to Se supply, able to accumulate up to 1200 µg Se kg^{-1} d. m. in case of $Secys_2$ treated plants, with 1.35 times lower values in case of selenate supplementation (Table 5).

A less pronounced effect was recorded in beetroot where Se accumulated predominantly in root peel. The lowest biofortification levels were recorded in root pulp reaching only 3-3.5 times (Figure 4).

Table 5: Selenium accumulation in beet roots under organic and inorganic Se supply (µg Se kg^{-1} d. m.)

Plant part	Treatment	'Nezhnost'	'Marusia'
Pulp	Control	33 ± 3 d	44 ± 4 c
	Se^{+6}	120 ± 10 ab	133 ± 13 a
	$SeCys_2$	100 ± 9 b	128 ± 11 a
Peel	Control	38 ± 3 d	50 ± 5 c
	Se^{+6}	345 ± 30 b	361 ± 33 b
	$SeCys_2$	458 ± 41 a	355 ± 31 b
Leaves	Control	29 ± 2 d	39 ± 3 c
	Se^{+6}	900 ± 79 b	910 ± 76 b
	$SeCys_2$	1220 ± 98 a	1235 ± 100 a

For each parameter, values with the same letters do not differ statistically according to Duncan test at $p < 0.05$.

3.6 CORRELATION ANALYSIS

The correlation analysis between the parameters tested indicated a significant beneficial effect of pulp monosaccharide content and peel Se levels on beet root yield and a positive effect of Se on betalain accumulation in pulp and peel (Table 6).

Furthermore, antioxidant activity of fat-soluble antioxidants and root yield were significantly affected by Se accumulation both in pulp and peel. Indeed, Se supply demonstrated a significant relationship with root yield,

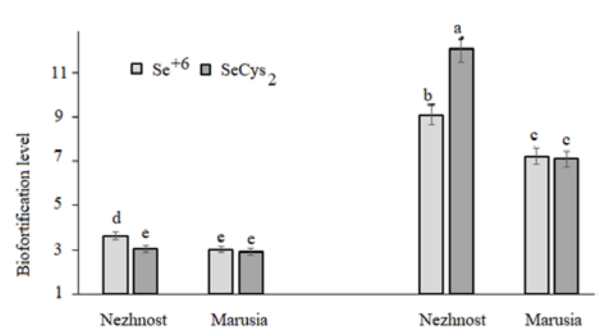


Figure 4: Selenium biofortification levels of beet root pulp (a) and peel (b). Values with the same letters do not differ statistically according to Duncan test at $p < 0.05$

Table 6: Correlations between beetroot quality parameters

	Betalains		Ms	AOA		Se	
Yield	pulp	peel	pulp	pulp	peel	pulp	peel
Yield	0.240	0.411	0.769 a	0.332	0.670 d	0.561 f	0.858 a
Pulp betalains		0.947 a	0.031	0.617 e	0.628 c	0.860 a	0.674 d
Peel betalains			0.044	0.464	0.605 e	0.880 a	0.742 b
Monosaccharides				0.505	0.578 f	0.241	0.644 e
Pulp AOA					0.888 a	0.717 c	0.685 d
Peel AOA						0.884 a	0.906 a
Se pulp							0.880 a

Ms: monosaccharides; AOA: total antioxidant activity; p: a) < 0.001; b) < 0.002; c) < 0.005; d) < 0.01; e) < 0.02; f) < 0.05.

betalain pigment accumulation and total antioxidant activity. It is interesting that antioxidant characteristics (including Se) of beet root peel directly correlate both with root yield values and accumulation of water-soluble betalain pigments, and indicate the importance of the 'edge' effect for plant adaptation (Golubkina et al., 2023).

4 CONCLUSION

From research carried out on selenium biofortification of beetroot, it arose that the organic and inorganic Se supply to beetroot plants significantly enhanced yield and quality of roots, compared to control plants, though showing low efficiency in producing beetroots with Se levels suitable to compensate Se deficiency in humans. Sodium selenate had a significantly higher beneficial effect on betalain pigment levels and fat-soluble antioxidant content, in comparison with $SeCys_2$, which led to higher root yield and monosaccharide levels. Overall, the outcome obtained from the present study allows to infer that Se biofortification of beetroot plants is a useful strategy to encourage yield and nutritional quality of roots.

5 FUNDING STATEMENT

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors that could be construed as a conflict of interest or that might have appeared to influence the work reported in this paper.

6 AUTHOR CONTRIBUTIONS

Conceptualization: N.G. and G.C.; formal analysis: N.G., A.S., O.C.M., and A.V.T.; investigation: N.G., V.Z., P.P. and Z.A.; methodology: N.G., A.S., O.C.M., and A.V.T.; validation, N.G., A.S., O.C.M., and G.C.; draft manuscript writing, N.G., O.C.M., and A.V.T.; manuscript revision and final editing, N.G., A.S., and G.C. All authors have read and agreed to the published version of the manuscript.

7 REFERENCES

Abedi-Firoozjah, R., Parandi, E., Heydari, M., Kolahdouz-Nasiri, A., Bahraminejad, M., Mohammadi, R., Rouhi, M., & Garavand, F. (2023). Betalains as promising natural colorants in smart/active food packaging. *Food Chemistry, 424*, 136408. https://doi.org/ 10.1016/j.foodchem.2023.136408.

Agic, R., Zdravkovska, M., Popsimonova, G., Dimovska, D., Bogevska, Z., & Davitkovska, M. (2018). Yield and quality of beetroot (*Beta vulgaris* ssp. esculenta L.) as a result of microbial fertilizers. *Contemporary Agriculture, 67*(1), 40-44.

Alfthan, G. V. (1984). A micromethod for the determination of selenium in tissues and biological fluids by single-test-tube fluorimetry. *Analitica Chimica Acta, 165,* 187–194.

Antoshkina, M., Golubkina, N., Poluboyarinov, P., Skrypnik, L., Sekara, A., Tallarita, A., & Caruso, G. (2023). Effect of sodium selenate and selenocystine on Savoy cabbage yield, morphological and biochemical characteristics under *Chlorella* supply. *Plants*, *12*(5), 1020. doi: 10.3390/plants12051020.

Brzezinska-Rojek, J., Sagatovych, S., Malinowska, P., Gadaj, K., Prokopowicz, M., & Grembecka, M. (2023). Antioxidant capacity, nitrite and nitrate content in beetroot-based dietary supplements. *Foods, 12,* 1017. https:// doi.org/10.3390/foods12051017.

Bucur, L., Aralunga, C., Schroder, V. (2016). The betalains content and antioxidant capacity of red beet (*Beta vulgaris* L. subsp.*vulgaris*). *Farmacia*, *64*(2), 198—201.

Calva-Estrada, S. J., Jiménez-Fernández, M., & Lugo-Cervantes, E. (2022). Betalains and their applications in food:

The current state of processing, stability and future opportunities in the industry, *Food Chemistry***,** *4*, 100089. https://doi.org/10.1016 /j.fochms.2022.100089.

Carter, M. R., & Gregorich, E. G. (ed.) (2008). *Soil sampling and methods of analysis*, 2d ed. Canadian Society of Soil Science. CRC Press Taylor & Francis Group.

Chikara, N., Kushwaha, K., Jaglan, S., Sharma, P., & Panghal, A. (2019). Nutrition, physicochemical, and functional quality of beetroot (*Beta vulgaris* L.) incorporated Asian noodles. *Cereal Chemistry, 96*, 154–161.

Czapski, J., Mikołajczyk, K., & Kaczmarek, M. (2009). Relationship between antioxidant capacity of red beet juice and contents of its betalain pigments. *Polish Journal of Food and Nutrition Sciences, 59*, 119–122.

da Silva, D. V. T., dos Santos Baião, D., de Oliveira, F., Silva, G., Alves, D., Perrone, D., Aguila, E. M. & Paschoalin V. M. M. (2019). Betanin, a natural food additive: stability. bioavailability, antioxidant and preservative ability assessments. *Molecules, 24*, 2403058.

de Oliveira, V. C., Faquin, V., Guimarães, K. C., Andrade, F. R., Pereira, J., & Guilherme, L. R. G. (2018). Agronomic biofortification of carrot with selenium. *Ciência e Agrotecnologia*, *42*(2), 138-147. http://dx.doi.org/10.1590/1413-70542018422031217.

Delgado-Vargas, F., Jiménez, A. R., & Paredes-López, O. (2000). Natural pigments: carotenoids, anthocyanins, and betalains characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition*, *40*, 173–289. doi : 10.1080/10408690091189257.

Dinh, Q. T., Wang, M., Tran, T. A. T., Zhou, F., Wang, D., Zhai, H., Peng, Q., Xue, M., Du, Z., Bañuelos, G. S., Lin, Z.-Q., & Liang, D. (2019). Bioavailability of selenium in soil-plant system and a regulatory approach, *Critical Reviews in Environmental Science and Technology, 49*(6), 443-517. doi: 10.1080/10643389. 2018.1550987.

dos S. Baião. D., da Silva D. V. T., & Paschoalin, V. M. F. (2020). Beetroot, a remarkable vegetable: Its nitrate and phytochemical contents can be adjusted in novel formulations to benefit health and support cardiovascular disease therapies. *Antioxidants, 9*, 960. doi:10.3390/antiox9100960.

El-Gamal, I. S., Abd El-Aal, M. M. M., El-Desouky, S. A., Khedr, Z. M., & Abo Shady, K. A. (2016). Effect of some growth substances on growth, chemical compositions and root yield productivity of sugar beet (*Beta vulgaris* L.) plant. *Middle East Journal of Agriculture Research, 5*(2), 171-185.

Fu, Y., Shi, J., Xie, S. Y., Zhang, T. Y., Soladoye, O. P., & Aluko, R. E. (2020). Red beetroot betalains: perspectives on extraction, processing, and potential health benefits. *Journal of Agricultural and Food Chemistry, 68*(42), 11595-11611. doi: 10.1021/acs.jafc.0c04241.

Golubkina, N., Kekina, H., & Caruso G. (2018). Yield, quality and antioxidant properties of Indian mustard (*Brassica juncea* L.) in response to foliar biofortification with selenium and iodine. *Plants, 7,* 80.

Golubkina, N., Skrypnik, L., Logvinenko, L., Zayachkovsky, V., Smirnova, A., Krivenkov, L., Romanov, V., Kharchenko, V., Poluboyarinov, P., Sekara, A., Tallarita, A., & Caruso G. (2023). The 'edge effect' phenomenon in plants: morphological, biochemical and mineral characteristics of border tissues. *Diversity, 15*(1), 123. https://doi.org/ 10.3390/d15010123.

Golubkina, N., Zamana, S., Seredin T., Poluboyarinov, P., Sokolov, S., Baranova. H., Krivenkov, L., Pietrantonio, L., & Caruso, G. (2019). Effect of selenium biofortification and beneficial microorganism inoculation on yield, quality and antioxidant properties of shallot bulbs. *Plants*, *8*, 102. doi: 10.3390/plants8040102.

Golubkina, N. A., Kekina, H. G., Molchanova, A. V., Antoshkina, M. S., Nadezhkin, S. M., & Soldatenko, A. V. (2020). *Plants Antioxidants and Methods of Their Determination,* Infra M: Moscow, (in Russian).

Hegedűsová, A., Hegedűs, O., Jakabová, S., Andrejiová, A., Šlosár, M., Mezeyová, I., & Golian, M. (2021). *Selenium Supplementation in Horticultural Crops*, first ed., Springer Nature, Cham.

Kavalcová, P., Bystrická, J., Tomáš, J., Kovarovič, J., & Lenková, M. (2015). The content of total polyphenols and antioxidant activity in red beetroot. *Potravinárstvo, 9*(1), 77–83.

Khan, Z., Thounaojam, T. C., Chowdhury, D., & Upadhyaya, H. (2023). The role of selenium and nano selenium on physiological responses in plant: a review. *Plant Growth Regulation, 100*, 409–433. https://doi.org/10.1007/s10725-023-00988-0.

Kujala, T. S., Vienola, M. S., Klika, K. D., Loponen, J. M., & Pihlaja, K. (2002). Betalain and phenolic compositions of our beetroot (*Beta vulgaris*) cultivars. *European Food Research and Technology, 214*, 505–510. doi: 10.1007/s00217-001-0478-6.

Liu, H., Xiao, C., Qiu, T., Deng, J., Cheng, H., Cong, X., Cheng, S., Rao, S., & Zhang, Y. (2023). Selenium regulates antioxidant, photosynthesis, and cell permeability in plants under various abiotic stresses: A review. *Plants, 12*, 44. https://doi.org/ 10.3390/plants12010044.

Lundberg, J. O., & Weitzberg, E. (2005). NO generation from nitrite and its role in vascular control *Arteriosclerosis, Thrombosis and Vascular Biology, 25,* 915-922.

Malagoli, M., Schiavon, M., dall'Acqua, S., & Pilon-Smits, E. A. H. (2015). Effects of selenium biofortification on crop nutritional quality. *Frontiers in Plant Science, 6*, 280. doi: 10.3389/fpls.2015.00280.

Ninfali, P., & Angelino, D. (2013). Nutritional and functional potential of *Beta vulgaris cicla* and *rubra*. *Fitoterapia, 89*, 188–199. doi: 10.1016/j.fitote.2013.06.004.

Pilon-Smits, E. A. H., & Quinn, C. F. (2010). Selenium metabolism in plants. In *Cell Biology of Metals and Nutrients*; Hell, R.; Mendel, R.R., Eds.; Springer: Berlin/Heidelberg, Germany.

Płatosz, N., Sawicki, T., & Wiczkowski, W. (2020). Profile of phenolic acids and flavonoids of red beet and its fermentation products. Does long-term consumption of fermented beetroot juice affect phenolics profile in human blood plasma and urine? *Polish Journal of Food Nutrition and Sciences, 70*, 55–65.

Poluboyarinov, P. A., Moiseeva, I. Y., Mikulyak, N. I., Golubkina, N. A., & Kaplun, A. P. (2022). New synthesis of cysteine and selenocystine enantiomers and their derivatives *News of Higher Educational Technologies. Series Chemistry and*

Chemical Technology, 65(2), 19-29. https://doi.org/ 10.6060/ivkkt.20226502.6466 (in Russian).

Rašovský, M., Pačuta, V., Ducsay, L., & Lenická, D. (2022). Quantity and quality changes in sugar beet (*Beta vulgaris* provar. *altissima* Doel) induced by different sources of biostimulants. *Plants, 11*(17), 2222. doi: 10.3390/plants11172222.

Sadowska-Bartosz, I., & Bartosz, G. (2021). Biological properties and applications of betalains. *Molecules, 26*(9), 2520. doi: 10.3390/molecules26092520.

Sami, F., Yusuf, M., Faizan, M., Faraz, A., & Hayat, S. (2016). Role of sugars under abiotic stress. *Plant Physiology and Biochemistry, 109,* 54-61. https://doi.org/10.1016/j.plaphy.2016.09.005.

Sentkowska, A., & Pyrzynska, K. (2020). Determination of selenium species in beetroot juices. *Heliyon, 6*(6), e04194. https://doi.org/10.1016/j.heliyon.2020.e04194.

Sentkowska, A., & Pyrzyńska, K. (2023). Old-fashioned, but still a superfood—red beets as a rich source of bioactive compounds. *Applied Science, 13,* 7445. https:// doi.org/10.3390/app13137445.

Slatnar, A., Štampar, F., Veberič, R., & Jakopič, J. (2015). HPLC-MS identification of betalains profile of different beetroot (*Beta vulgari* L. ssp. *vulgaris*) parts and cultivars. *Journal of Food Science***,** *80,* 1952–1958.

Swamy, P. M. (2008). *Laboratory Manual on Biotechnology*; Rastogi Publications: Meerut, India, 617.

Wruss, J., Waldenberger, G., Huemer, S., Uygun, P., Lanzerstorfer, P. Müller, U., Höglinger, O., & Weghuber, J. (2015). Compositional characteristics of commercial beetroot products and beetroot juice prepared from seven beetroot varieties grown in Upper Austria. *Journal of Food Composition and Analysis, 42*, 46-55.

Zhang, Y., Nan, J., & Yu, B. (2016). OMICS technologies and applications in sugar beet. *Frontiers in Plant Science, 7,* 1–11.

doi:10.14720/aas.2025.121.1.21409 Original research article / izvirni znanstveni članek

Bionomija in načini zatiranja japonskega hrošča (*Popillia japonica* Newman, 1841, Coleoptera: Scarabaeidae)

Luka BATISTIČ [1], Stanislav TRDAN [1, 2], Špela MODIC [3], Žiga LAZNIK [1]

Received: December 12, 2024; accepted February, 24, 2025
Delo je prispelo 12 december 2024, sprejeto 24. februar 2025

Bionomija in načini zatiranja japonskega hrošča (*Popillia japonica* Newman, 1841, Coleoptera: Scarabaeidae)

Izvleček: Japonski hrošč (*Popillia japonica* Newman, 1841), ki je bil leta 2024 prvič ugotovljen v Sloveniji, je pomemben invazivni škodljivec, ki lahko povzroča obsežno škodo v kmetijstvu. Zaradi sposobnosti hitrega širjenja in prehranjevanja z več kot 300 vrstami rastlin predstavlja resno grožnjo na območjih, kjer se pojavlja in širi, vključno z Evropo in ZDA. Odrasli osebki so škodljivi zaradi skeletiranja listov, medtem ko ogrci, ki so talni škodljivci, objedajo korenine trav in poljščine. V preglednem članku predstavljamo možnosti zatiranja japonskega hrošča. Kemične metode so učinkovite, a na insekticide škodljivec lahko razvije odpornost, škodljivi pa so tudi neciljnim organizmom. Biotično zatiranje škodljivca s parazitoidi, entomopatogenimi bakterijami, glivami in ogorčicami, kaže potencial, vendar njihova učinkovitost ni vedno zadovoljiva. Za učinkovito zatiranje japonskega hrošča so potrebne integrirane strategije, ki temeljijo na okoljskih prilagoditvah in nadaljnjih raziskavah.

Ključne besede: japonski hrošč, invazivni škodljivec, polifagni škodljivec, biotično varstvo rastlin, kemično varstvo rastlin, entomopatogene ogorčice, integrirano varstvo rastlin

Bionomics and control methods of the Japanese beetle (*Popillia japonica* Newman, 1841, Coleoptera: Scarabaeidae)

Abstract: The Japanese beetle (*Popillia japonica* Newman, 1841), which was first recorded in Slovenia in 2024, is an important invasive pest that can cause extensive damage in agriculture. Due to its ability to spread rapidly and feed on more than 300 plant species, it poses a serious threat in areas where it occurs and spreads, including Europe and the United States. Adults are harmful by skeletonizing the leaves, while white grubs, which are soil pests, eat the roots of grasses and field crops. This review paper presents the methods for controlling the Japanese beetle. Chemical methods are effective, but the pest can develop resistance to insecticides, and they are also harmful to non-target organisms. Biological control of this scarab beetle with parasitoids, entomopathogenic bacteria, fungi and nematodes has potential, but their effectiveness is not always satisfactory. Integrated strategies based on environmental adaptations and further research are needed for effective control of the Japanese beetle.

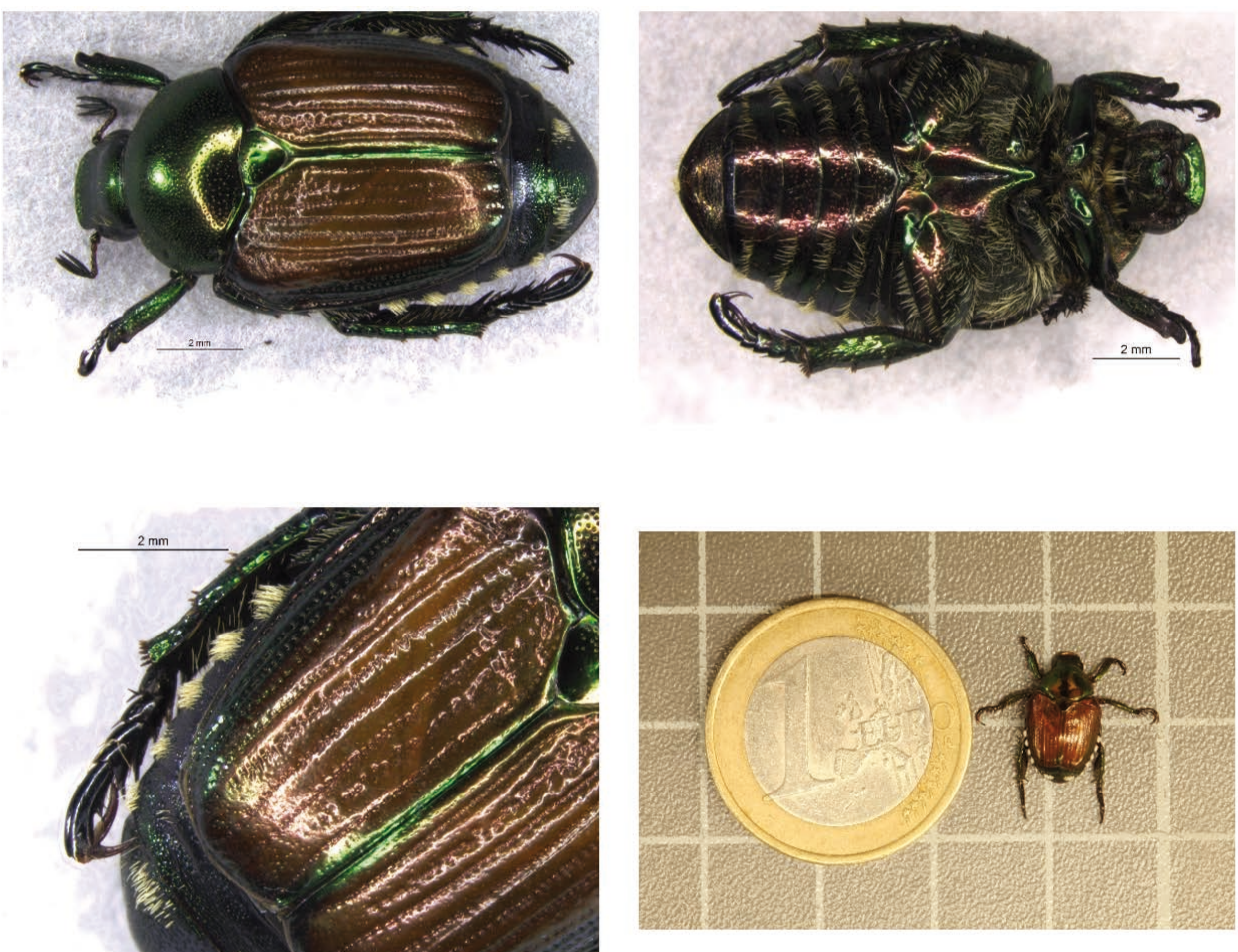
Key words: Japanese beetle, invasive pest, polyphagous pest, biological control, chemical control, entomopathogenic nematodes, integrated pest management

1 Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za agronomijo, Jamnikarjeva 101, SI-1000 Ljubljana
2 korespondenčni avtor: stanislav.trdan@bf.uni-lj.si
3 Kmetijski inštitut Slovenije, Hacquetova 17, SI-1000 Ljubljana

1 STATUS ŠKODLJIVCA IN NJEGOVA BIONOMIJA

Japonski hrošč (*Popillia japonica* Newman, 1841) izvira iz Japonske, kjer ne velja za škodljivca (Clausen et al., 1927). Ta žuželčja vrsta je pomemben invazivni škodljivec, ki predstavlja resno grožnjo kmetijstvu na območjih, kjer se pojavlja in širi. Uvrščen je med prednostne karantenske škodljivce v Evropski uniji in v številnih drugih državah (Marianelli et al., 2019; Graf et al., 2023). V ZDA je bil vnesen v začetku 20. stoletja (Clausen et al., 1927), iz New Jerseyja pa se je razširil proti zahodni obali, v Kanado ter proti jugu ZDA (Potter in Held, 2002; Frank, 2016). Od leta 2014 je zastopan tudi v celinski Evropi (Marianelli et al., 2019; Graf et al., 2023), kjer se je iz severne Italije (Piemont in Lombardija) razširil v južno Švico (Ticino). Obe državi sta določili območja pojavljanja in uvedli fitosanitarne ukrepe za omejevanje njegovega širjenja (Marianelli et al., 2019; Graf et al., 2023). O najdbah japonskega hrošča v Evropi so doslej poročali tudi iz Portugalske, Rusije in Slovenije, vendar le na manjšem številu lokacij, kjer so širjenja škodljivca uspeli omejiti. V Sloveniji je bil škodljivec prvič ugotovljen leta 2024, ko so bili odrasli osebki julija najdeni v feromonskih pasteh na dveh avtocestnih počivališčih - Barje v Ljubljani in Lukovica v smeri jug. Pasti na obeh lokacijah so bile postavljene v okviru programa preiskave, ki se v skladu z EU zahtevami vsako leto izvaja na celotnem območju Slovenije.

Prehranjevalne navade japonskega hrošča se med odraslimi osebki in ličinkami močno razlikujejo, vendar oba razvojna stadija povzročata estetsko škodo in izgube pridelka pri okrasnih rastlinah, travah in gojenih vrstah rastlin (Graf et al., 2023). Odrasli hrošči so izrazito polifagni in se prehranjujejo z listi, cvetovi in plodovi več kot 300 rastlinskih vrst, vključno z lesnatimi oz. okrasnimi



Slika 1: Odrasel osebek japonskega hrošča: hrbtna stran (zgoraj levo), trebušna stran (zgoraj desno), pet šopov belih dlak na levi strani zadka (spodaj levo), velikostna primerjava s kovancem za 1 evro (foto: Š. Modic). Hrošč je bil najden 10. julija 2024 v feromonski lijakasti pasti z zelenim pregradnim križem, pokrovom in lijakom ter prozornim vedrom (Pherobank, Nizozemska, št. art. 30251) na avtocestnem počivališču Lukovica.

rastlinami (breza, brest, javor, jerebika, vrtnica, cinija), sadnimi vrstami (jablana, marelica, češnja, sliva), jagodičjem (borovnica, malina), zelenjadnicami (šparglji), vinsko trto ter poljščinami (koruza, soja) (Fleming, 1972; Ladd, 1987, 1989). Poškodbe, ki jih povzročijo odrasli osebki, so hitro prepoznavne, saj se hranijo z mehkimi deli listov in za seboj puščajo le listne žile (t. i. skeletiranje listov). Odrasli hrošči lahko z objedanjem zmanjšujejo tudi kakovost plodov nekaterih rastlinskih vrst (Bragard et al., 2018).

Japonski hrošč je prepoznaven po svoji velikosti (8-11 mm dolžine in 5-7 mm širine) (Slika 1, spodaj desno) ter značilni barvi – kovinsko zeleni glavi in oprsju ter bakreno obarvanih pokrovkah, ki zadka ne pokrivajo v celoti (Slika 1, zgoraj levo). Na obeh bočnih straneh zadka ima pet šopov značilnih belih dlačic (Slika 1, spodaj levo) ter dva šopa belih dlačic na pigidiju. Zaradi teh lastnosti ga splošna javnost zlahka prepozna kot škodljivca. Samce lahko ločimo od samic po obliki golenskih trnov, pri čemer so trni samcev bolj koničasti kot pri samicah (Fleming, 1972). Odrasel osebek japonskega hrošča je podoben vrtnemu hrošču (*Phyllopertha horticola* [L., 1758]), vendar se od slednjega loči po zgoraj navedenih morfoloških znakih.

Ličinke (ogrci), ki prebivajo v tleh, se prehranjujejo s koreninami številnih rastlinskih vrst, vključno s pleveli, okrasnimi rastlinami in poljščinami, vendar najraje napadajo korenine trav (Graf et al., 2023). Prehranjevanje ličink s koreninami lahko povzroči škodo na tratah, igriščih za golf, travnikih in pašnikih. Ličinke, ki v dolžino ne presežejo 25 mm, so značilno bele, so značilno zavite v obliko črke C, imajo rumenorjavo glavo in so podobne ličinkam drugih evropskih predstavnikov družine pahljačnikov (Scarabaeidae) le da so nekoliko manjše. Leta 2002 je bila škoda, ki jo je japonski hrošč povzročil na tratah in okrasnih rastlinah v ZDA, ocenjena na kar 450 milijonov ameriških dolarjev (Potter in Held, 2002).

Japonski hrošč je v večini območij njegovega naravnega habitata in območij, kjer se je razširil naknadno, univoltilna vrsta, čeprav lahko njegov razvojni krog na severnejših območjih traja tudi dve leti (Clausen et al., 1927). Odrasli hrošči navadno prilezejo iz tal konec junija ali v začetku julija in živijo do 40 dni (Hadley in Hawley, 1934). Samci in samice se združujejo in zadržujejo na gostiteljskih rastlinah, ki jih prepoznajo kot ustrezne za prehrano na podlagi hlapnih snovi, ki jih rastline sproščajo ob poškodbah zaradi hranjenja, ter snovi, ki jih izločajo sami hrošči, vključno z agregacijskimi feromoni (Loughrin et al., 1995, 1996; Sara et al., 2013). Samice se lahko parijo večkrat v rastni dobi, pri čemer parjenje s samci lahko traja tudi do dve uri (Barrows in Gordh, 1978). Jajčeca odlagajo posamezno v zgornjo plast tal do 7,5 cm globoko, pri čemer vsaka samica v življenju odloži do 60 jajčec (Fleming, 1972; Dalthorp et al., 2000). Iz jajčec se izležejo ličinke po približno dveh tednih (Fleming, 1972). Ličinke se hranijo s koreninami in se levijo, dokler ne dosežejo tretje razvojne stopnje. Aktivnost ličink se ustavi, ko temperatura tal doseže približno 10 °C. Oktobra se začnejo pripravljati na prezimovanje in se zarijejo v tla na globino 20-25 cm. Spomladi se vrnejo na površje, ponovno začnejo s hranjenjem, se zabubijo ter nato izletijo kot odrasli hrošči.

2 ZATIRANJE JAPONSKEGA HROŠČA

Zatiranje japonskega hrošča je zahtevna naloga (Potter in Held, 2002; Shanovich et al., 2019) iz več razlogov: (i) škodo na gostiteljskih rastlinah povzročajo tako ličinke kot odrasli hrošči, (ii) ličinke in odrasli hrošči naseljujejo različne habitate, (iii) velike skupine mobilnih odraslih osebkov lahko hitro povzročijo obsežno škodo, in (iv) na območjih, ki jih je škodljivec na novo naselil, navadno ni njegovih učinkovitih naravnih sovražnikov. Zatiranje japonskega hrošča temelji na kombinaciji kemičnih, mehanskih in biotičnih strategij, katerih cilj je zmanjšati število ličink in odraslih hroščev pod raven, ki bi lahko povzročila gospodarsko pomembno škodo.

2.1 KEMIČNO ZATIRANJE

Tuja literatura navaja, da je bilo za zatiranje japonskega hrošča v nekaterih državah registriranih več insekticidov, med drugim tudi tisti, ki vsebujejo aktivne snovi, kot so acetamiprid, klorantraniliprol, ciantraniliprol, fenpropatrin, imidakloprid, novaluron in fosmet (Gagnon in sod., 2023); vendar nekatere od teh aktivnih snovi v EU niso več dovoljene. V Sloveniji so na voljo pripravki na podlagi aktivnih snovi, ki so po podatkih iz Italije učinkoviti proti odraslim hroščem: piretrin, deltametrin, klorantraniliprol, acetamiprid in lambda cihalotrin, vendar bi bilo zanje treba pridobiti dovoljenje oziroma razširitve dovoljenja za zatiranje japonskega hrošča.

Za zatiranje ličink ali ogrcev insekticide uporabljamo od sredine julija do sredine septembra, ko se ličinke hranijo s koreninami rastlin blizu površja tal, pri čemer je potrebno tudi naknadno namakanje. Ti širokospektralni insekticidi zmanjšajo populacije ličink japonskega hrošča, vendar imajo lahko škodljive učinke tudi na druge neciljne organizme. Odrasle osebke lahko ciljno zatiramo z insekticidi prek foliarnih nanosov (Gotta in sod., 2023).

Eden od načinov zatiranja japonskega hrošča je tudi uporaba ‚LLIN‘s‘ ali dolgo trajajočih z insekticidom tretiranih mrež. Največkrat so te tretirane s piretroidi, so tudi bolj selektivne (manj vpliva na ostale koristne ali druge vrste). Uporabijo se lahko ob napadenih območjih in nasadih. Največkrat so postavljene skupaj z rastlinskim privabilom ter feromonom, ki privabi samce japonskega hrošča na mrežo (Gotta in sod., 2023).

Na poljščinah, kot sta soja in koruza, sta že določena oba gospodarska pragova škodljivosti (Russin, 1989; Steckel in sod., 2013). Učinkovitost kemičnega zatiranja se lahko tekom uporabe zmanjša zaradi nenadne selitve in migracije odraslih hroščev na netretirano območje (Potter in Held, 2002; Calderwood in sod., 2015).

Ogrci japonskega hrošča so že razvili odpornost na več vrst aktivnih snovi, ki se uporabljajo za namene varstva rastlin (Niemczyk in Lawrence, 1973; Ahmad in Ng, 1981). Zato morajo pridelovalci izvajati kemično zatiranje v sklopu integriranega varstva rastlin, kar omogoča omejevanje hroščev in ličink na okolju prijaznejši način ter preprečuje razvoj odpornosti na preostale aktivne snovi, ki so še v uporabi (Shanovich in sod., 2019).

2.2 MEHANSKO ZATIRANJE

Kljub temu, da so ličinke in odrasli osebki vrste *P. japonica* polifagi, do določene mere izkazujejo preferenco do nekaterih rastlinskih vrst in kultivarjev (Fleming, 1972; Potter in Held, 2002). Vzorčenje na terenu je pokazalo pomembne razlike v številčnosti odraslih osebkov in stopnji defoliacije med različnimi sortami jablan v Minnesoti, ZDA (Shanovich in sod., 2021).

Mehansko zatiranje so doslej večinoma preizkušali v kontekstu zatiranja ogrcev v travni ruši, obravnavanja v poskusih pa so vključevala različne načine namakanja, gnojenja, oranja in puščanja višje travne ruše po vsaki košnji (Potter in Held, 2002). Gotta in sod. (2023) poročajo o preučevanju učinkovitosti različnih zastirk pri zatiranju plevela. V raziskavi so ugotavljali katera od zastirk lahko samicam vrste *P. japonica* prepreči ovipozicijo v loncih, v katerih je bila posajena vinska trta. Preizkušali so zastirke iz kokosovih vlaken, jute in lesnih sekancev, pri čemer se je kokosova zastirka izkazala za najbolj učinkovito, saj je znatno zmanjšala odlaganje jajčec in razvoj ličink japonskega hrošča (Gotta in sod., 2023).

Kot zgled mehanskega zatiranja japonskega hrošča lahko izpostavimo tudi močno napadeno drevesnico v Lombardiji, kjer so preizkušali tri tipe mrež: navadno protitočno mrežo, protitočno mrežo, obdelano s permetrinom, in protiinsektno mrežo z manjšim premerom odprtin. Mreže so služile kot fizične ovire za zaščito vinske trte v loncih pred odraslimi hrošči vrste *Popillia japonica*. Vse vrste mrež so učinkovito ščitile rastline skozi celotno trajanje poskusa, pri čemer so se navadne protitočne mreže izkazale za najgospodarnejšo rešitev (Gotta in sod., 2023).

2.3 NAČRTNO SPREMLJANJE IN MNOŽIČNO LOVLJENJE

Večina znanja o širjenju japonskega hrošča izhaja iz raziskav, izvedenih v ZDA, ki kažejo na različne stopnje širjenja, ki so odvisne od trajanja napada in ustreznosti okolja. Zgodnje študije poročajo o povprečni hitrosti širjenja škodljivca od 16 do 24 km/leto po prvem pojavu, medtem ko poznejše raziskave navajajo hitrost širjenja med 3 in 24 km/leto (Fox, 1932). Sodobni modeli kažejo, da se hitrost širjenja s časom povečuje, pri čemer na hitrejše širjenje vpliva ustreznost habitata (Borgogno Mondino in sod., 2022). Poleg tega na razporeditev in dinamiko populacije škodljivca pomembno vplivajo lastnosti tal in podnebje (Régnière in sod., 1979; Simonetto in sod., 2022).

Za spremljanje pojavnosti, širjenja ter številčnosti japonskega hrošča poznamo zelo učinkovite feromone in pasti, ki so že v uporabi. Pasti so namenjene spremljanju populacij japonskega hrošča in množičnemu lovljenju. Na pasti se namesti sintetični spolni feromon samic ([R,Z]-5-[1-decenil] dihidro-2[3H]-furanon, znan kot »Japonilure«) (Tumlinson in sod., 1977; Doolittle in sod., 1980), ki je kombiniran s prehranskimi vabami, sestavljenimi iz mešanice fenetil propionata, evgenola in geraniola v razmerju 3:7:3 (Ladd in sod., 1981). Pasti so zelo učinkovite pri lovljenju samcev in samic v velikem številu (Ladd in McGovern, 1980) ter zagotavljajo zanesljive ocene številčnosti populacij in drugih parametrov, kot je parazitizem ulovljenih hroščev (Legault in sod., 2024).

Prehranske vabe, še posebno tiste, ki vsebujejo cvetni prah, lahko povzročijo nenameren odlov opraševalcev (Hamilton in sod., 1970), zlasti kadar pasti vključujejo komponente, ki so bele barve (Hamilton in sod., 1971; Sipolski in sod., 2019) ali ko so populacije japonskega hrošča majhne ali jih sploh ni. Na nekaterih območjih v Alberti (Kanada), kjer vrsta *P. japonica* še ni bila ugotovljena, so v omenjene pasti na teden ujeli več kot 1000 opraševalcev na past (Brodeur in sod., 2024). Nasprotno pa je bil v Quebecu (Kanada), zaradi velikih populacij japonskega hrošča, ulov ostalih opraševalcev praktično ničen (Brodeuin sod., 2024).

Zaradi splošne učinkovitosti pasti je bila metoda množičnega lovljenja obravnavana kot ena izmed potencialnih metod zatiranja japonskega hrošča, vendar bo za dokončno potrditev ustreznosti te metode potrebnih še več raziskav. Množično lovljenje je bilo kot metoda zatiranja različnih vrst škodljivih organizmov v kmetijskih ekosistemih preizkušano že v tridesetih (Langford in sod., 1940), sedemdesetih (Hamilton in sod., 1971) in osemdesetih letih prejšnjega stoletja (Gordon in Potter, 1985, 1986). V nekaterih primerih je bila ta metoda uspešna (Langford in sod., 1940), kljub očitnim težavam pri izvajanju lovljenja na večjih območjih in v daljših časovnih obdobjih. Pasti, ki so namenjene masovnemu lovljenju japonskega hrošča, so učinkovite tudi za načrtno spremljanje oz. monitoring (npr. ena past za spremljanje pojavnosti).

Rezultati več raziskav pa nas opozarjajo, da zaradi privabljanja hroščev v pasti lahko vplivamo na večjo škodo na rastlinah v njihovi bližini (Gordon in Potter, 1985, 1986; Switzer in sod., 2009). Ena izmed možnih težav je tudi t. i. „*spillover*“ učinek, kjer prepolne pasti privabljajo hrošče, a v njih ni več prostora za ulov, zato ostanejo na rastlinah v bližini pasti (Piñero in Dudenhoeffer, 2018). Piñero in Dudenhoeffer (2018) z nedavno raziskavo na borovnicah ugotavljata, da kombinacija dveh standardnih vab z velikimi 12-litrskimi posodami omogoča ulov velikega števila odraslih osebkov japonskega hrošča, pri čemer je škoda na okoliškem pridelku minimalna.

2.4 BIOTIČNO ZATIRANJE

V njegovi izvorni domovini na Japonskem ima japonski hrošč številne naravne sovražnike, kar pripomore k ohranjanju majhnih populacij škodljivca in majhne škode na rastlinah (Clausen et al., 1927; Fleming, 1968). V ZDA je bilo za zatiranje japonskega hrošča vnesenih skoraj 50 vrst naravnih sovražnikov iz Azije in Avstralije (Fleming, 1968; Potter in Held, 2002). Med njimi sta trenutno najbolj učinkoviti parazitoidni osici *Tiphia vernalis* Rohwer, 1924 (Hymenoptera: Tiphiidae) in *Istocheta aldrichi* (Mesnil, 1953) (Diptera: Tachinidae) (Potter in Held, 2002). Prva vrsta je parazitoid ličink tretje stopnje, druga vrsta pa je parazitoid odraslih osebkov.

Clausen in sod. (1927) ter Fleming (1968) poročajo, da je vrsta *P. japonica* na Japonskem gospodarsko manj pomembna. To pripisujejo biotičnemu zatiranju, ki ga na Japonskem izvajajo naravni sovražniki. Med njimi kot najučinkovitejša izstopa parazitoidna osica *I. aldrichi*. Avtorji so ugotovili, da se letne stopnje parazitizma gibljejo med 20 in 90 % na otoku Hokkaido ter okoli 50 % na otoku Honšu, kar kaže, da populacije vrste *P. japonica* vsaj delno uravnava parazitoidna osica *I. aldrichi*.

Izpusti vrste *I. aldrichi* iz Japonske v zvezne države New Jersey, Connecticut in Pennsylvanijo v ZDA so potekali med letoma 1920 in 1931, pozneje pa so se dodatni izpusti parazitoidne osice, namnožene na ameriških populacijah japonskega hrošča, na severovzhodu ZDA občasno nadaljevali vse do leta 1950 (Fleming, 1968; Potter in Held, 2002). Po teh izpustih so ugotovili, da se je vrhunec izvalitve osic zgodil približno tri tedne pred razvojem hroščev, kar je pomenilo, da so bile parazitirane (do 28 %; Fleming, 1968) le tiste ličinke hroščev, ki so se razvile predčasno. Osice so bile izpuščene tudi v severnejših državah, da bi izboljšali sinhronizacijo med izvalitvijo parazitoidov in njihovimi gostitelji, vendar od Flemingovega poročila (1968) te težave niso bile temeljito raziskane.

Kumulativne stopnje parazitizma v Minnesoti so bile v letih 2017 in 2018 relativno nizke (manj kot 12 %; Shanovich in sod., 2021), medtem ko sta Klein in McDonald (2007) poročala o 20- do 70-odstotnem parazitizmu japonskega hrošča v Severni Karolini v letih po načrtnih izpustih v to regijo (2000–2005).

Poleg koristnih žuželk, kot so plenilske stenice in parazitoidne osice, lahko tudi drugi biotični agensi pomembno vplivajo na zmanjševanje populacije japonskega hrošča. Te metode temeljijo na uporabi mikroorganizmov, kot so entomopatogene bakterije, entomopatogene glive in entomopatogene ogorčice, ki okužujejo različne razvojne stadije hrošča ter lahko znatno zmanjšajo njegovo populacijo na trajnosten način (Andreadis in Hanula, 1987; Lacey et al., 1994; Potter in Held, 2002).

Med entomopatogenimi bakterijami sta najpogosteje uporabljeni vrsti *Paenibacillus popilliae* Dutky, 1941 in *Bacillus thuringiensis* Berliner, 1915. Vrsta *Paenibacillus popilliae* je bakterija, ki povzroča t. i. „mlečno bolezen“, bolezen, ki okuži ličinke japonskega hrošča in povzroči njihovo smrt. Spore bakterije se v tleh širijo in okužijo ličinke, kar sčasoma zmanjša populacijo hroščev (Pettersson et al., 2002). *Bacillus thuringiensis* podvrsta *galleriae* Sakanian et al. 1983 pa je učinkovit bioinsekticid, ki specifično cilja na ličinke hrošča s svojimi kristalnimi toksini, ki poškodujejo črevesje hrošča in povzročijo njegovo smrt (Tanada & Kaya, 1993).

Entomopatogene glive, kot sta *Metarhizium anisopliae* (Metschn.) Sorokīn in *Beauveria bassiana* (Bals.-Criv.) Vuill., imajo pomembno vlogo v biotičnem zatiranju odraslih osebkov in ličink japonskega hrošča. Te glive okužijo gostitelja preko kutikule, sprožijo razgradnjo tkiv in povzročijo smrt. Gliva *Metarhizium anisopliae* se je izkazala kot učinkovita pri okužbi ličink, zlasti v vlažnih tleh, kjer se spore gliv hitreje širijo (Shapiro-Ilan et al., 2002). Gliva *Beauveria bassiana* deluje podobno, okuži gostitelja in povzroči njegovo smrt skozi razgradnjo notranjih organov. Obe glivi sta priljubljeni zaradi svoje dolgotrajnosti in učinkovite penetracije v gostiteljevo telo (Lacey et al., 1994).

Entomopatogene ogorčice so pokazale velik potencial kot biotični agensi pri zatiranju populacij japonskega hrošča, vendar pa njihova učinkovitost v naravnih razmerah pogosto niha. V laboratorijskih razmerah je bila smrtnost ogrcev vrste *P. japonica* ob izpostavljenosti vrsti *Heterorhabditis bacteriophora* Poinar, 1976 večja kot ob izpostavljenosti vrsti *Steinernema scarabaei* Stock & Koppenhöfer (Simões et al., 1993; Marianelli et al., 2017; Renkema in Parent, 2021). Ta razlika je lahko posledica bioloških značilnosti vrst, kot sta prilagoditev na gostitelja in sposobnost preživetja v različnih okoljskih razmerah.

Kljub obetavnim laboratorijskim rezultatom se učinkovitost entomopatogenih ogorčic v naravnem okolju zmanjša zaradi številnih dejavnikov. Eden ključnih je temperatura, saj so nekatere vrste bolj občutljive na temperaturne spremembe. Na primer, ogorčica *H. bacteriophora* se bolje obnese v toplejših razmerah, medtem ko višje temperature negativno vplivajo na učinkovitost *S. scarabaei* (Koppenhöfer et al., 2006). Poleg temperature ima velik pomen tudi vlažnost tal. Velika vlažnost je ključna za preživetje in gibanje ogorčic, saj jim omogoča lažjo pot do gostitelja (Koppenhöfer in Fuzy, 2007). Sušna obdobja in majhna vlažnost tal pa drastično zmanjšajo učinkovitost ogorčic.Tip tal je še en pomemben dejavnik, ki vpliva na učinkovitost entomopatogenih ogorčic. Peščena tla omogočajo boljše prehajanje ogorčic, vendar slabše ohranjajo vlago, kar negativno vpliva na dolgoročno učinkovitost zatiranja. Po drugi strani glinena tla bolje zadržujejo vlago, vendar otežujejo gibanje ogorčic do gostitelja (Koppenhöfer et al., 2006).

Poleg okoljskih dejavnikov omejuje širšo uporabnost entomopatogenih ogorčic v kmetijski pridelavi tudi visoka cena pripravkov. Stroški množičnega gojenja in uporabe entomopatogenih ogorčic so še vedno relativno visoki v primerjavi z drugimi metodami zatiranja škodljivcev, kar otežuje njihovo širšo uporabo v praksi (Georgis et al., 2006). Kljub tem izzivom raziskovalci iščejo načine za izboljšanje učinkovitosti ogorčic, na primer z optimizacijo formulacij in tehnik uporabe ter z integracijo z drugimi metodami biotičnega zatiranja, kot so entomopatogene glive in entomopatogene bakterije (Lacey et al., 1994; Marianelli et al., 2017). Entomopatogene ogorčice imajo torej velik potencial, vendar njihova uporaba zahteva natančno prilagoditev okoljskim razmeram in nadaljnje raziskave za optimizacijo njihove učinkovitosti v naravnih razmerah.

V raziskavah uporabnosti simbiotskih gliv iz rodu *Epichloe* (Clavicipitaceae) na travni ruši, na območju zastopanosti ogrcev japonskega hrošča (Hartley in Gange, 2009), so ugotovili, da glive niso imele opaznega učinka na vitalnost ličink in odraslih osebkov *P. japonica* (Potter in sod., 1992; Richmond in sod., 2004), so pa povečale občutljivost ličink na napad entomopatogenih ogorčic (Grewal in sod., 1995).

3 ZAKLJUČEK

Japonski hrošč velja za gospodarsko škodljivo invazivno žuželčjo vrsto, ki povzroča obsežno škodo v kmetijstvu in okrasnem vrtnarstvu. Zaradi njegove sposobnosti hitrega širjenja in prehranjevanja na širokem spektru rastlinskih vrst predstavlja resno grožnjo v številnih državah. Odrasli osebki povzročajo poškodbe s skeletiranjem listov, medtem ko se ličinke hranijo s koreninami trav na tratah, travnikih in pašnikih ter poljščinami. Zatiranje japonskega hrošča je kompleksno, saj zahteva kombinacijo kemičnih, biotičnih in mehanskih metod. Kemične strategije so sicer učinkovite, a imajo negativne vplive na okolje in povzročajo razvoj odpornosti pri škodljivcih. Sledijo mehanske metode, ki vključujejo uporabo najrazličnejših načinov zatiranja z uporabo zastirk, protiinsektnih mrež, različnih agrotehničnih ukrepov, ipd., ter uporabo feromonskih in prehranskih pasti, ki lahko pripomorejo k boljšemu razumevanju načina širjenja japonskega hrošča, v primeru množičnega pojava škodljivca pa tudi v množično lovljenje odraslih osebkov. Biotične metode, ki vključujejo uporabo naravnih sovražnikov, kot so parazitoidne osice, entomopatogene bakterije, entomopatogene glive in entomopatogene ogorčice, kažejo obetavne rezultate, vendar njihova učinkovitost v naravnih razmerah pogosto niha. Uspešno zatiranje japonskega hrošča bo tako zahtevalo nadaljnje raziskave in razvoj celovitih strategij, ki vključujejo prilagoditev metod okolju in integracijo različnih tehnik zatiranja škodljivcev, da bi dosegli trajnostno in učinkovito zmanjševanje njegovega gospodarskega pomena.

4 ZAHVALA

Prispevek je nastal v okviru projekta Euphresco »Methods for outbreak management of *Popillia japonica* in line with EU plant protection legislation (2023-C-423)«, ki je del Programa strokovnih nalog s področja zdravstvenega varstva rastlin, ki ga financira Ministrstvo za kmetijstvo, gozdarstvo in prehrano, Uprava Republike Slovenije za varno hrano, veterinarstvo in varstvo rastlin), in projektov J4-50135, P4-0431 ter programske skupine Hortikultura (P4-0013), financiranih s strani Javne agencije za znanstvenoraziskovalno in inovacijsko dejavnost Republike Slovenije.

5 VIRI

Ahmad, S., Ng, Y. (1981). Further evidence for chlorpyrifos tolerance and partial resistance by the Japanese beetle (Coleoptera: Scarabaeidae). *Journal of the New York Entomological Society*, *89*, 34–39.

Andreadis, T. G. & Hanula, J. L. (1987). Ultrastructural study and description of *Ovavesicula popilliae* N.G., N.Sp. (Microsporida: Pleistophoridae) from the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). *The Journal of Protozoology*, *34*, 15–21.

Barrows, E. M. & Gordh, G. (1978). Sexual behavior in the Japanese beetle, *Popillia japonica*, and comparative notes on sexual behavior of other scarabs (Coleoptera: Scarabaeidae). *Behavioral Biology*, *23*, 341–354.

Borgogno Mondino, E., Lessio, F., Bianchi, A., Ciampitti, M., Cavagna, B., & Alma, A. (2022). Modelling the spread of *Popillia japonica* Newman (Coleoptera: Scarabaeidae) from a recently infested area. *Entomologia Generalis*, *42*. https://doi.org/10.1127/entomologia/2022/1370

Bragard, C., Dehnen-Schmutz, K., Di Serio, F., Gonthier, P., Jacques, M. A., Miret, J. A. J., Justesen, A. F., Magnusson, C. S., Milonas, P., Navas-Cortes, J. A., Parnells, S., Potting, R., Reignault, P. L., Thulke, H., van Der Werf, W., Civera, A. V., Yuen, J., Zappalà, L., Czwienczek, E. & Macleod, A. (2018). Pest categorisation of *Popillia japonica*. *European Food Safety Authority Journal*, *16*, e05438

Brodeur, J., Doyon, J., Abram, P.K., & Parent, J.-P. (2024). *Popillia japonica* Newman, Japanese Beetle / Scarabée japonais (Coleoptera: Scarabaeidae). In: Mason, P.G., Gillespie, D.R. (Eds.), *Biological Control Programmes in Canada* (pp. 343–347). CABI. https://doi.org/10.1079/9781800623279.0037

Calderwood, L.B., Lewins, S.A., & Darby, H.M. (2015). Survey of northeastern hop arthropod pests and their natural enemies. *Journal of Integrated Pest Management*, *6*, 18.

Clausen, C.P., King, J.L., & Teranishi, C. (1927). *The parasites of Popillia japonica in Japan and Chosen (Korea) and their introduction into the United States (Department Bulletin 1429)*. United States Department of Agriculture, Washington, DC, USA

Dalthorp, D., Nyrop, J. & Villani, M. G. (2000). Spatial ecology of the Japanese beetle, Popillia japonica. *Entomologia Experimentalis et Applicata*, *96*, 129–139.

Doolittle, R.E., Tumlinson, J.H., Proveaux, A.T., & Heath, R.R. (1980). Synthesis of the sex pheromone of the Japanese beetle. *Journal of Chemical Ecology*, *6*, 473–485.

Fleming, W. E. (1968). Biological control of the Japanese beetle. *Technical Bulletin*, *1383*. Washington, DC, USA: United States Department of Agriculture, Forest Service

Fleming, W. E. (1972). Biology of the Japanese beetle. *Technical Bulletin*, *1449*. Washington, DC, USA: United States Department of Agriculture, Forest Service

Fox, H. (1932). The Distribution of the Japanese Beetle in 1930 and 1931, With Special Reference to the Area of Continuous Infestation1. *Journal of Economic Entomology*, *25*(2), 396–407. https://doi.org/10.1093/jee/25.2.396

Frank, K.D. (2016). Establishment of the Japanese beetle (*Popillia japonica* Newman) in North America near Philadelphia a century ago. *Entomological News*, *126*, 153–174.

Gagnon, M.-E., Doyon, J., Legault, S., Brodeur, J. (2023). The establishment of the association between the Japanese beetle (Coleoptera: Scarabaeidae) and the parasitoid *Istocheta aldrichi* (Diptera: Tachinidae) in Québec, Canada. *The Canadian Entomologist*, *155*, e32.

Georgis, R., Koppenhöfer, A. M., Lacey, L. A., Bélair, G., Duncan, L. W., Grewal, P. S., Samish, M., Tan, L., Torr, P., van Tol, R. W. H. M. (2006). Successes and failures in the use of parasitic nematodes for pest control. *Biological Control*, *38*(1), 103–123. https://doi.org/10.1016/j.biocontrol.2005.11.005

Gordon, F.C., & Potter, D.A. (1985). Efficiency of Japanese beetle (Coleoptera: Scarabaeidae) traps in reducing defoliation of plants in the urban landscape and effect on larval density in turf. *Journal of Economic Entomology*, *78*, 774–778.

Gordon, F.C., & Potter, D.A. (1986). Japanese beetle (Coleoptera: Scarabaeidae) traps: evaluation of single and multiple arrangements for reducing defoliation in urban landscapes. *Journal of Economic Entomology*, *79*, 1381–1384.

Gotta, P., Ciampitti, M., Cavagna, B., Bosio, G., Gilioli, G., Alma, A., Battisti, A., Mori, N., Mazza, G., Torrini, G., Paoli, F., Santoiemma, G., Simonetto, A., Lessio, F., Sperandio, G., Giacometto, E., Bianchi, A., Roversi, P. F., & Marianelli, L. (2023). *Popillia japonica* – Italian outbreak management. *Frontiers in Insect Science*, *3*. https://doi.org/10.3389/finsc.2023.1175138

Graf, T., Scheibler, F., Niklaus, P.A., & Grabenweger, G. (2023). From lab to field: biological control of the Japanese beetle with entomopathogenic fungi. *Frontiers in Insect Science*, *3*, 1138427. doi: 10.3389/finsc.2023.1138427

Grewal, S.K., Grewal, P.S., & Gaugler, R. (1995). Endophytes of fescue grasses enhance susceptibility of *Popillia japonica* larvae to an entomopathogenic nematode. *Entomologia Experimentalis et Applicata*, *74*, 219–224.

Hadley, C. H. & Hawley, I. M. (1934). General information about the Japanese beetle in the United States. *Circular*, *332*. Washington, DC, USA: United States Department of Agriculture, Forest Service

Hamilton, D.W., Schwartz, P.H., & Townshend, B.G. (1970). Capture of bumble bees and honey bees in traps baited with lures to attract Japanese beetles. *Journal of Economic Entomology*, *63*, 1442–1445.

Hamilton, D.W., Schwartz, P.H., Townshend, B.G., & Jester, C.W. (1971). Effect of color and design of traps on captures of Japanese beetles and bumblebees. *Journal of Economic Entomology*, *64*, 430–432.

Hartley, S.E., & Gange, A.C. (2009). Impacts of plant symbiotic fungi on insect herbivores: mutualism in a multitrophic context. *Annual Review of Entomology*, *54*, 323–342.

Klein, M. G. & McDonald, R. C. (2007). Recent IPM advances using Japanese beetle parasitoids. *Entomological Society America Conference, San Diego, California, USA*

Koppenhöfer, A. M. & Fuzy, E. M. (2007). Soil moisture effects on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei*, *S. glaseri*, *Heterorhabditis zealandica*, and *H. bacteriophora*. *Applied Soil Ecology*, *35*, 128–139.

Koppenhöfer, A. M., Grewal, P. S. & Fuzy, E. M. (2006). Virulence of the entomopathogenic nematodes *Heterorhabditis bacteriophora*, *Heterorhabditis zealandica*, *Steinernema scarabaei* against five white grub species (Coleoptera: Scarabaeidae) of economic importance in turfgrass in North America. *Biological Control*, *38*, 397–404.

Lacey, L. A., Martins, A. & Ribeiro, C. (1994). The pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* for adults of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). *European Journal of Entomology*, *91*, 313–319.

Ladd, T.L. Jr., Klein, M.G., & Tumlinson, J.H. (1981). Phenethyl propionate C eugenol C geraniol (3:7:3) and japonilure: a highly effective joint lure for Japanese beetles. *Journal of Economic Entomology*, *74*, 665–667.

Ladd, T. L. Jr (1987). Japanese beetle (Coleoptera: Scarabaeidae): influence of favored food plants on feeding response. *Journal of Economic Entomology*, *80*, 1014–1017.

Ladd, T. L. Jr (1989). Japanese beetle (Coleoptera: Scarabaeidae): feeding by adults on minor host and nonhost plants. *Journal of Economic Entomology*, *82*, 1616–1619.

Ladd, T.L. Jr., & McGovern, T.P. (1980). Japanese beetle: a superior attractant, phenethyl propionate C eugenol C geraniol 3:7:3. *Journal of Economic Entomology*, *73*, 689–691.

Langford, G.S., Crosthwait, S.L., & Whittington, F.B. (1940). The value of traps in Japanese beetle control. *Journal of Economic Entomology*, *33*, 317–320.

Legault, S., Doyon, J., & Brodeur, J. (2024). Reliability of commercial traps to estimate population parameters of Japanese beetle (Coleoptera: Scarabaeidae) and parasitism by *Istocheta aldrichi*. *Journal of Pest Science*, *97*, 575–583.

Loughrin, J. H., Potter, D. A. & Hamilton-Kemp, T. R. (1995). Volatile compounds induced by herbivory act as aggregation kairomones for the Japanese beetle (*Popillia japonica* Newman). *Journal of Chemical Ecology*, *21*, 1457–1467.

Loughrin, J. H., Potter, D. A., Hamilton-Kemp, T. R. & Byers, M. E. (1996). Role of feeding-induced plant volatiles in aggregative behavior of the Japanese beetle (Coleoptera: Scarabaeidae). Environmental Entomology, *25*, 1188–1191.

Marianelli, L., Paoli, F., Torrini, G., Mazza, G., Benvenuti, C., Binazzi, F., Sabbatini Peverieri, G., Bosio, G., Venanzio, D., Giacometto, E., Priori, S., Koppenhöfer, A. M. & Roversi, P. F. (2017). Entomopathogenic nematodes as potential biological control agents of *Popillia japonica* (Coleoptera, Scarabaeidae) in Piedmont Region (Italy). *Journal of Applied Entomology*, *142*, 311–318.

Marianelli, L., Paoli, F., Sabbatini Peverieri, G., Benvenuti, C., Barzanti, G.P., Bosio, G., Venanzio, D., Giacomett, E., & Roversi, P.F. (2019). Long-lasting insecticide-treated nets: a new integrated pest management approach for *Popillia japonica* (Coleoptera: Scarabeidae). *Integrated Environmental Assessment and Management*, *15*, 259–265.

Niemczyk, H.D., & Lawrence, K.O. (1973). Japanese beetle: evidence of resistance to cyclodiene insecticides in larvae and adults in Ohio. *Journal of Economic Entomology*, *66*, 520–521.

Pettersson, M., Hagstrum, D., Smith, L., & Jones, T. (2002). *Paenibacillus popilliae* in controlling *Popillia japonica*. *Journal of Applied Microbiology*, *92*(4), 582–590.

Piñero, J.C., & Dudenhoeffer, A.P. (2018). Mass trapping designs for organic control of the Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae). *Pest Management Science*, *74*, 1687–1693.

Potter, D.A., Patterson, C.G., & Redmond, C.T. (1992). Influence of turfgrass species and tall fescue endophyte on feeding ecology of Japanese beetle and southern masked chafer grubs (Coleoptera: Scarabaeidae). *Journal of Economic Entomology*, *85*, 900–909.

Potter, D.A., & Held, D.W. (2002). Biology and management of the Japanese beetle. *Annual Review of Entomology*, *47*, 175–205.

Régnière, J., Rabb, R. L., & Stinner, R. E. (1979). *Popillia japonica* (Coleoptera: Scarabaeidae): a mathematical model of oviposition in heterogeneous agroecosystems. *The Canadian Entomologist*, *111*(11), 1271–1280. https://doi.org/10.4039/Ent1111271-11

Renkema, J. M. & Parent, J.-P. (2021). Mulches used in highbush blueberry and entomopathogenic nematodes affect mortality rates of third-instar *Popillia japonica*. *Insects*, *12*, 907.

Richmond, D.S., Grewal, P.S., & Cardina, J. (2004). Influence of Japanese beetle *Popillia japonica* larvae and fungal endophytes on competition between turfgrasses and dandelion. *Crop Science*, *44*, 600–606.

Russin, J.S. (1989). Severity of soybean stem canker disease affected by insect-induced defoliation. *Plant Disease*, *73*, 144.

Sara, S. A., McCallen, E. B. & Switzer, P. V. (2013). The spatial distribution of the Japanese beetle, *Popillia japonica*, in soybean fields. *Faculty Research & Creative Activity*, *13*, 1–9.

Shanovich, H. N., Dean, A. N., Koch, R. L. & Hodgson, E. W. (2019). Biology and management of Japanese beetle (Coleoptera: Scarabaeidae) in corn and soybean. *Journal of Integrated Pest Management*, *10*, 1–14.

Shanovich, H. N., Ribeiro, A. V. & Koch, R. L. (2021). Seasonal abundance, defoliation, and parasitism of Japanese beetle (Coleoptera: Scarabaeidae) in two apple cultivars. *Journal of Economic Entomology*, *114*, 811–817.

Shapiro-Ilan, D. I., Gouge, D. H., & Koppenhöfer, A. M. (2002). Biological control of insects with entomopathogenic nematodes and fungi. *Journal of Nematology*, *34*(3), 245–252.

Simões, N., Laumond, C. & Bonifassi, E. (1993). Effectiveness of *Steinernema* spp. and *Heterorhabditis bacteriophora* against *Popillia japonica* in the Azores. *Journal of Nematology*, *25*, 480–485.

Simonetto, A., Sperandio, G., Battisti, A., Mori, N., Ciampitti, M., Cavagna, B., ... & Gilioli, G. (2022). Exploring the main factors influencing habitat preference of *Popillia japonica* in an area of recent introduction. *Ecological Informatics*, *70*, 101749.

Sipolski, S.J., Datson, S.W., Reding, M., Oliver, J.B., & Alm, S.R. (2019). Minimizing bee (Hymenoptera: Apoidea) bycatch in Japanese beetle traps. *Environmental Entomology*, *48*, 1203–1213.

Steckel, S., Stewart, S.D., & Tindall, K.V. (2013). Effects of Japanese beetle (Coleoptera: Scarabaeidae) and silk clipping in field corn. *Journal of Economic Entomology*, 106, 2048–2054.

Switzer, P.V., Enstrom, P.C., & Schoenick, C.A. (2009). Behavioral explanations underlying the lack of trap effectiveness for small-scale management of Japanese beetles (Coleoptera: Scarabaeidae). *Journal of Economic Entomology*, *102*, 934–940.

Tanada, Y. & Kaya, H. K. (1993*). Insect Pathology*. Academic Press, San Diego, USA

Tumlinson, J.H., Klein, M.G., Doolittle, R.E., Ladd, T.L., & Proveaux, A.T. (1977). Identification of the female Japanese beetle sex pheromone: inhibition of male response by an enantiomer. *Science*, *197*, 789–792.