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RESEARCH PAPER

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EVALUATION OF DIVERSITY IN QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF DIFFERENT WHITE EGGPLANT GENOTYPES UNDER CLIMATIC CONDITIONS OF KARAJ, IRAN

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ABSTRACT

The eggplant (*Solanum melongena* L.) is one of the most consumed and healthiest vegetables in the world. This plant is important both nutritionally and medicinally. This research, based on a randomized complete block design, investigated the quantitative and qualitative traits of nine inbred lines (11111, 11121, 11122, 13411, 13421, 13511, 13521, 24111, and 51311) and one commercial cultivar of white eggplant (Aretussa) in two growing seasons (2021–2022, and 2022–2023) in the climatic conditions of Karaj, Iran. The analysis of variance showed that the interaction of year and genotypes was significant for all studied traits, as plant height, leaf length and width, fruit yield, and content of minerals (P, Ca, K, Fe, Zn, Mg), protein, vitamin C, dry matter, crude fat, crude fiber, and total carbohydrates in the fruit. The comparison of means revealed that genotype 13511 had the tallest plants. Aretussa was the best genotype in terms of yield, vitamin C, crude fiber, and protein, and genotypes 51311 and 11121 were the best in P and K. The variation range of the genotype was not wide in qualitative traits, but as a summary of the two years, the three genotypes of 13421, 51311, and Aretussa can be recommended as the best genotypes in terms of fruit yield per ha, while there were close to one another in fruit quality.

Keywords: crude fiber, mineral elements, protein, *Solanum melongena* L., vitamin C

INTRODUCTION

Vegetables have a special place in the human food regime due to their nutritional value and antioxidants. Eggplant (*Solanum melongena* L.) from the Solanaceae family is native to Southeast Asia and has spread in hot and semi-hot regions [Arivalagan et al. 2013, Bidaramali et al. 2020]. According to FAO's statistics, in the years 2020–2021 the leading eggplant-producing countries were China, India, Egypt, Turkey, Indonesia, and Iran [FAOSTAT 2024].

Eggplant is important nutritionally and medicinally. Its fruits contain phenolic compounds, antioxidants, good quantities of fiber, minerals (mainly Ca, K, P, Fe, Zn, Mg, and Na), vitamins (particularly A, B, C, D, E, and K), proteins, carbohydrates, and small quantities of calories and fats [Turhan and Kuscu 2019, Bidaramali et al. 2020, Yarmohammadi et al. 2021]. It is among the top ten vegetables as a nutritional and antioxidant source and is a healthy food abundant globally, especially in Asian and developing countries. It is also a substitution for meat in vegetarians' food regimes [Bidaramali et al. 2020, Kameli et al. 2020].

The willingness to consume eggplants is increasing due to their effectiveness in health preservation. Thus, various cultivars and genotypes of this plant have been

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produced that are highly diverse in shape (oval, spherical, spear-shaped, and elongated), size (small to big), color (green, white, purple, violet, black, pink, and so on), spine status (spiny and spineless), and fruit yield, as well as in nutritional value and biologically active compounds in addition to their morphological diversity [Arivalagan et al. 2012, Fallahi et al. 2023].

Bidaramali et al. [2020] explored the food value of 20 eggplant genotypes. They reported that the cultivars with white fruits were richer in crude fiber and those with violet fruits were richer in proteins. Sharma and Kaushik [2021] revealed that the local cultivars were richer in minerals (P, Ca, K, Fe, Zn, and Mg) than the commercial cultivars. A research study was conducted in 2014 to assess the food and mineral value of Chinese, Filipino, American, Indian, and Thai eggplant cultivars produced in Mexico. The results showed that the Thai cultivar was richer in proteins and fiber and the Indian cultivar was richer in minerals (P, Mg, Zn, Ca, and K) and vitamin C [Guillermo et al. 2014].

In addition to genotype, the environmental and cultivation conditions of vegetables, influence the concentrations and percentage of primary and secondary compounds and nutritional and organoleptic properties of their edible parts. Therefore, the investigation of the interaction of genetic and ecological conditions in different years can affect the food value and organoleptics of eggplants [San José et al. 2014]. In this regard, the present research investigated the morphological and biochemical variations of 10 different white eggplant genotypes during two growing sesons in the Karaj region, Iran.

MATERIALS AND METHODS

Experimental time and location

The research was conducted in the Seed and Plant Improvement Institute of Karaj (35°55' N, 50°45' E, and Alt. 1312.5 m from sea level) in 2021–2022 and 2022–2023. Karaj is a mountain city with hot and dry weather in summer and cold and dry in winter.

Plant material

The plant materials used in this research were 10 white eggplant genotypes, including nine inbred lines (11111, 11121, 11122, 24111, 13411, 13421, 13511, 13521, 51311), and a commercial cultivar Aretussa. Table 1 presents the features of these genotypes.

Experiment description

To grow the seedlings, the seeds of the target genotypes were cultivated in greenhouse conditions

Table 1. The characteristics of the genotypes explored in the present work

Genotype	Spiny/ spineless	Growth type	Fruit shape	Seed content	Purple color intensity	Edge form	Calyx
Aretussa	spineless	semi-erect	cosh-shaped	very low	moderate to dark	moderately toothed	moderate to small
11111	spineless	semi-erect	pear-shaped	low	moderate to light	moderately toothed	moderate
11121	spineless	erect	spherical	moderate	moderate	moderately toothed	moderate
11122	spineless	semi-erect	spherical	high	moderate	moderately toothed	moderate
13411	spineless	erect	spherical	low	moderate	moderately toothed	moderate
13421	slightly spiny	prostrate	pear-shaped	moderate	moderate	moderately toothed	moderate
13511	spineless	semi-erect	elongated elliptical	moderate	light	moderately toothed	moderate
13521	spineless	semi-erect	ovoid	moderate	moderate to light	lowly toothed	moderate
24111	spineless	prostrate	spherical	moderate	light	moderately toothed	small
51311	spineless	semi-erect	ovoid	moderate	moderate	moderately toothed	moderate

(17–24 °C, 65% relative humidity, and 16/8 hours of day/night photoperiod). In May, when the seedlings were at the 4-leaf stage with an approximate height of 10 cm, they were transplanted in the main farm, which soil characteristics are given in Table 2. The seedlings were transplanted on the basis of a randomized complete block design with three replications. The experimental blocks were 3 × 5 m plowed and fertilized parcels. The rows were spaced by 75 cm, and the plants in the rows were spaced by 70 cm. After transplanting, they were irrigated (by a drip system during the experiment) every other day, and fertilized at two stages: 50 kg ha^{-1} N at flower initiation and 3 kg h^{-1} full fertilizer (WOPROFERT NPK 20-20-20 + TE, Syngenta Co., Swithzerland) at fruit formation as foliar application. The farm was weeded by hand five times during the growth period. The quantitative and qualitative traits were measured with the initiation of fruit formation.

Assessment of traits

Quantitative traits. Plant height, leaf length and width were measured with a ruler, and fruit yield was determined with a 0.001-g high-precision digital scale.

Qualitative traits

Minerals. To measure minerals, 10 g of the fresh fruit tissue was first converted into ash at 550 °C. It was then extracted by concentrated nitric acid. Then, the P content was measured by spectrophotometry (Metash spectrophotometr, UV-6100, China), and the Ca, K, Fe, Zn, and Mg contents were determined by an atomic absorption device (Varian Spectra AA220FS, Gemini BV) [Guillermo et al. 2014].

Fruit protein. The fruit protein content was determined by the Kjeldahl method. First, the N content of the samples was estimated. Then, it was put in the following equation to yield fruit protein content in percent (AOAC International, 2016):

$$\text{Protein (\%)} = \text{N} \times 6.25$$

Vitamin C. The vitamin C content was determined in mg100 g^{-1} fresh weight (FW) by the titration with 2,6-dichlorophenolindophenol using the following equation [Mazumdar and Majumdar 2003]:

$$\text{Vitamin C} = \frac{\text{e} \times \text{d} \times \text{b}}{\text{c} \times \text{a}} \times 100$$

Where: *a* represents the sample weight, *b* represents the volume of the metaphosphoric used for extraction, *c* represents the volume of the solution taken for titration, *e* represents the volume of the dye solution consumed for each sample, and *d* represents the dye factor that was obtained by the following equation:

$$\text{d} = \frac{0.5}{\text{The amount of dye solution used for the titration of the standard sample}}$$

Table 2. Physico-chemical properties of the soil in the experimental site

Parameter	Value	Unit
Electrical conductivity	4.31	dS m^{-1}
Saturated paste acidity (pH)	7.7	–
Organic carbon	0.58	%
Absorbable potassium	274	mg kg^{-1}
Moisture percentage (w/w) at 0.33 atmospheres	19.93	%
Moisture percentage (w/w) at 15 atmospheres	9.30	%
Apparent density	1.66	g cm^{-1}
Clay content	26	%
Silt content	42	%
Sand content	32	%
Texture	loamy	–

Crude fiber. A 100-g sample of the fruit was extracted with chemicals, including sulfuric acid 0.3 N and sodium hydroxide 1.5 N, on a heater. The resulting sample was washed twice with hot water, sulfuric acid, and ethanol alcohol 70%, dried at 105 °C for 6 hours, and weighed (*a*). Then, it was converted to ash at 550 °C, and its weight was recorded (*b*). Finally, the crude fiber percentage was calculated by the following equation [Aryapak and Ziarati 2014]:

$$\text{Crude fiber (\%)} = \frac{a}{b} \times 100$$

Crude fat. Crude fat content was determined using the Soxhlet extraction method. Briefly, 10 g of eggplant fruit powder was placed in a cellulose thimble and extracted with 200 mL of n-hexane for 1 hour using a Soxhlet apparatus. The solvent flask was heated to 55–60°C with an electric heater, allowing the n-hexane to evaporate, condense, and continuously reflux over the sample. The extracted fat was collected in the solvent flask, and after completion, the n-hexane was evaporated under controlled conditions. The remaining fat was dried to a constant weight, and the crude fat content was calculated as a percentage of the initial sample weight.

Total carbohydrates. It was determined by the phenol sulfuric acid method. After the extract was prepared, the absorbance was read at 490 nm with a spectrophotometer (Metash spectrophotometr, UV-6100, China), and the total carbohydrate content was determined in percentage using the glucose standard curve [Nadeeshani et al. 2021].

Experimental design and data analysis. The research was conducted using a randomized complete block design with three replications in two consecutive years. After the traits were measured at the farm and in the laboratory, the data were subjected to the analysis of variance and the comparison of means by the SAS software package. Duncan's multiple range test compared the means.

RESULTS

Morphological traits

The results in Tables 3 and 4 show that leaf length and width, and fruit yield were significantly ($P < 0.01$) higher in the first year than in the second year. The simple effect of the genotype was not significant on fruit yield and average fruit number per plant, but the genotypes differed in plant height, leaf length and width, yield per plant, and fruit weight significantly. The interaction of year and genotype was also significant for yield and the recorded morphological traits (Table 3). The significant effect of the year and genotype on the recorded traits implies the high diversity in the germplasms of the studied eggplants, so the genotypes responded even to year variations.

The plants were taller in the second year than in the first year of the study. Genotypes 13511 and 51311 had the highest 63.70 cm and the lowest 47.66 cm plant heights, respectively. The comparison of means

Table 3. Combined ANOVA of plant height, leaf length and width, fruit yield, fruits number, and average fruit weight of analysed white eggplant genotypes

Source of variance	df	Plant height	Leaf length	Leaf width	Fruit yield	Plant yield	Fruits no/ plant	Fruit weight
Year	1	2143**	161**	135**	3393**	17685510**	3.901ns	126.1ns
Replication × year	4	21.4	6.41	5.54	147.9**	1030087**	52.12	874.1**
Genotype	9	219**	12.58*	8.64*	71.2ns	456078*	42.78ns	715.4**
Year × genotype	9	355**	23.4**	9.33*	147.8**	602869**	45.5*	293.1*
Error	36	42.4	2.93	2.11	43.4	203211	24.0	108
CV	–	10.91	11.23	14.2	20.3	19.2	18.43	9.3

*, ** – significant at $P < 0.05$, $P < 0.01$, respectively; ns – insignificant based on Duncan's test

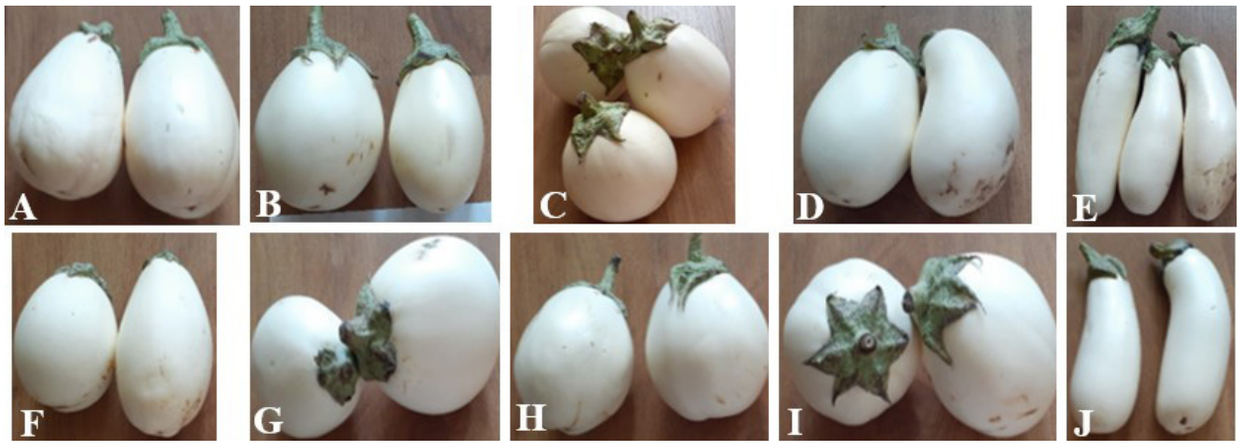

Fig. 1. Morphological characteristics of the genotypes analyzed in the present study. A: 13421, B: 13521, C: 11121, D: 11111, E: 13511, F: 24111, G: 13411, H: 51311, I: 11122, and J: Aretussa

for the interaction of year × genotype revealed that the plant height of the genotypes was in the range 45.9–63.8 cm in the first year and 56.33–79.22 cm in the second year. In both years, the lowest and the highest plant heights were recorded for genotypes 13411 and 24111, respectively (Table 4).

The leaf length of the studied genotypes was higher in the first year than in the second year. The longest leaves 16.66 cm were produced by genotype 13521, but it did not differ from the other genotypes significantly, except for genotype 24111 whose leaves were indeed the shortest 13.65 cm. The mean leaf length of the 10 studied genotypes was higher in the first year than in the second year. In both years, the lowest leaf length was recorded by genotype 24111. Genotypes 13521 and 13421 had the highest leaf length in the first and second year, respectively. They did not differ from the other genotypes significantly except for genotype 24111 (Table 4).

Leaf width was significantly smaller in the second year. Genotypes 13511 and 11121 had the highest (11.40 cm) and lowest (8.28 cm) leaf width, respectively. The comparison of means showed that leaf width was in the range 9.66–14.0 cm in the first year and 7.15–9.28 cm in the second year, showing a decline in this trait in the second year. Genotype 13521 had the highest leaf width in the first year, but four genotypes (13521, 13511, 51311, and Aretussa) were significantly different than genotype 11121 (with the lowest leaf width). In the second year, the highest leaf width was observed in genotypes 13421 and 13511. This year, genotype 13521 exhibited the lowest leaf width (Table 4).

Fruit yield and mean yield per plant were significantly higher in the first year than in the second year. The best three genotypes in fruit yield were 13421 (53.76 t ha^{-1}), 51311 (51.22 t ha^{-1}), and Aretussa (48.77 t ha^{-1}), respectively. The worst genotype was 11122 (33.51 t ha^{-1}) whose yield was (15 t ha^{-1}) lower than that of Aretussa in the same conditions. In terms of mean yield per plant, the highest was related to genotypes Aretussa, 13421, and 51311, and the lowest was 11111. The largest number of fruits per plant was recorded by 13411, and then by 13511, whereas genotypes 51311 and Aretussa produced the heaviest fruits. The results for the interaction of year × genotype revealed that the yield of all genotypes, except for 11122, declined in the second year versus the first year. The range of yield variations in the first and second years was 28.2–53.4 and 15.7–31.13, showing the loss of yield of the studied genotypes in two consecutive years in the same region, which is not optimal. Genotype 13421 had the highest yield in the first year, not differing significantly from genotypes Aretussa, 51311, and 24111. The lowest yield in the second year was recorded by genotype 13421, which was the most successful in yield in the first year. The highest yield in the second year was recorded by Aretussa and then by genotypes 11122 and 13411, but it was no significantly different from genotypes 51311, 13511, 11121,

Table 4. The comparison of means for the effect of year and genotypes on the quantitative traits

Year and genotypes	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Fruit yield (t ha^{-1})	Plant yield (g)	Fruits no/ plant	Fruit weight (g)
Year							
First	54.5b	17.48a	12.52a	39.8a	2889a	26.36a	110.5a
Second	66.3a	12.86b	8.34b	24.8b	1803b	25.97a	113.4a
Genotype							
Aretussa	60.00ab	14.52ab	9.29bc	48.77ab	2608a	113.0bcd	30.38ab
11111	53.19cde	14.82ab	9.15bc	37.15bc	1835c	107.5cd	24.21c
11121	55.44bcd	14.86ab	8.28c	39.65abc	1959bc	115.0ad	25.38bc
11122	50.55de	14.49ab	9.97abc	33.51c	1999abc	90.1e	26.4abc
13411	48.54e	15.55ab	9.42abc	45.08abc	2397abc	126.16a	26.16bc
13421	56.20bcd	15.90ab	9.66abc	53.76a	2493ab	117.167abc	24.66bc
13511	63.70a	16.59a	11.40a	44.95abc	2203abc	124.8ab	24.55bc
13521	59.80ab	16.66a	11.00ab	41.09abc	2031abc	103.6d	25.71bc
24111	57.29bc	13.65b	9.75abc	45.14abc	2198abc	118.1abc	26.26bc
51311	47.66e	16.40a	10.23abc	51.22ab	2375ab	103.8d	32.4a
First year × genotype							
Aretussa	55.60bc	19.00ab	13.33a	44.63ab	3441ab	32.27ab	106.7b–d
11111	50.10cd	17.30ab	13.0ab	37.13bc	2648c–f	25.13a–f	105.7b–d
11121	56.20abc	16.00ab	9.66b	33.83bc	2392d–g	21.53ef	110.7b–d
11122	56.96ab	18.00ab	12.60ab	28.20c	2396d–g	28.00a–e	85.7e
13411	45.90d	16.30ab	12.30ab	39.06bc	2996a–d	22.77d–f	132.7a
13421	49.90cd	17.60ab	12.30ab	53.40a	3605a	30.13a–d	119.3ab
13511	55.50bc	18.00ab	13.66a	39.46bc	2749b–e	23.07d–f	119.3ab
13521	56.60abc	19.66a	14.00a	37.06bc	2595c–f	24.47b–f	106.7b–d
24111	62.86a	15.66b	11.0ab	41.30abc	2877a–e	24.33b–f	118.3a–c
51311	54.50bc	17.30ab	13.30a	44.70ab	3194a–c	31.90a–c	100.0c–e
Second year × genotype							
Aretussa	73.33ab	13.61ab	8.6abc	31.13a	2309d–h	28.50a–e	119.3ab
11111	71.60abc	13.11ab	7.89bc	19.8bc	1414ij	26.30a–f	101.7c–e
11121	74.11ab	13.22ab	7.67bc	25.06ab	1774g–j	29.23a–e	119.3ab
11122	60.90bc	12.0ab	8.44abc	29.50a	2367d–g	24.80b–f	94.7de
13411	56.33c	12.27ab	7.9bc	29.33a	2150e–i	29.57a–d	119.7ab
13421	60.40bc	13.94a	9.28a	15.70c	1075j	19.20f	115.0a–c
13511	74.10ab	13.33ab	9.22a	27.30ab	1911f–i	26.03a–f	130.3a
13521	72.78abc	12.17ab	7.15c	19.70bc	1423ij	23.97c–f	108.3b–d
24111	79.22a	11.55b	8.01bc	23.10abc	1624hij	28.20a–e	118.0a–c
51311	71.60abc	13.44ab	8.71ab	27.80ab	1989f–i	32.90a	107.7b–d

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan's test.

and 24111 (Table 4). Also, the mean yield per plant varied from 3605 g for genotype 13421 to 2392 g for genotype 11121 in the first year, and from 2367 g for genotype 11122 to 1075 g for genotype 13421 in the second year. The highest fruit weight in the first year was observed in Aretussa 32.27 g, which was not significantly different from genotypes 13421, 51311, and 11111. The highest and the lowest fruit number in the second year was observed in genotypes 51311 (32.90 fruits) and 13421 (19.20 fruits), respectively. The fruit weight of the genotypes in the first year varied from 32.7 g (genotype 13411) to 85.7 g (genotype 11122). The highest and lowest fruit weights in the second year were recorded by genotypes 13511 (130.3 g) and 11222 (94.7 g) (Table 4).

Among the studied genotypes of the eggplant, genotype 11122 had the lowest range of yield variations. Although this genotype was one of those with the lowest yield, it preserved its yield in the second year, making its way into the list of suitable genotypes.

Qualitative traits

The analysis of variance for the qualitative traits (dry matter, carbohydrate, protein, crude fiber, crude fat, and vitamin C) showed that the simple effect of year was significant ($P < 0.01$) only on total carbohydrates and vitamin C, whereas the simple effect of genotype was significant on all qualitative traits, except for vitamin C. The interaction of year × genotype was significant for dry matter, crude fiber, and vitamin C at the $P < 0.05$ level and for carbohydrates, proteins, and crude fat at the $P < 0.01$ level (Table 5).

The mean comparison showed that total carbohydrates and vitamin C were higher in the first year than in the second year of the study (Table 6). Among the genotypes, the highest dry matter was observed in 13521 and Aretussa, which were among the best in terms of crude fiber. Aretussa had the lowest and genotype 13411 had the highest fruit carbohydrates. The highest (0.181%) and lowest (0.133%) protein content was related to the genotype Aretussa and 13411, respectively. The highest crude fat content was noted in genotypes 11121 and 13521, and the lowest in genotype 13421 (Table 6).

It was revealed by the comparison of means for the interaction of year × genotype that genotypes 11111 and Aretussa were superior in dry matter content in both years. The lowest dry matter content in the first year (5.93%) was recorded by genotypes 13411 and 51311, whereas the lowest in the second year (6.30%) was observed in genotype 51311 (Table 6).

The total carbohydrate content varied from 1.94% for genotype Aretussa to 3.12% for genotype 13411 in the first year. However, in the first year, no genotype, except for Aretussa, significantly differed from genotype 13411, whose total carbohydrate content was the highest. In the second year, the lowest total carbohydrate content was recorded by Aretussa (2.17%), and the highest was obtained from genotype 13521 (3.43%) (Table 6).

The protein content in the studied genotypes was in the range 0.130–0.183% and 0.137–0.180% in the first and second year, respectively. In the first year, Aretussa and 13521 had the first and second-highest protein content among the studied genotypes, but they did not differ significantly from genotypes 11121, 11122, 24111, 13421, 13511, and 51311. In the second year, although the highest protein content was recorded for

Table 5. Analysis of variance for the effect of year and genotype on the qualitative traits

Source of variance	df	Dry matter	Carbohydrate	Protein	Crude fiber	Crude fat	Vitamin C
Year	1	1.16ns	1.5843**	0.594ns	0.064ns	0.035ns	60.88**
Replication × year	4	0.904	0.1286	0.102	0.5109	0.0223	5.52
Genotype	9	9.66**	1.585**	34.37**	5.682**	1.379**	6.21ns
Year × genotype	9	0.369*	0.508**	1.107**	1.631*	0.679**	21.67*
Error	36	0.632	0.168	0.284	0.568	0.0138	7.94
CV (%)	–	10.05	15.76	3.13	11.55	4.7	16.9

*, ** and ns – significant at $P < 0.05$, $P < 0.01$, and insignificant based on Duncan's test, respectively

Table 6. The comparison of means for the effect of year and genotypes on the content of dry matter, carbohydrates, protein, crude fiber, crude fat, and vitamin C

Year and genotypes	Dry matter (%)	Carbohydrate (%)	Protein (%)	Crude fiber (%)	Crude fat (%)	Vitamin C (mg 100 g^{-1} FW)
			Year			
First	7.77a	3.07a	0.163a	6.68a	0.259a	1.553a
Second	8.04a	2.22b	0.161a	6.53a	0.242a	1.118b
			Genotype			
Aretussa	9.60a	2.06b	0.181a	8.40a	0.228bcd	1.661a
11111	8.23b	2.93a	0.171abc	6.34bc	0.190cd	1.261a
11121	8.16b	2.61ab	0.153cd	7.09b	0.303a	1.190a
11122	8.22b	2.99a	0.176ab	7.26b	0.296ab	1.116a
13411	6.66cd	2.97a	0.133d	4.71d	0.213cd	1.488a
13421	7.51bc	2.50ab	0.168abc	5.65cd	0.185d	1.566a
13511	6.40d	2.55ab	0.155bcd	4.91d	0.288ab	1.355a
13521	9.91a	2.53ab	0.151cd	8.38a	0.303a	1.348a
24111	8.26b	2.72ab	0.165abc	7.16b	0.255abc	1.180a
51311	6.11d	2.58ab	0.165abc	6.20bc	0.243a–d	1.186a
			First year × genotype			
Aretussa	9.60abc	1.94c	0.183a	5.30e–g	0.233bcd	1.696abc
11111	10.11a	2.45abc	0.147bcd	8.32a	0.310abc	1.596a–e
11121	8.24d	2.78abc	0.153a–d	6.87bcd	0.303abc	1.316c–i
11122	8.10d	2.95abc	0.173ab	7.26abc	0.326ab	1.43c–g
13411	5.93g	3.12ab	0.130d	4.93g	0.263abc	1.946a
13421	7.30def	2.81abc	0.173ab	6.45b–e	0.153d	1.866ab
13511	6.35fg	2.93abc	0.157a–d	5.02g	0.270abc	1.490b–f
13521	8.01d	2.44abc	0.180a	7.50ab	0.160d	1.440c–g
24111	8.10d	2.74abc	0.167abc	7.28ab	0.243a–d	1.336c–i
51311	5.93g	2.55abc	0.170ab	6.02c–g	0.330a	1.410c–h
			Second year × genotype			
Aretussa	9.61abc	2.17bc	0.180a	5.41e–g	0.223cd	1.626a–d
11111	9.71ab	2.61abc	0.157a–d	8.36a	0.296abc	1.100f–j
11121	8.08d	2.45abc	0.153a–d	6.36b–f	0.303abc	1.063g–j
11122	8.34cd	3.02abc	0.180a	6.95bcd	0.266abc	0.803j
13411	7.39def	2.82abc	0.137cd	6.84bc	0.163d	1.030h–j
13421	7.71de	2.18bc	0.163abc	6.33b–f	0.216cd	1.266d–i
13511	6.45e–g	2.18bc	0.153a–d	5.87d–g	0.306abc	1.220e–i
13521	8.45bcd	3.43a	0.163abc	7.04bcd	0.220cd	1.083g–j
24111	8.42cd	2.70abc	0.163abc	7.24abc	0.266abc	1.023h–j
51311	6.30fg	2.61abc	0.160a–d	5.20g	0.156d	0.960ij

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using Duncan's test.

genotypes Aretussa and 11122 (both 0.180%), they were no significantly different from the other genotypes except for 13411, which had the lowest total protein content in both years (Table 6).

The crude fat content in various eggplant genotypes was in the range 0.153–0.330% in the first, and 0.156–0.306% in the second year, respectively. The three genotypes 13421, 13521, and Aretussa had lower crude fat content than the other genotypes. The highest crude fat content in the first year was related to genotype 51311, while it had the lowest crude fat content (0.156%) in the second year. The crude fat content of genotypes 13411 and 51311 was lower in the second year than in the first year, but that of genotypes 13521 and 13421 was higher in the second year than in the first year.

The comparison of means revealed that the amount of crude fiber in the studied genotypes was 4.93–8.32% in the first year and 5.20–8.36% in the second year. The highest crude fiber content was observed in genotypes 11111 and 13521 in the first year. Genotypes 13411 and 13511 were the weakest in this trait in the first year. In the second year, crude fiber was the lowest in genotype 51311, while genotypes 11111 and 24111 were the best in this trait (Table 6).

The vitamin C content was higher in all genotypes in the first year than in the second year. It was in the ranges of 1.316–1.946 and 0.803–1.626 mg 100 g^{-1} FW in the studied genotypes in the first and second year, respectively. The highest vitamin C content was recorded by genotype 13411 in the first year, but it did not differ from genotypes Aretussa, 11111, and 13421, significantly. The lowest in the first year was recorded by genotypes 11121 and 24111. In the second year, the highest was related to Aretussa, and the lowest to genotypes 11122 and 51311 (Table 6).

Minerals

Table 7 presents the analysis of variance for the simple and interactive effects of year and genotype on the minerals of eggplant fruits.

Table 8 shows that the Ca and Fe contents of the fruits were higher in the first year than in the second year, but the K, P, Zn, and Mg contents were higher in the second year. Among the genotypes, 11121 had the highest amounts of K and P. Genotypes 11122 and 51311 had the highest Ca content, and genotypes 13421 and 11111 had the highest Zn content. Genotype 13511 was the richest in Mg, whereas genotypes 13511, 11121, 24111, and 13521 were the richest in Fe (Table 8).

The comparison of means for the interaction of year and genotype revealed significant differences among genotypes in Ca content (1.26–2.807 mg kg^{-1}), K content (0.22–0.292 mg kg^{-1}), P content (26.76–37.16 mg kg^{-1}), Zn content (0.133–0.286 mg kg^{-1}), Fe content (0.704–1.57 mg kg^{-1}), and Mg content (14.47–17.70 mg kg^{-1}). In the first year, genotype 11121 had the highest amounts of Ca, K, and P, and genotypes 13421, 13511, 11121 had the highest amounts of Zn, Fe, and Mg (Table 8). In the second year, the studied genotypes exhibited various ranges of Ca (1.130–2.40 mg kg^{-1}), K (0.235–0.298 mg kg^{-1}), P (21.1–40.46 mg kg^{-1}), Zn (0.200–0.290 mg kg^{-1}), Fe (0.496–1.257 mg kg^{-1}), and Mg (14.93–24.73 mg kg^{-1}). Genotypes 11122, 13521, 11111, and 13511 outperformed the other genotypes in Ca, Zn, Fe, and Mg. Genotype 11121 was the best in K and P content in the second year (Table 8).

Table 7. Analysis of variance for the effect of year and genotype on the mineral content in eggplant fruit

Source of variance	Df	Ca	K	P	Fe	Zn	Mg
Year	1	0.0133**	3.71**	0.0112*	13172**	400.4**	0.00925**
Replication × Year	4	0.00019	0.0051	0.002	26.352	2.328	0.00033
Genotype	9	0.0119**	0.731**	0.0378**	4590**	64.28**	0.000086ns
Year × Genotype	9	0.0073**	0.0918**	0.0181**	116**	6.72**	0.00045*
Error	36	0.0011	0.0132	0.00226	37.74	1.098	0.00016
CV (%)		15.6	9.013	14.16	5.36	5.3	8.6

*, ** and ns – significant at $P < 0.05$, $P < 0.01$, and insignificant based on Duncan's test, respectively

Table 8. The comparison of means for the effect of year and genotypes on the mineral content of the eggplant fruit

Year and genotypes	Ca (mg kg^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	Zn (mg kg^{-1})	Fe (mg kg^{-1})	Mg (mg kg^{-1})
Year						
First	2.00a	0.223b	28.25b	0.203b	1.38a	16.32b
Second	1.76b	0.326a	37.62a	0.248a	1.01b	19.18a
Genotype						
Aretussa	1.345d	0.230d	32.26bc	0.206ab	0.673ab	16.21b
11111	1.195d	0.238cd	32.55abc	0.255a	0.828ab	16.58b
11121	2.248ab	0.295a	38.81a	0.216ab	1.483a	18.41ab
11122	2.518a	0.267ab	35.88ab	0.223ab	1.178ab	17.83ab
24111	1.926bc	0.266bc	30.30bc	0.208ab	1.426a	17.70ab
13411	1.528cd	0.285ab	31.81bc	0.183b	1.330ab	19.0ab
13421	2.180ab	0.268ab	33.43abc	0.265a	0.976ab	18.130ab
13511	1.876bc	0.264bc	32.90abc	0.238ab	1.486a	20.35a
13521	1.565cd	0.268ab	28.88c	0.226ab	1.381a	16.70b
51311	2.473a	0.283ab	32.55abc	0.233ab	1.228ab	16.60b
First year × genotype						
Aretussa	1.283fg	0.226f	32.70abc	0.210ab	0.704k	17.50c–e
11111	1.667e–g	0.269a–e	26.76c	0.220ab	1.44bc	14.47e
11121	2.807a	0.292ab	37.16ab	0.233ab	1.505ab	17.70b–e
11122	2.637ab	0.2733a–e	35.16abc	0.230ab	1.499c	17.03c–e
24111	2.420a–d	0.268a–f	29.60bc	0.196bc	1.53ab	15.73c–e
13411	1.670e–g	0.2813abc	31.50abc	0.133c	1.295d	15.90c–e
13421	1.993b–e	0.245c–f	33.03abc	0.286a	1.051gh	17.37c–e
13511	1.780d–f	0.251b–f	32.16abc	0.223ab	1.57a	15.97c–e
13521	1.260fg	0.232ef	32.10abc	0.220ab	0.918ij	15.33c–e
51311	2.553a–c	0.280a–c	32.30abc	0.210ab	1.397c	16.20c–e
Second year × genotype						
Aretussa	1.407e–g	0.235d–f	31.8abc	0.203b	0.496l	14.93de
11111	1.463e–g	0.267a–f	25.00d	0.233ab	1.257de	18.93b–d
11121	1.690e–g	0.298a	40.46a	0.200b	1.223f	19.13b–d
11122	2.400a–d	0.262a–f	36.60ab	0.216ab	1.187ef	18.63b–e
24111	1.433e–g	0.264a–f	31.00abc	0.220ab	1.151fg	19.67bc
13411	1.387e–g	0.289ab	21.10d	0.202b	1.012hi	22.10ab
13421	2.367a–d	0.292ab	33.84abc	0.243ab	0.837j	18.90b–d
13511	1.973c–e	0.276a–d	33.63abc	0.253ab	1.189ef	24.73a
13521	1.130g	0.2446c–f	33.0abc	0.290a	0.664k	17.83c–e
51311	2.393a–d	0.287ab	32.8abc	0.256ab	0.943i	17.00c–e

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using Duncan's test.

Correlation of trait

According to Table 9, the eggplant fruit yield as the most important economic trait had a positive and significant correlation with plant height, leaf length and width, dry matter, and total carbohydrates, whereas its correlation was significant but negative with the minerals, including Fe, K, and Mg, as well as proteins. Therefore, increasing the yield will entail a decline in minerals and protein in the studied genotypes. Significant and positive correlations were found between plant height and Zn and Mg content, between leaf length and Fe content, total fat, and proteins, and between leaf width and Fe content and vitamin C.

DISCUSSION

Fallahi et al. (2023) recorded the plant height of 13 genotypes of white, purple, and green eggplant between 33.41 and 94 cm. In our experiment, among the white eggplant genotypes, the highest plant height (63.70 cm) was recorded for genotype 13511, and the genotype 51311 had the shortest height (47.66 cm). Khaleghi et al. (2019) measured the plant height of 13 local eggplant cultivars between 48 to 71.2 cm. Since plant height is important for producers in terms of management and mechanized harvesting, shorter genotypes with desirable performance are the most suitable choice for commercial cultivation.

Table 9. The correlation of the recorded traits of the different white eggplant genotypes

Measured traits	Crude fibre	Zn	Fe	P	K	Mg	Ca	Vitamin C	Fat	Protein	Carbo-hydrate	Dry matter	Plant height	Leaf length	Leaf width	Yield
Crude fibre	1.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Zn	0.215	1.000	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Fe	0.242*	–0.253*	1.000	–	–	–	–	–	–	–	–	–	–	–	–	–
P	–0.312*	0.131	0.024	1.000	–	–	–	–	–	–	–	–	–	–	–	–
K	0.035	–0.180	0.458**	0.078	1.000	–	–	–	–	–	–	–	–	–	–	–
Mg	0.018	0.241*	–0.080	–0.048	0.361*	1.000	–	–	–	–	–	–	–	–	–	–
Ca	–0.051	0.071	0.538**	0.407**	0.540**	–0.043	1.000	–	–	–	–	–	–	–	–	–
Vitamin C	–0.315*	–0.268*	0.023	–0.041	–0.378**	–0.516**	–0.109	1.000	–	–	–	–	–	–	–	–
Fat	0.178	–0.254*	0.620**	0.249*	0.322*	0.046	0.317*	–0.029	1.000	–	–	–	–	–	–	–
Protein	0.032	0.321*	–0.362**	0.472**	–0.654**	–0.276*	0.073	0.011	–0.164	1.000	–	–	–	–	–	–
Carbohydrate	0.153	0.066	0.313*	–0.019	0.103	–0.112	0.085	–0.155	–0.004	–0.281*	1.000	–	–	–	–	–
Dry matter	0.572**	0.071	–0.265*	–0.166	–0.390**	–0.188	–0.325*	–0.036	0.096	0.321*	–0.271*	1.000	–	–	–	–
Plant height	0.076	0.291*	–0.337*	0.139	0.035	0.422**	–0.277**	–0.643**	0.043	0.138	–0.152	0.198	1.000	–	–	–
Leaf length	–0.109	–0.134	0.265*	0.078	–0.364*	–0.573**	0.108	0.738**	0.034	0.249*	–0.171	–0.066	–0.728**	1.000	–	–
Leaf width	–0.152	–0.281*	0.395**	–0.001	–0.323*	–0.557**	0.118	0.728**	0.077	0.194	–0.125	–0.179	–0.756**	0.956**	1.000	–
Yield	–0.09	0.024	–0.310*	–0.126	–0.314*	–0.270*	–0.197	0.184	–0.225*	0.376**	–0.006	0.923**	0.392**	0.310*	0.185	1.000

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

Cultivars and genotypes that can preserve their mean optimal yields in different climatic conditions and undergo less fluctuation are more valuable and stable, so various cultivars and landraces are evaluated in various locations and years [Hakim et al. 2021]. Weather variations and annual fluctuations of variables like precipitation, moisture, and temperature, and even the occurrence of environmental stresses influence plant yields remarkably. The cultivation of plants, especially new genotypes, in regions that are characterized by climatic and environmental variations can change their growth patterns and yields. Therefore, producers focus on developing cultivars and genotypes with optimal traits for new geographical regions with diverse weather conditions, hoping these genotypes can preserve their economic and yield advantages for many years [Owuor et al. 2011].

The eggplant is a vegetable with low carbohydrate content suitable for diabetics [Gurbuz et al. 2018]. The carbohydrate content has been reported at various levels in different eggplant cultivars. For example, it was recorded at 2.80–6.82% in 20 genotypes by Bidaramali et al. [2020] while at 4.27–6.63% in 10 genotypes by Quamruzzaman et al. [2020]. The highest level of carbohydrates in the present research was 3.43%, as recorded by genotype 13521 in the second year. In total, the present and past research results show that eggplant cultivars and genotypes differ in carbohydrates considerably.

Eggplant fruits contain little protein, and cultivars with purple fruits have higher protein content than those with green or white fruits [Bidaramali et al. 2020]. Sharma and Kaushik [2021] estimated the protein content of fresh eggplants at 0.98% and Rosa-Martínez et al. [2021] reported it in 10 eggplant varieties at 8.1–20.8 g kg^{-1} FW. Likewise, Guillermo et al. [2014] showed that the protein content of five eggplant cultivars was 0.65–0.9%, whereas Rodriquez-Jimenz et al. [2018] demonstrated it in a range of 12.55–12.77%. A protein content of 0.85–1.54% in 10 eggplant cultivars [Quamruzzaman et al. 2020] and 13.85–16.98% in 20 genotypes [Bidaramali et al. 2020] are other results, showing that the protein content of eggplant fruit depends on cultivar and genotype, as well as the environmental and growth conditions.

The eggplant is poor in fat [Agoreyo et al. 2012]. Previous researchers have recorded the fat content at 0.02–0.4% in 10 eggplant cultivars [Quamruzzaman et al. 2020] and 0.24–0.42% in four eggplant cultivars [Ossamulu et al. 2014]. The fat content of *Solanum melongena*, *S. torvum*, and *S. melongena* Insanum was estimated at 0.23%, 0.82%, and 0.7%, respectively [Nadeeshani et al. 2021], showing that our studied genotypes were analogous to *S. melongena* in fat content, but had lower fat than *S. torvum* and *S. melongena* Insanum. So, these genotypes are suitable for people suffering from diabetes and obesity [Nadeeshani et al. 2021].

The fiber content of eggplant fruits greatly contributes to better food digestion and the disposal of toxins and wastes. It also reduces the risk of colon and gastric cancers [Gurbuz et al. 2018]. Nadeeshani et al. (2021) reported the amount of crude fiber in *S. melongena*, *S. melongena* Insanum, and *S. torvum* were 4.85%, 3.91%, and 3.81%, respectively. Ossamulu et al. [2014] found that four eggplant species e.g. *Solanum macrocarpon* (round), *Solanum atheopicum*, *Solanum gilo*, and *S. macrocapon* (oval) had 2.21–3.07% of crude fiber. In another study, the crude fiber content of 10 eggplant cultivars varied from 1.01 to 2.48% [Quameuzzanan et al. 2020]. So, the genotypes we studied outperformed the cultivars reported in this literature regarding crude fiber.

The eggplant is a good source of antioxidants, including vitamin C. Research have reported various ranges for the vitamin C content of different eggplant genotypes. For example, Sharma and Kaushik [2021] reported it at 1.8–2.2 mg 100 g^{-1} FW, which is consistent with the vitamin C content of genotypes 13411 (1.94 mg 100 g^{-1} FW) and 13511 (1.86 mg 100 g^{-1} FW) in our study. In Nadeeshani et al.'s [2021] research, it was found to be lower than 20 mg 100 g^{-1} FW for the *S. melongena*, *S. melongena* Insanum, and *S. torvum*, among which the latter had the highest amount. Other researchers have reported values like 0.66–3.53 mg 100 g^{-1} FW [Bidaramali et al. 2020], 3.9–1.4 mg 100 g^{-1} FW [Shabetya et al. 2020], 6.57–17.21 mg 100 g^{-1} FW [Quamruzzaman et al. 2020], and 0.3–1 g kg^{-1} FW [Rosa-Martínez et al. 2020]. Thus, different eggplant species and cultivars can meet a part of the human body's daily need for vitamin C [Rosa-Martínez et al. 2020].

The eggplant is a good source of minerals (K, Fe, Ca, P, Zn, and Mg), which is more economical as

a cheaper source of food than the other mineral-rich nutrients in addition to its availability throughout the year [Yarmohammdi et al. 2021]. Nadeeshani et al. [2021] reported the amount of Mg, K, Ca, Fe, and Zn in three eggplant species at 23.8–49.6, 427–632, 60.5–329, 1.07–1.85, and 0.34–1 mg kg^{-1}, respectively. This means that the genotypes studied in our research were almost similar to *S. melongena* Insanum in terms of Fe (1.07 mg kg^{-1}) and Zn (0.34 mg kg^{-1}). In terms of Mg, genotype 13411 (22.10 mg kg^{-1}) was similar to *S. melongena* (23.8 mg kg^{-1}) [Nadeeshani et al. 2021]. Since the recommended level of daily intake of Ca, K, P, Fe, Mg, and Zn is 1000 mg, 100 mg, 4000 mg, 18 mg, 400 mg, and 15 mg [Arivalagan et al. 2012], the daily consumption of 100 g of the studied white eggplant genotypes can only provide a small fraction of the body requirements.

A positive correlation between the studied traits is a helpful index to select a genotype with more desirable characteristics for the development of its cultivation and consumption [Kameli et al. 2020].

CONCLUSION

Based on the results, most genotypes exhibited higher yield, leaf length, and width but lower plant height in the second year. Aretussa was in the first rank in protein, crude fiber, and vitamin C and was one of the best in plant height, leaf length, plant yield, dry matter, Fe, and Zn. Genotype 11121 outperformed the other genotypes in P, K, and Fe content, and was one of the best in Ca, Zn, and Mg. Genotype 13421 produced the highest yield (53.4 t ha^{-1}) in the first year, but its yield sharply declined in the second year. Aretussa showed a decline in yield in the second year versus the first year, but its yield was the highest in the second year and it was among the best genotypes in terms of vitamin C in both years. Genotype 11121 was the best in P and K in both years and in Ca and Mg in the first year. The best crude fiber and dry matter genotype in both years was 11111. The studied genotypes differed in quantitative and qualitative traits, but their differences had no specific pattern. However, the summary of the results for the two years shows that the three genotypes of 13421, 51311, and Aretussa were the best in terms of the economical trait (fruit yield per ha) and performed acceptably in the qualitative traits, so this research recommends them as the best genotypes for mass production to meet the consumer needs.

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REFERENCES

AOAC (2010). Official Methods of Analysis. Association of Official Analytical Chemists, Washington DC.

AOAC International (2016). Official Methods of Analysis of AOAC International (20th ed.). Gaithersburg, MD: AOAC International.

Agoreyo, B.O., Obansa, E.S., Obanor, E.O. (2012). Comparative nutritional and phytochemical analyses of two varieties of *Solanum melongena.* Sci. World J., 7(1), 5–8.

Arivalagan, M., Bhardwaj, R., Gangopadhyay, K.K., Prasad, T.V., Sarkar, S.K. (2013). Mineral composition and their genetic variability analysis in eggplant (*Solanum melongena* L. germplasm. J. Appl. Bot. Food Qal., 86(1), 99–103. https://doi.org/10.5073/JABFQ.2013.086.014

Arivalagan, M., Gangopadhyay, K.K., Kumar, G., Bhardwaj, R., Prasad, T.V., Sarkar, S.K., Roy, A. (2012). Variability in mineral composition of Indian eggplant (*Solanum melongena* L.) genotypes. J. Food Compos. Anal., 26(1–2), 173–176.

Aryapak, S., Ziarati, P. (2014). Nutritive value of Persian walnut (*Juglans regia* L.) orchards. Am.-Euras. J. Agric. Environ. Sci., 14(11), 1228–1235.

Bidaramali, V., Akhtar, S., Das, A. (2020). Proximate composition and bioactive compounds in diverse eggplant genotypes. Curr. J. Appl., 39(4), 113–121.

Fallahi, F., Abdossi, V., Bagheri, M., Ghanbari Jahromi, M., Mozaffari, H. (2023). Evaluation of morphological and phytochemical diversity of some white eggplant genotypes. J. Crop. Improv., 25(2), 485–504. https://doi.org/10.22059/jci.2022.341315.2696

FAOSTAT (2024). Countries by commodity. Available: https://www.fao.org/faostat/en/#rankings/countries_by_commodity [date of access: 21.05.2024].

Guillermo, N.M., Dolores, M.R., Gardea-Bejar, A., Gonzalez-Aguilar, G., Heredia, B., Manuel, B.S., De La Rocha, R.V. (2014). Nutritional and nutraceutical components of commercial eggplant types grown in Sinaloa,

Mexico. Not. Bot. Horti Agrobot. Clu- Napoca, 42(2), 538–544.

Gurbuz, N., Uluişik, S., Frary, A., Frary, A., Doğanlar, S. (2018). Health benefits and bioactive compounds of eggplant. Food Chem., 268, 602–610. https://doi.org/10.1016/j.foodchem. 2018.06.093

Hakim, M.A., Biswas, B.K., Hasanuzzaman, M., Matin, M.Q.I., Banu, M.B., Barma, N.C.D., Joshi, A.K. (2021). Genotype environment interaction (G × E) of heat tolerant wheat genotypes over locations and years. Am. J. Plant Sci., 12(11), 1633–1645.

Kameli, A.M., Kiani, G., Kazemitabar, S.K. (2020). The Evaluation of phenotypic diversity in eggplant (*Solanum melongena* L.) genotypes. J. Veg. Sci., 3(2), 31–41. https://doi.org/10.22034/iuvs.2020.114655.1071

Khaleghi, S., Mobli, M., Baninasab, B., Majidi, M.M. (2019). Study of variation of yield and morphological traits of some local varieties of Iran's eggplant (*Solanum melongena* L.). J. Crop Prod. Process., 9(1), 15–32. http://dx.doi.org/10.29252/jcpp.9.1.15

Mazumdar, B.C., Majumdar, K. 2003. Methods on physicochemical analysis of fruits. University College of Agriculture, Calcutta University, 108–109.

Nadeeshani, H., Samarasinghe, G., Wimalasiri, S., Silva, R., Hunter, D., Madhujith, T. (2021). Comparative analysis of the nutritional profiles of selected *Solanum* species grown in Sri Lanka. J. Food Compos. Anal., 99, 103847.

Owuor, P.O., Kamau, D.M., Kamunya, S.M., Msomba, S.W., Uwimana, M.A., Okal, A.W., Kwach, B.O. (2011). Effects of genotype, environment and management on yields and quality of black tea. In: Lichtfouse, E. (ed.). Genetics, biofuels and local farming systems. *Sustain. Agric. Rev*., Springer, Dordrecht, 277–307. https://doi.org/10.1007/978-94-007-1521-9_10

Ossamulu, I.F., Akanya, H.O., Jigam, A.A. and Egwim, E.C. (2014). Evaluation of nutrient and phytochemical constituents of four eggplant cultivars. Food Sci., 73, 26424–26428.

Quamruzzaman, A.K.M., Khatun, A., Islam, F. (2020). Nutritional content and health benefits of Bangladeshi eggplant cultivars. Eur. J. Agric. Food Sci., 2(4), 1–7. http://dx.doi.org/10.24018/ejfood.2020.2.4.76

Rodriguez-Jimenez J.R., Amaya-Guerra C.A., Baez-Gonzalez J.G., Aguilera-Gonzalez C., Urias-Orona V., Nino-Medina G. (2018). Physicochemical, functional, and nutraceutical properties of eggplant flours obtained by different drying methods. Molecules, 23, 3210. https://doi.org/10.3390/molecules23123210

Rosa-Martínez, E., García-Martínez, M.D., Adalid-Martínez, A.M., Pereira-Dias, L., Casanova, C., Soler, E., Figàs, M.R., Raigón, M.D., Plazas, M., Soler, S. and Prohens, J. (2021). Fruit composition profile of pepper, tomato and eggplant varieties grown under uniform conditions. Food Res., 147, 110531.

San José, R., Sánchez-Mata, M.C., Cámara, M., Prohens, J. (2014). Eggplant fruit composition as affected by the cultivation environment and genetic constitution. J. Sci. Food Agric., 94(13), 2774–2784.

Shabetya, O.N., Kotsareva, N.V., Nasser, A.M., Katskaya, A.G. and Al-Maidi, A.A. (2020). Biochemical composition of eggplant and its change during storage. Plant Arch., 20, 385–388.

Sharma, M., Kaushik, P. (2021). Biochemical composition of eggplant fruits. A review. Appl. Sci., 11(15), 7078. https://doi.org/10.3390/app11157078

Turhan, A., Kuscu, H. (2019). [Effects of salinity stress on water use efficiency, yield components, leaf chlorophyll and carotenoid content of eggplant (*Solanum melongena* L.)]. Yuzuncu Yil Univ. J. Agric. Sci., 29(1), 60–68. In Turkish. https://doi.org/10.29133/yyutbd.462094

Yarmohammadi, F., Ghasemzadeh Rahbardar, M., Hosseinzadeh, H. (2021). Effect of eggplant (*Solanum melongena*) on the metabolic syndrome. A review. Iran J. Basic Med. Sci., 24(4), 420–427. https://doi.org/10.22038/ijbms.2021.50276.11452

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EFFECTS OF BACTERIAL INOCULANTS AND IRRIGATION REGIMES ON YIELD, MYCORRHIZAL COLONISATION, AND PHOTOSYNTHETIC EFFICIENCY IN STRAWBERRY CULTIVARS

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ABSTRACT

Strawberries (*Fragaria* × *ananassa*) are a globally significant fruit crop with high nutritional and economic value. However, their shallow roots and high water demands make them vulnerable to water stress. The effects of microbial inoculants and irrigation regimes on the yield, root colonisation by arbuscular mycorrhizal fungi (AMF), and photosynthetic efficiency of strawberry cultivars Rumba and Honeoye were investigated. Field and pot experiments were conducted, where plants were subjected to 100% and 50% water supply conditions. The application of Inoculum 1 (C09EX – *Pseudomonas* sp., Ps 150AB *Pseudomonas* sp.) and Inoculum 2 (JAFGU – *Lysobacter* sp.) were applied to evaluate their potential to enhance plant growth and resilience under these conditions.

A full irrigation regime (100% water supply) significantly increased fruit yield per plant in both cultivars compared to a reduced irrigation regime (50% water supply). Both inoculants positively affected yield, with Inoculum 1 showing the best results under full irrigation and Inoculum 2 under reduced irrigation. Mycorrhizal colonisation of roots was significantly improved by both inoculants, with the highest colonisation levels observed in plants treated with Inoculum 2. Photosynthetic efficiency parameters, such as the maximum quantum yield of PSII (F_V/F_M) and quantum efficiency of photochemical reaction in PSII (ΦPSII), declined under reduced irrigation, particularly in Honeoye, but microbial inocula mitigated these effects and enhanced performance under both regimes.

These findings suggest that microbial inoculants can alleviate the adverse effects of water stress on strawberry plants, enhancing yield and physiological performance. Future research should explore the underlying mechanisms of these interactions and evaluate the long-term benefits of microbial inocula in different environmental conditions.

Keywords: bacterial inoculants, mycorrhiza, photosynthetic efficiency, irrigation regimes

INTRODUCTION

Strawberries are one of the most popular berry-like fruits and can be widely produced in almost all regions of the world due to their delicious taste and nutritional value. The strawberry (*Fragaria* × *ananassa*) is a result of crossbreeding between two wild strawberries, *Fragaria virginiana* and *Fragaria chiloensis*.

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Although the parent species are native to the Americas, the hybrid we know today was developed in Europe using imported specimens [Morais et al. 2019].

Strawberries have a prominent position among the fruit-bearing plants in the world. Consumers prefer it because it is the first fruit to ripen in the spring when no other fruit is available, and it is one of the most profitable fruits due to its nutritional value and benefits for human health [Morais et al. 2019]. For this reason, fruits can find buyers at high prices until other fruits reach the market. The demand for marketing fresh strawberries is very high in the world market because strawberries are widely used either fresh or in processed foods, such as fruit juices, jams, jellies, ice creams, chocolates, pies, syrups, pastries, and many beverages [Sahana et al. 2020, Sharma et al. 2023, Azam et al. 2019]. According to Food and Agriculture Organization [FAO 2023] data, in 2022, world strawberry production was 10,2 million tons. While China ranks first with 4 million tons of production, the USA ranks second with 1,3 million tons, and Turkey ranks third with 728 thousand tons. Poland holds the eighth position in this ranking with 199 thousand tons. In terms of cultivation area, Poland ranks third with 31.3 thousand hectares [FAO 2023].

Several challenges occur in strawberry cultivation in Poland. Among the most significant are climatic constraints, such as drought, which adversely affect both plant growth and fruit quality. Additionally, the Polish climate is highly variable, with unpredictable winter and spring conditions. Late frosts can damage strawberry flowers and newly formed fruit, impacting yield and quality.

In light of these challenges, recent studies emphasize the potential of combining microbial inoculants with different irrigation regimes as a strategy to enhance strawberry physiology and productivity. Chiomento et al. [2024] demonstrated that combinations of mycorrhizal fungi (AMF), *Trichoderma harzianum*, and *Ascophyllum nodosum* improved strawberry fruit weight and synchrony under variable water regimes. Similarly, Haghshenas et al. [2024] showed that inoculated strawberry plants cultivated in nutrient-limited substrates exhibited increased root colonization and enhanced yield parameters.

The beneficial effects of microbial inoculants have also been documented in other horticultural crops. In organic sweet pepper systems, AMF inoculation combined with drip irrigation led to improved soil microbial activity and plant health [Jamiołkowska et al. 2020]. Likewise, Chen et al. [2017] observed that cucumber seedlings inoculated with consortia of AMF exhibited superior photosynthetic performance, biomass accumulation, and phosphorus uptake.

Further support comes from a review by Shahrajabian et al. [2023], who compiled successful applications of microbial biostimulants across various horticultural crops, including tomato and pepper, highlighting their role in improving plant productivity and stress tolerance. In strawberries, Todeschini et al. [2018] reported that beneficial microbes enhanced fruit yield, nutritional value, and photosynthetic pigment composition. Moreover, Cecatto et al. [2016] linked increased AMF colonization with elevated levels of phytochemicals and enhanced antioxidant capacity in strawberry fruit.

The results obtained by Pérez-Moncada et al. [2024] provided additional field-based evidence that the integration of AMF and *Bacillus* spp. contributes to improved fruit development, chlorophyll biosynthesis, and plant tolerance to water stress in strawberry cultivation under semi-controlled conditions.

The application of endophytic bacteria in strawberry production has garnered interest. Mei et al. [2021] explored the potential of endophytic bacteria in improving strawberry growth and yield. Endophytes are bacteria that reside within plant tissues without causing harm, and their presence has been associated with enhanced plant growth, nutrient uptake, and disease resistance. These researchers demonstrated that certain endophytic bacteria positively influenced strawberry growth parameters and exhibited potential as biocontrol agents against *Botrytis cinerea*, a common fungal pathogen affecting strawberries.

Water deficit is a significant challenge in strawberry production, and researchers have investigated the potential of bacterial inoculants in mitigating its negative effects. Paliwoda et al. [2022] studied the effects of rhizosphere bacteria on strawberry plants under water deficit conditions. The results showed that certain bacterial strains enhanced plant growth, photosynthetic efficiency, and antioxidant enzyme activities, thus improving the ability of plants to withstand water stress.

Furthermore, the combined effects of bacterial inoculants with other agricultural practices have been investigated. Oregel-Zamudio et al. [2017] explored the impact of candelilla wax edible coatings combined with biocontrol bacteria on strawberry quality during shelf-life. Findings from the study indicated that the treatments improved the postharvest quality and extended the shelf-life of strawberries by reducing decay and maintaining fruit firmness.

Understanding the interactions between bacterial inoculants and strawberry plants is crucial for developing sustainable agricultural practices. Further research is needed to unravel the specific mechanisms by which bacterial inoculants promote strawberry growth and to optimise their application methods. By exploiting the potential of bacterial inoculants, strawberry growers can enhance crop productivity, reduce reliance on chemical inputs, and contribute to more sustainable and environmentally friendly strawberry cultivation.

The objective of the current study was to evaluate the effects of bacterial inoculants on the yield, mycorrhizal colonisation, and photosynthetic efficiency of strawberry plants in field and pot cultivation under different irrigation regimes. By examining the results of studies conducted in the field, valuable insights can be gained into the mechanisms underlying the positive effects of bacterial inoculation and their potential applications in strawberry production.

MATERIALS AND METHODS

Field and pot experiments on strawberry plants of Rumba and Honeoye cultivars were carried out in 2021, at the National Institute of Horticultural Research in Skierniewice in Ecological Experimental Field (Central Poland, latitude 51.914210 N, longitude 20.111524 E, 128 meters above sea level).

For the field experiment, due to ecological cultivation and the elimination of chemical weed control methods, the plants were planted on agrotextile (50 cm wide in experimental rows) and irrigated using a drip system. The experiment was conducted in four replicates, with each replicate including 75 plants. Strawberry plants were purchased from a licensed nursery and were planted in the field during the third decade of October 2018.

In the pot experiment, strawberry plants were planted in vases also during the third decade of October 2018 filled with podzolic soil, each 40 cm in diameter. Each vase was inserted in the soil of the Ecological Experimental Field and 25 cm from each other. The pot experiment was planned in seven replications (vases), each containing three plants**.**

In the first half of May of 2019, 2020, and 2021, a certified organic fertiliser, Bioilsa 6-5-13 (NPK), was applied as a soil application at a dose of 9 g/plant (350 kg/ha).

The experiment consisted of two blocks irrigated by drip technique; under one of them, the plants received the full dose of water (100% irrigated), and under the second block, the plants received irrigation with 50% water supply. The water requirements were adjusted according to the indications of tensiometers installed in the root zone. Irrigation was scheduled each morning at 07:00 h based on soil-water tension recorded by tensiometers in the open-field strawberry crop. When the tensiometer reading reached 30 cbar (≈ 30 kPa), the irrigation valve in the 100% block was opened fully, and in the 50% block it was opened to exactly half its maximum flow rate, thereby delivering 100 % and 50% of the target water supply, respectively. During the flowering and fruiting phases, each irrigation event lasted 30 minutes; in all other developmental stages, it lasted 20 minutes. This daily, valve-based adjustment maintained the intended water regimes under open-field conditions. Each block comprised the same three treatments. All treatments under each block were repeated four times.

– Control plants treated with organic fertiliser Bioilsa 6-5-13 (9 g/plant) as a soil application.
– Plants treated with Inoculum 1 of microorganisms with 9 g/plant of Bioilsa 6-5-13.
– Plants treated with Inoculum 2 with 9 g/plant of Bioilsa 6-5-13.

Bacterial strains used in inoculants

For the experiments, three non-rhizosphere strains of bacteria were selected and included in two different inocula.

I. Inoculum 1: strains:

– C10C09 – *Pseudomonas* sp. strain isolated from non-rhizosphere soil under an apple tree in Rowiska, Poland (Fig. 1),

– AF70AC – *Pseudomonas* sp., strain isolated from non-rhizosphere soil under an apple tree in the Pieprzowe Mountains, Poland (Fig. 2).

Pseudomonas strains exhibit plant growth-promoting potential through mechanisms such as siderophore production and calcium phosphate solubilisation. Additionally, these bacterial strains demonstrate antagonistic activity against *Verticillium*, *Fusarium*, *Botrytis*, and *Colletotrichum*.

II. Inoculum 2: strain JAFGU – *Lysobacter* sp.; strain isolated from non-rhizosphere soil in a strawberry cultivation field, Skierniewice, Poland.

Lysobacter sp. bacterial strain exhibits antagonistic properties against *Verticillium*, *Fusarium*, *Botrytis*, and *Colletotrichum*.

The bacteria chosen for the study produced secondary metabolites toxic to *Verticillium*, *Fusarium*, *Botrytis* and *Colletotrichum*. Additionally, the AF70AC and C10C09 produced the siderophores and were able to dissolve calcium phosphate in *in vitro* conditions. The strains from inoculum 1 did not have antagonistic activity toward each other.

The composition of the medium per litre for culturing bacterial strains consisted of 0.25 g glucose, 0.3 g soy peptone, 1.7 g casein peptone, 0.5 g NaCl and 0.25 g K_2HPO_4.

The bacteria were identified by sequencing the gene encoding the 16S rRNA subunit. The GeneMatrix Bacterial & Yeast Genomic DNA Purification Kit (EURx) was used to isolate DNA, and the 16S rRNA gene was amplified using the primer pair 27F/1492R [Lane 1991]. The obtained DNA sequences were compared to NCBI data using the BLAST tool (National Center for Biotechnology Information, https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

All strains used in this study are preserved in a sterile solution consisting of glycerol (30%), peptone (0.5%), yeast extract (0.3%), and distilled water (69.2%) and stored at –80 °C in SYMBIO-BANK microorganism collection maintained by the National Institute of Horticultural Research, Department of Microbiology and Rhizosphere, Skierniewice, Poland.

Microbial inoculants were applied in 2019, 2020 and 2021 to the soil under the plants in strawberry cultivation using tractor-mounted sprayers equipped with 'dropleg' lances. The mounting of the "dropleg" lances allows for the adjustment of their position to match the row spacing of the crops and their tilt from the vertical according to the plant size. In the strawberry plantation, TF10 nozzles were used at a pressure of 3.4 bar. In the pot experiment, the microbial inoculants were applied to the soil using a backpack sprayer.

The present experiment was conducted in 2021 following two consecutive annual applications of the microbial inoculants (2019–2020). Although positive effects of microbial inoculation can be detected already in

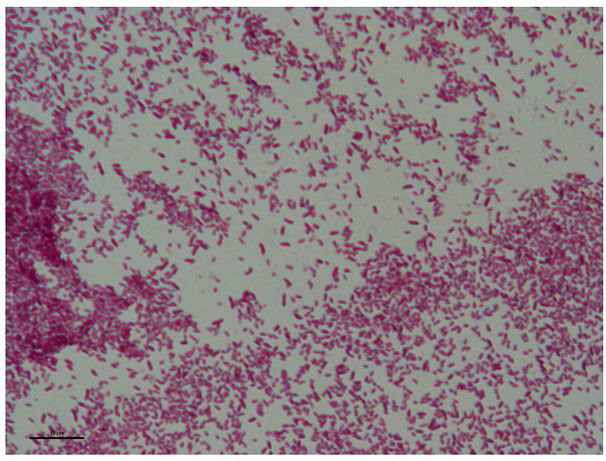


Fig. 1. Strain C10C09 – Gram staining. Material taken from a 24-hour culture (PCA medium)

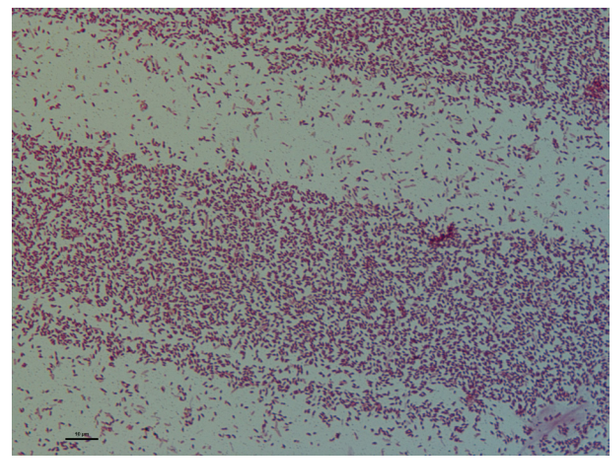


Fig. 2. Strain AF70AC – Gram staining. Material taken from a 48-hour culture (PCA medium)

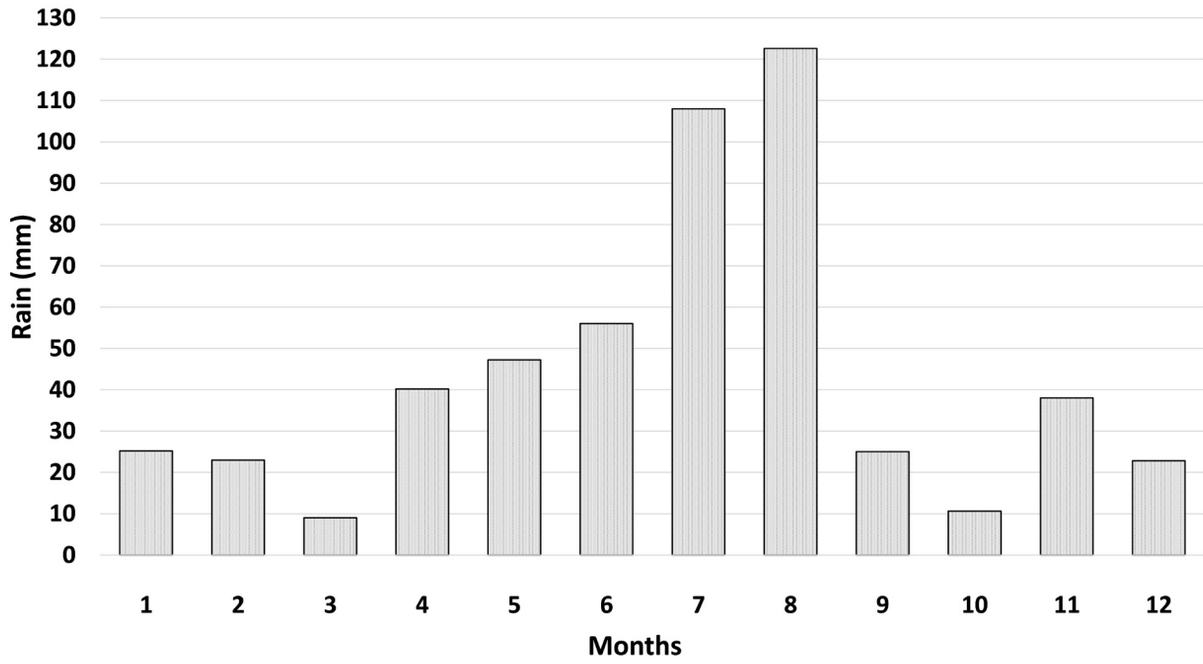


Fig. 3. Monthly precipitation at the experimental site (data from the weather station located in the Experimental Orchard)

the first year, both our investigations and international studies on orchard crops, including strawberry, demonstrate that the most pronounced and reproducible effects manifest in the second and third years of treatment.

The evaluation of strawberry plant roots for the presence of arbuscular mycorrhizal fungi growing under field and growth pot conditions

In September 2021, the root systems of Rumba and Honeoye strawberry plants were sampled from field and pot experiment (growth vases) to analyse the degree of mycorrhizal colonisation. Microbiological analyses of rhizosphere soil samples were performed on the same dates as fluorescence measurements (July and August 2021). The plants were treated with bacterial strains included in Inoculum 1 (plant growth-promoting potential) – C10C09, AF70AC (*Pseudomonas* spp.), and Inoculum 2 (antagonistic properties against pathogens) – JAFGU (*Lysobacter* sp.).

Segments of the root systems of strawberry (10 g from each replication) were collected from experiments and stained using the method developed at the Rhizosphere Laboratory of the National Institute of Horticulture Research in Skierniewice [Derkowska et al. 2015]. Subsequently, microscopic preparations were made and analysed using a Nikon 50i microscope (objectives at magnifications: 20x, 40x, 60x, 100x), and photographic documentation of observed mycorrhizal structures was conducted. The assessment of root colonisation by arbuscular mycorrhizal fungi naturally present in the soil was performed using the Trouvelot method [1986]. Based on the obtained results, mycorrhizal frequency (F%), mycorrhizal intensity (m%, M%), and arbuscule abundance (a%, A%) were calculated using the MYCOCALC computer program, available on the website: http://www2.dijon.inra.fr/mychintec/Mycocalc-prg/MYCOCALC.EXE. The following parameters were observed: F – mycorrhizal frequency, M – relative mycorrhizal intensity, m – absolute mycorrhizal intensity, a – absolute abundance of arbuscular, and A – relative abundance of arbuscules.

Evaluation of strawberry fruits yield

The fruits of the strawberry varieties Rumba and Honeoye were harvested at full maturity twice a week for three weeks. The field experiment was planned in

four replications, each comprising 75 plants. The pot experiment was planned in seven replications (vases), each containing 3 plants.

Chlorophyll a fluorescence measurements

Chlorophyll fluorescence was determined during the 2021 growing season. Measurements were carried out in July and August 2021, corresponding to the fruit development and ripening stages (generative phase) of the crop. All fluorescence readings were taken in the morning between 08:30 and 11:00 h, immediately following scheduled irrigation events; thus, soil moisture was at or very near field capacity at the time of measurement.

Chlorophyll fluorescence was measured after fruit harvest during July–August 2021 in an open-field crop. Readings were taken in the morning (08:30–11:00 h) following irrigation, when soil moisture was at field capacity.

Chlorophyll fluorescence parameters were recorded on fully expanded leaves using a portable Pulse Amplitude Modulation (PAM) Chl fluorometer (FMS-1, Hansatech Instruments Ltd., King's Lynn, Norfolk, United Kingdom). The measurements were performed in 3 replications, each containing 10 plants. For measurements one leave was taken for each plant. The measurements were taken at the same time of day. The fibre optic of the FMS-1 was positioned using the PPF/temperature leaf clip at a 60° angle from the upper surface of the leaf, and the distance between the leaf surface and the fiber optic was kept constant for all measurements. Before chlorophyll a fluorescence measurements, the leaves were dark-adapted for 30 min to obtain oxidoreduction equilibrium of PSII--PSI electron transport carriers.

The following parameters of chlorophyll fluorescence after dark adaptation were measured: F_0 – minimum fluorescence or initial fluorescence. This parameter indicates the excitation energy loss during its transmission from the energetic antennas to the PSII reaction centre, F_M – maximum fluorescence is attained when the dark-adapted sample is exposed to an intense saturating pulse of light. F_V – variable fluorescence; $F_V = F_M - F_0$. The value of this parameter depends on the maximum quantum yield of PSII.

– F_V/F_M – the maximum potential photochemical reaction efficiency in PS II,
– F_V/F_0 – the activity of PS II – the maximum efficiency of water decomposition on the donor side of PSII.

The following parameters of light-adapted leaves were measured:

– Φ_{PSII} – quantum yield of photosystem II photochemistry is directly associated with the electron transfer rate in PSII toward biochemical processes. This parameter measures the proportion of the light absorbed by PSII that is used in photochemistry and provides the rate of linear electron transport and so indicates overall photosynthesis,
– qP – photochemical quenching,
– qNP – non-photochemical quenching,
– ETR – electron flow rate through photosystems,
– Rfd – vitality index. A measure of potential photosynthetic activity under given light conditions and the interaction of the light-phase reaction with biochemical reactions in the dark phase of photosynthesis.

Statistical analyses

The experiments for determining strawberry fruit yield in the field experiment, the presence of arbuscular mycorrhizal fungi and chlorophyll fluorescence, were performed in four replications. The least significant differences (LSD) were calculated at the level of $p = 0.05$ for all experimental data. The results were statistically analysed by one-way analysis of variance in a random block design. Multiple comparisons of means for the combinations were performed with Tukey's test at a significance level of $\alpha = 0.05$ using STATISTICA v.13.3 software [TIBCO Software Inc., 2017].

RESULTS

Fruit yield of strawberry

The obtained results showed that irrigation with 100% supply of strawberry plants grown in the field significantly increased the fruit yield per plant in cultivars Rumba and Honeoye in comparison to irrigation with 50% water supply (Fig. 4). Additionally, the application of Inoculum 1 or 2 significantly increased the yield of the strawberry plant. The most beneficial effects were observed after the applications of Inoculum 1 and in Rumba plants grown under 100% water supply. Due to such treatments, the strawberry fruit

yield per plant increased by 8% compared to control plants. Under 50% water supply conditions, the most profitable result was noted after the treatment of Inoculum 2 with antagonistic properties against pathogens. Due to such treatment, the fruit yield increased by 4% compared to control. In the case of the cultivar Honeoye, the application of Inoculum 1 and 2 showed a tendency to increase the fruit yield of plants grown under irrigation with 100% water supply.

In the pot experiments, the yield of strawberry fruits of cultivars Rumba and Honeoye also depended on the irrigated regime (Fig. 5). Plants grown in fully irrigated (100% water supply) pots yielded much higher than plants irrigated with 50% water supply. Under the conditions of 100% water supply, the application of Inoculum 2 was the most advantageous compared to control. It concerned both Rumba and Honeoye plants. However, plants grown in conditions with a 50% water supply yielded significantly higher after Inoculum 1 and 2 applications than those grown in the control.

The effects of bacterial inoculants on the presence of arbuscular mycorrhizal fungi growing in different irrigation regimes

The laboratory analyses of Rumba strawberry plants conducted in the field experiment showed that the roots treated with Inoculum 2 and grown under conditions of 50% of water supply were the most frequently and intensely colonised by arbuscular mycor-

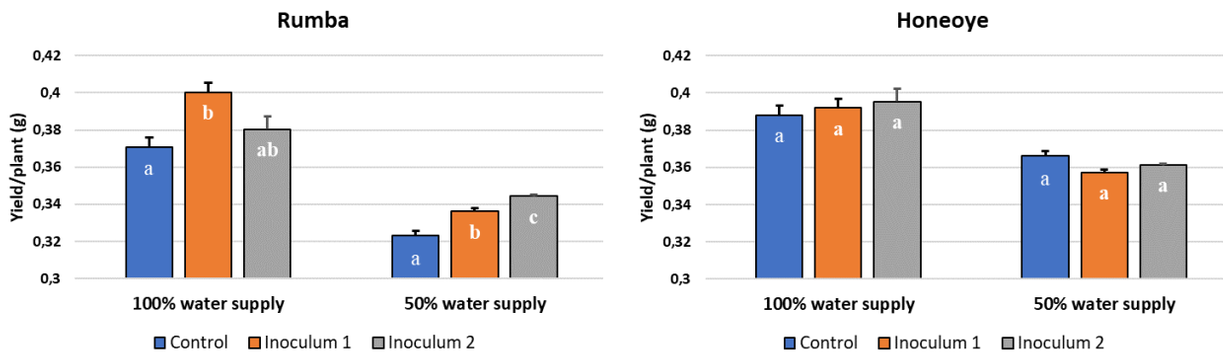


Fig. 4. In the field experiment (Ecological Experimental Field), the effect of microbiological inocula on the yield of Rumba and Honeoye cultivars of strawberry plants. The data within the variety and the irrigation regime, as well as with the same letter, do not differ significantly according to Tukey's test (5%). Values are the means of four replications, each comprising 75 plants

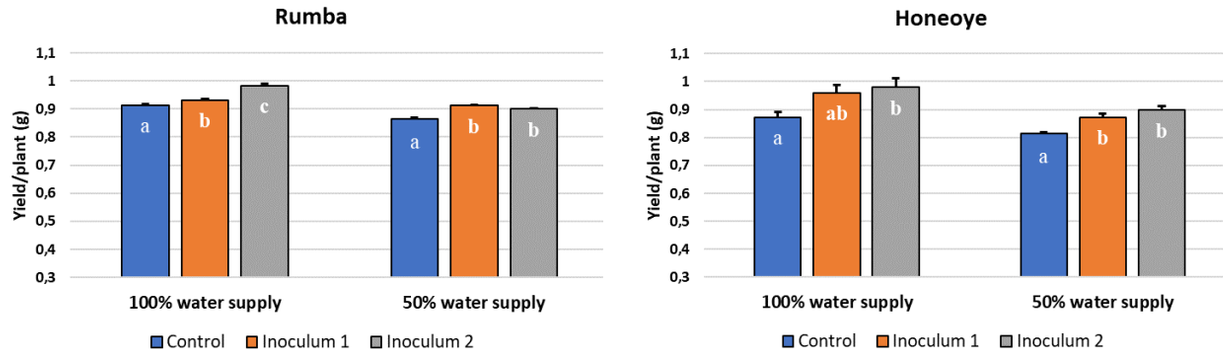


Fig. 5. In the pot experiment, the effect of microbiological inocula on the yield of Rumba and Honeoye cultivars of strawberry plants. The data within the variety and the irrigation regime, as well as with the same letter, do not differ significantly according to Tukey's test (5%). Values are the means of four replications, each comprising 75 plants

rhizal fungi (AMF – 62.22%) – Table 1, Figs 6–9. The application of Inoculum 1 also resulted in increased root colonisation by mycorrhizal fungi (52.22%). Similar beneficial results were also observed, but to a lesser extent, under 100% of water supply. After the application of Inoculum 2 and 1, the degree of mycorrhizal frequency (F%) increased to 51.11 and 42.22%, respectively.

As a result of the conducted laboratory analyses, it was observed – Figures 6–9.

Similar results were obtained in the case of Honeoye plants grown in the pot experiment (Table 2). The most pronounced effect was the application of Inoculum 2 with antagonistic properties against pathogens, both under 100% and 50% water supply. Due to such treatment, the colonisation of roots by arbuscular mycorrhizal fungi increased to 65.56 and 64.44%, respectively. The slight difference between the two conditions of water regimes (100% and 50% water supply) suggests that water supply did not affect the colonisation of roots by the arbuscular mycorrhizal fungi. Inoculum 2 had the most significant impact on mycorrhizal intensity in the roots of strawberry plants of the Honeoye variety. The application of Inoculum 1 also remarkably improved this parameter under 100% and 50% water supply. Due to such treatment, the colonisation of roots by arbuscular mycorrhizal fungi increased to 52.22 and 53.33%, respectively.

Table 1. The effect of treating strawberry plants of Rumba variety with microbial inocula, grown under various water conditions, on the degree of mycorrhizal frequency (F%), mycorrhizal intensity (m%, M%), and arbuscular abundance (a%, A%) in the field experiment. The data within the column and with the same letter, do not differ significantly according to Tukey's test (5%)

Treatment	100% water					50% water				
	F%	M%	m%	a%	A%	F%	M%	m%	a%	A%
Control	27.78a	2.33a	8.39a	0	0	35.56ab	2.22ab	6.23a	0	0
Inoculum 1	42.22b	3.02a	7.17a	0	0	52.22c	3.34ab	6.34a	0	0
Inoculum 2	51.11c	3.71ab	7.29a	0	0	62.22d	4.74b	7.59a	0	0

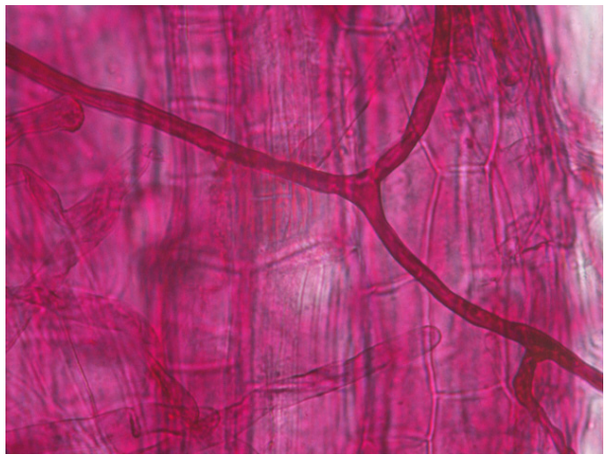


Fig. 6. Mycorrhizal hyphae in the roots of Rumba strawberry plants grown under 100% water supply conditions and treated with Inoculum 2 (field experiment)

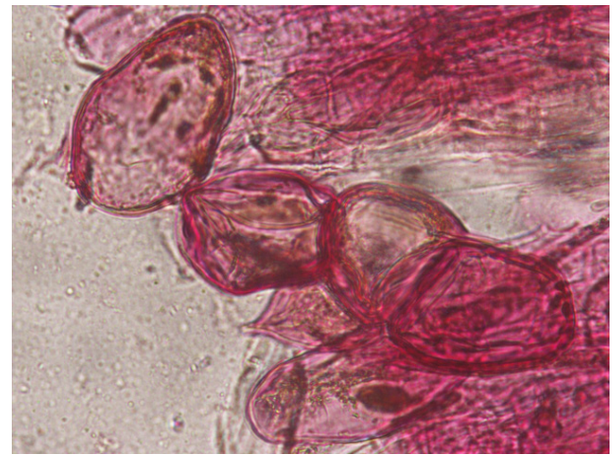


Fig. 7. Vesicle in the roots of Rumba strawberry plants grown under 100% water supply conditions and treated with Inoculum 2 (field experiment)

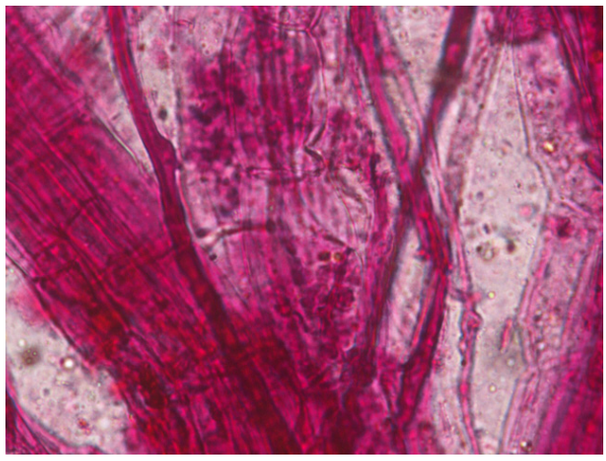


Fig. 8. Mycorrhizal hyphae in the roots of Rumba strawberry plants grown under 50% water supply and treated with Inoculum 2 (field experiment)

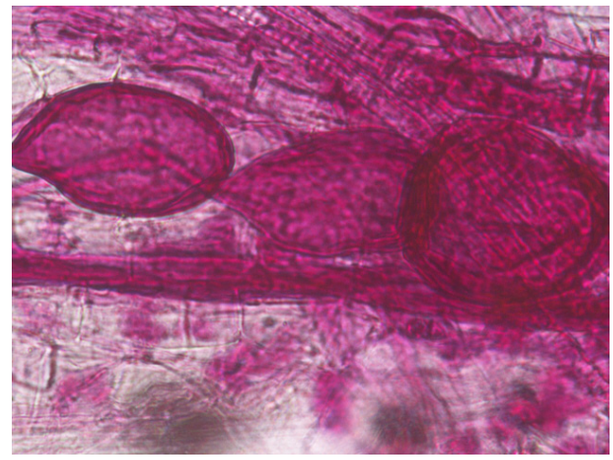


Fig. 9. Vesicles in the roots of Rumba strawberry plants grown under 50% water supply and treated with Inoculum 2 (field experiment)

Table 2. The influence of treating strawberry plants of the Honeoye variety with microbial inocula, grown under various water conditions, on the degree of mycorrhizal frequency (F%), mycorrhizal intensity (m%, M%), and arbuscular abundance (a%, A%) in the field experiment. The data within the column and with the same letter, do not differ significantly according to Tukey's test (5%)

Treatment	100% water			50% water		
	F%	M%	m%	F%	M%	m%
Control	36.67a	3.43a	9.31a	34.45a	2.68a	7.81a
Inoculum 1	52.22b	4.50a	8.59a	53.33b	3.68a	6.90a
Inoculum 2	65.56c	4.67a	7.10a	64.44c	4.33a	6.67a

As a result of the conducted laboratory analyses, it was observed – Figures 10–13.

The laboratory results obtained from the pot experiment with Rumba demonstrated the highest abundance of arbuscula in the root cells due to the application of Inoculum 1 (Table 3). After treatment with these inocula, the abundance of arbuscula in the root cells increased both under 100% and 50% water supply to 40,96 and 29,09%, respectively. Additionally, the application of Inoculum 2 increased the degree of mycorrhizal association in plant roots both under 100% and 50% water supply. As a result of water restriction, the application of Inoculum 2 increased the degree of mycorrhizal association in their roots. Under the influence of Inoculum 1 application, Rumba strawberry plant roots formed arbuscules with the highest abundance.

Similar results were obtained in the case of Honeoye plants grown in the pot experiment (Table 4, Figs 10–13). Inoculum 2 significantly increased the colonisation of roots by arbuscular mycorrhizal fungi. The applied plant watering method did not influence the obtained results. The enhancement of AMF colonisation and abundance of arbuscules in Rumba and Honeoye strawberries was observed particularly with Inoculum 2 under examined water regimes.

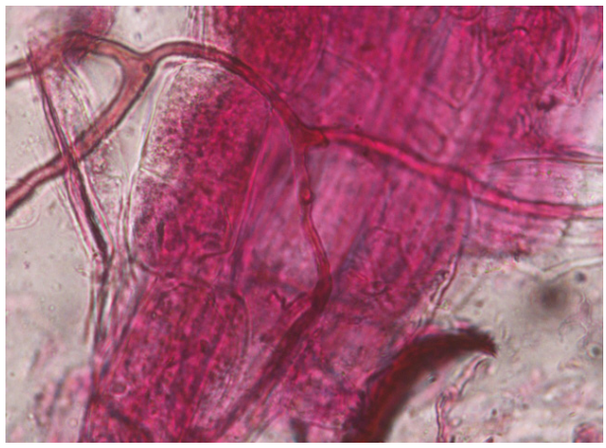


Fig. 10. Mycorrhizal hyphae in the roots of strawberry plants of Honeoye treated with Inoculum 2, grown under 100% water supply conditions (pot experiment)

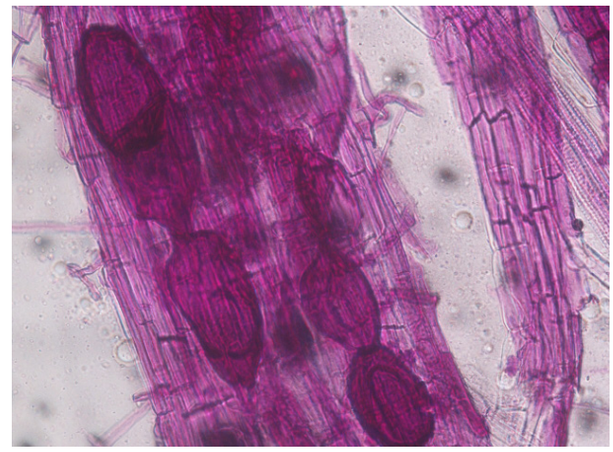


Fig. 11. Vesicles in the roots of strawberry plants of Honeoye treated with Inoculum 2, grown under 100% water supply conditions (pot experiment)

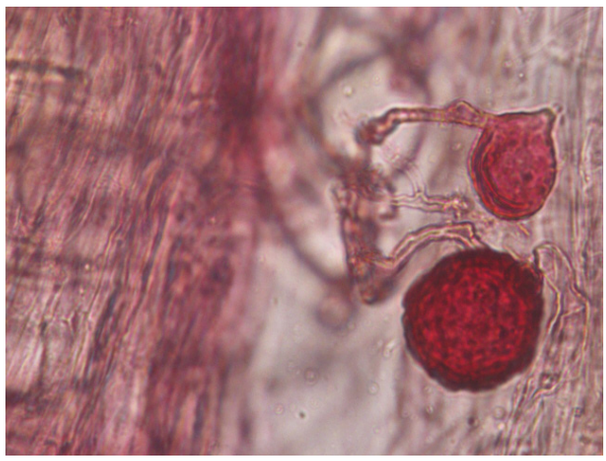


Fig. 12. Spores in the roots of strawberry plants of Honeoye variety treated with Inoculum 2, grown under 50% water supply conditions (pot experiment)

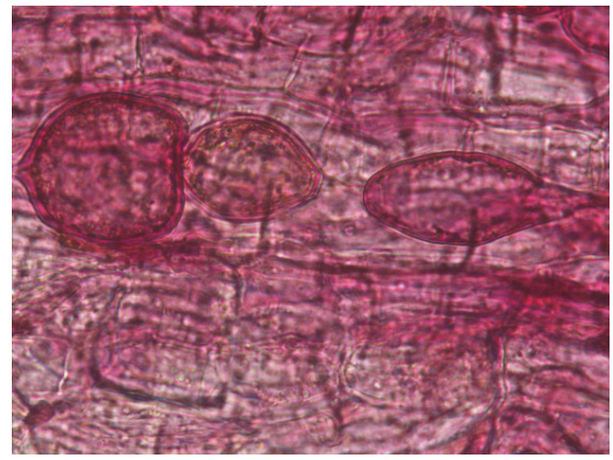


Fig. 13. Vesicles in the roots of strawberry plants of Honeoye variety treated with Inoculum 2, grown under 50% water supply conditions (pot experiment)

Table 3. Effect of treating Rumba variety strawberry plants with inocula of microorganisms growing in different water conditions on the presence of arbuscular mycorrhizal fungi in the roots in the pot experiment. The data within the column and with the same letter, do not differ significantly according to Tukey's test (5%)

Treatment	100% water					50% water				
	F%	M%	m%	a%	A%	F%	M%	m%	a%	A%
Control	71.11a	6.24a	8.95a	34.05ab	2.23a	50.0a	4.48a	8.81a	14.19a	0.75a
Inoculum 1	68.69a	6.91a	10.01a	40.96b	2.90a	58.89a	4.98a	8.34a	29.09ab	1.45a
Inoculum 2	66.67a	6.25a	9.28a	39.0b	2.56a	66.67a	5.56a	8.43a	20.63ab	1.15a

Table 4. Effect of treating Honeoye variety strawberry plants with inocula of microorganisms growing in different water conditions on the presence of arbuscular mycorrhizal fungi in the roots in the pot experiment. The data within the column and with the same letter, do not differ significantly according to Tukey's test (5%)

Treatment	100% water					50% water				
	F%	M%	m%	a%	A%	F%	M%	m%	a%	A%
Control	47.78a	3.77a	7.87a	9.70a	0.39a	47.78a	3.21a	6.63a	12.66a	0.39a
Inoculum 1	61.11bc	4.81ab	7.86a	30.62ab	1.48a	60.0ab	4.43ab	7.37a	16.78a	0.73a
Inoculum 2	78.89 c	7.65 b	9.69a	55.88 b	4.23 b	68.89bc	6.18ab	8.69a	37.94ab	2.48ab

The effects of bacterial inoculants application on the photosynthesis efficiency in strawberry leaves

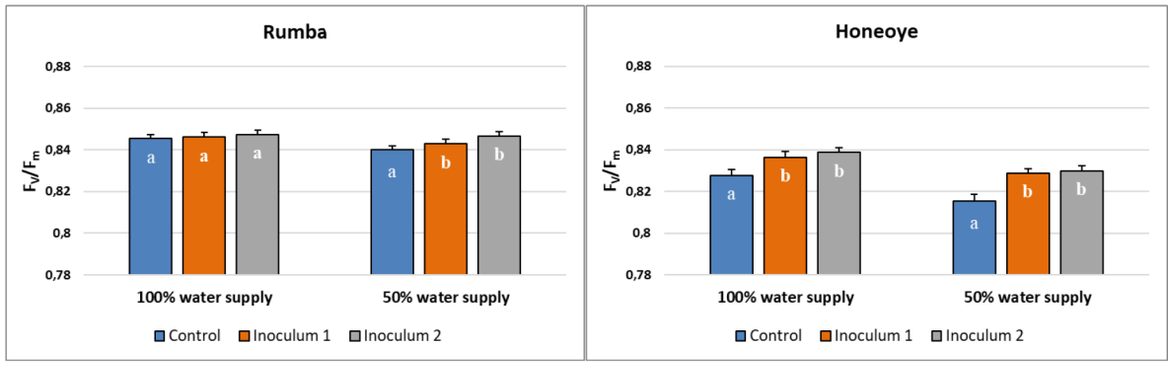
In the field experiment with strawberry plants, the irrigation in 50% water supply negatively affected the maximum quantum yield of PSII (F_V/F_M) and quantum efficiency of photochemical reaction in PSII (Φ_{PSII}), photochemical quenching (qP) and the vitality index compared to plants irrigated in 100% supply (RDF) – Fig. 14. The most harmful effect of reduced irrigation was observed in Honeoye cultivar, which produced a significant decrease in F_V/F_M, ΦPSII, qP and RDF.

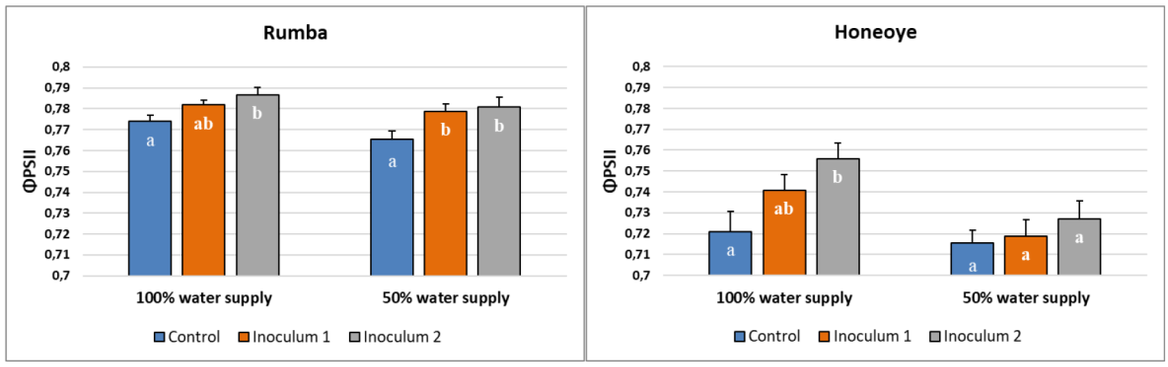
The application of Inoculum 1 and 2 positively affected Honeoye plants in both irrigated with 100% and 50% water supply plots. Its beneficial effects were visible in regard to the maximum efficiency of photochemical reaction (F_v/F_m), the quantum efficiency of photochemical reaction in PSII (Φ_{PSII}) and the maximum efficiency of water decomposition in PSII (F_V/F_0). In Rumba plants, the beneficial effect of both inocula applications was visible in irrigated in 50% water supply plots. Due to such treatments, the maximum quantum yield of PSII (F_V/F_M) and the maximum efficiency of water decomposition in PSII (F_V/F_0) were improved.

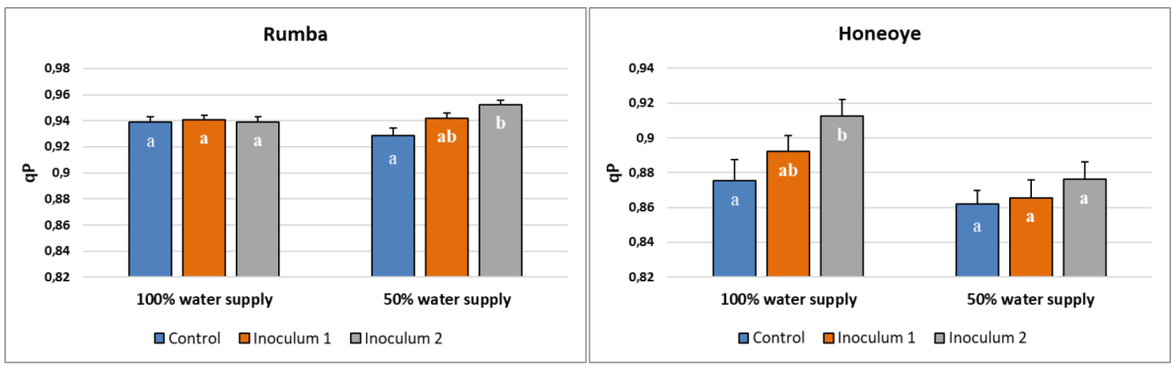
In the pot experiment, the irrigation regimes did not affect the parameters of fluorescence activity in leaves (RDF) – Fig. 15. However, the application of inocula positively affected the photosynthesis efficiency in both Rumba and Honeoye strawberry plants. Plant treatments with Inoculum 1, in most cases, stimulated the maximum efficiency of photochemical reaction (F_V/F_M), quantum efficiency of photochemical reaction in PSII (Φ_{PSII}), photochemical quenching (qP), electron flow rate through photosystems (ETR), maximum efficiency of water decomposition in PSII (F_V/F_0) and the vitality index (Rfd). In the case of 50% water supply, the best effect was observed after Inoculum 2. Especially in Honeoye plants. Due to such treatment, maximum efficiency of photochemical reaction (F_V/F_M), quantum efficiency of photochemical reaction in PSII (Φ_{PSII}), photochemical quenching (qP), maximum efficiency of water decomposition in PSII (F_V/F_0) and the vitality index (Rfd).

DISCUSSION

The widespread occurrence of drought stress poses a significant threat to crop yields worldwide, leading to a substantial decrease in the productivity of many crops due to water scarcity [Song et al. 2023, Mazurek-Kusiak et al. 2021]. Moreover, drought stands out as one of the most significant environmental factors limiting the agricultural production of strawberries globally, adversely affecting the anatomical, physiological, and enzymatic characteristics of plants [Fig, 3; Khan 2023]. In strawberry plants, reduced irrigation typically leads to smaller fruit size and lower yield due to their shallow root system, large leaf area, and succulent texture. As a result, they are highly vulnerable to water deficiency-induced damage, which ultimately reduces biomass and crop yield [Zahedi et al. 2023]. Furthermore, plants are highly susceptible to mineral deficiencies caused by inadequate soil moisture and reduced mobilisation of minerals within plant tissues [Zahedi et al. 2023]. The results obtained from the present study clearly indicated that reducing the water supply to 50% significantly decreased the fruit yield per strawberry plant in cultivars Rumba and Honeoye


Rumba
Honeoye
F_v/F_m
0,88
0,86
0,84
0,82
0,8
0,78
a
a
a
a
b
b
a
b
b
a
b
b
100% water supply
50% water supply
Control
Inoculum 1
Inoculum 2


Rumba
Honeoye
ΦPSII
0,8
0,79
0,78
0,77
0,76
0,75
0,74
0,73
0,72
0,71
0,7
a
ab
b
a
b
b
a
ab
b
a
a
a
100% water supply
50% water supply
Control
Inoculum 1
Inoculum 2


Rumba
Honeoye
qP
0,98
0,96
0,94
0,92
0,9
0,88
0,86
0,84
0,82
0,94
0,92
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0,88
0,86
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a
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b
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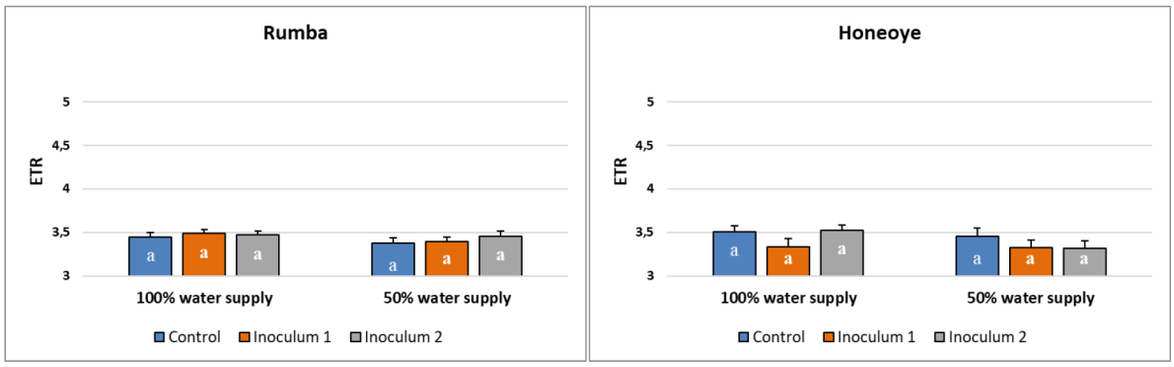
a
100% water supply
50% water supply
Control
Inoculum 1
Inoculum 2


Rumba
Honeoye
ETR
5
4,5
4
3,5
3
a
a
a
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a
a
a
a
100% water supply
50% water supply
Control
Inoculum 1
Inoculum 2

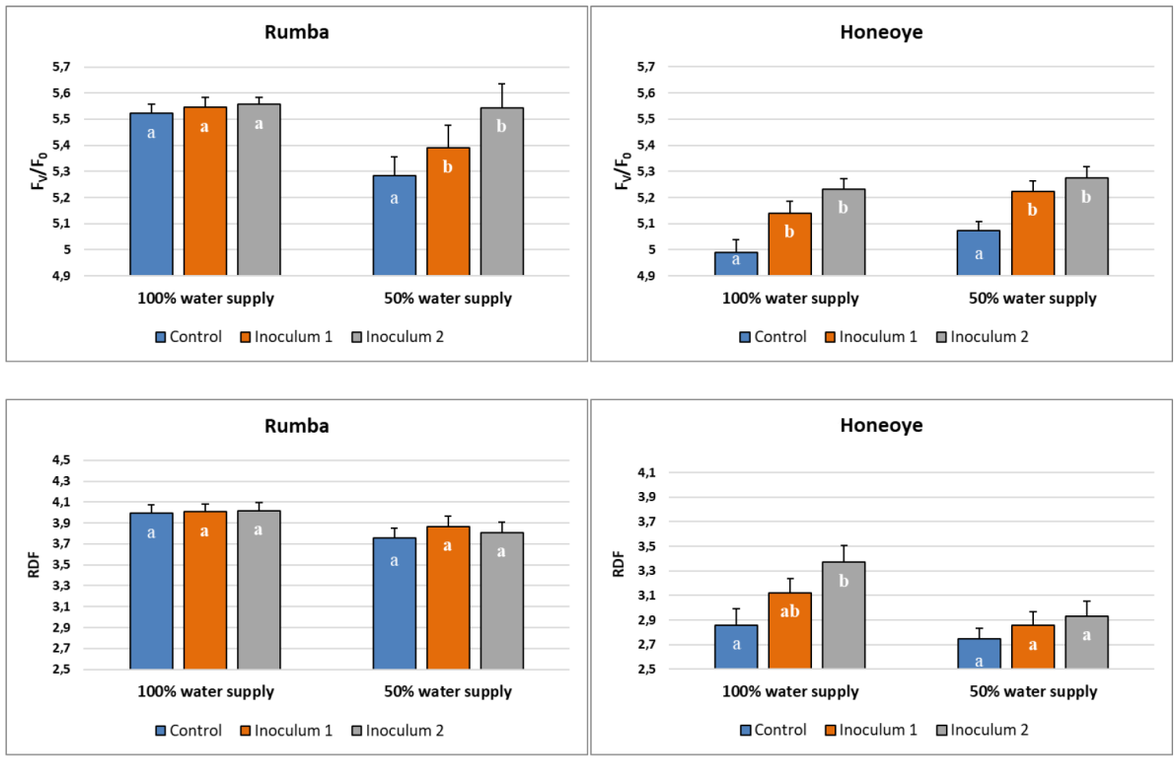
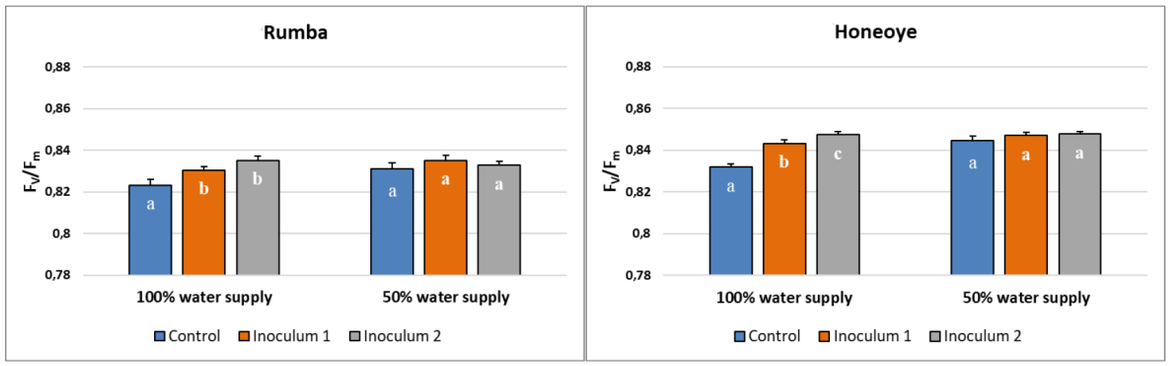
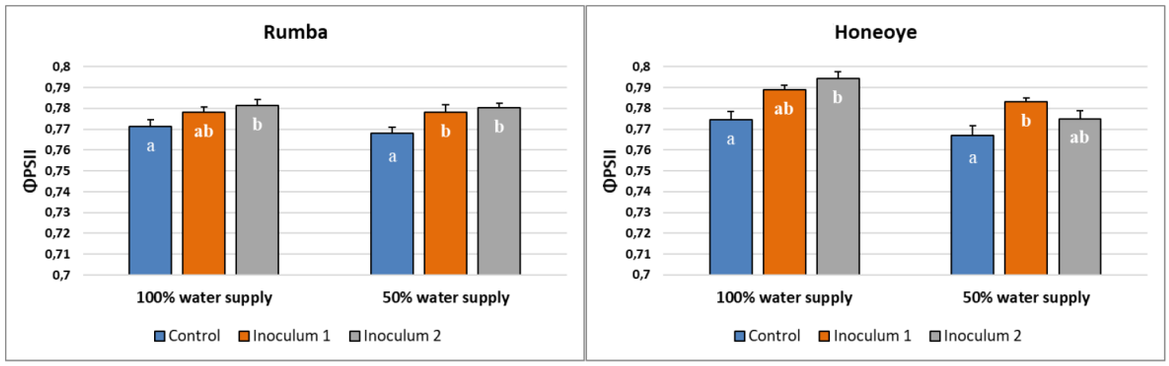


Fig. 14. The effect of microbiological inocula application in the field experiment on the photosynthesis efficiency of Rumba and Honeoye cultivars of strawberry plants in the field experiment. Maximum efficiency of photochemical reaction (F_V/F_M), quantum efficiency of photochemical reaction in PSII (Φ_{PSII}), photochemical quenching (qP), non-photochemical quenching (qNP), electron flow rate through photosystems (ETR), maximum efficiency of water decomposition in PSII (F_V/F_0) and the vitality index (Rfd). The data within the variety and with the same letter do not differ significantly according to Tukey's test (5%). Values are the means of four replications, each comprising 75 plants

compared to irrigation with 100% water supply. Furthermore, the diminishing water supply also caused a decrease in photosynthesis efficiency expressed by fluorescence activity in leaves. This was observed both in the field and pot conditions.

However, the present study showed that the application of Inoculum 1 (C09EX – *Pseudomonas* sp., Ps150AB *Pseudomonas* sp.) or 2 (JAFGU – *Lysobacter* sp.) significantly increased the fruit yield of strawberry plants. The most beneficial effects were observed after applying Inoculum 1 to Rumba plants grown under 100% water supply. Under 50% water supply conditions, the most profitable outcome was observed following treatment by Inoculum 2. Applying Inoculum 1 and 2 increased fruit yield in Honeoye cultivar plants grown with 100% water supply. Previous research also showed that the treatments of strawberry roots with SP116AC and JaFGU (*Lysobacter* sp.) resulted in a significant increase in the total leaf surface, the total length of roots, and their total surface area [Trzciński et al. 2021]. In pot experiments, the application of Inoculum 2 was beneficial under full irrigation, while the applications of Inoculum 1 and 2 increased yields similarly under 50% irrigation. Wang et al. [2024] also reported that *Pseudomonas fluorescens* could enhance biofilm formation and rhizosphere colonisation, which was probably essential in promoting strawberry growth.


Rumba
Honeoye
F_v/F_m
0,88
0,86
0,84
0,82
0,8
0,78
a b b
a a a
a b c
a a a
100% water supply
50% water supply
Control
Inoculum 1
Inoculum 2


Rumba
Honeoye
ΦPSII
0,8
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0,76
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0,72
0,71
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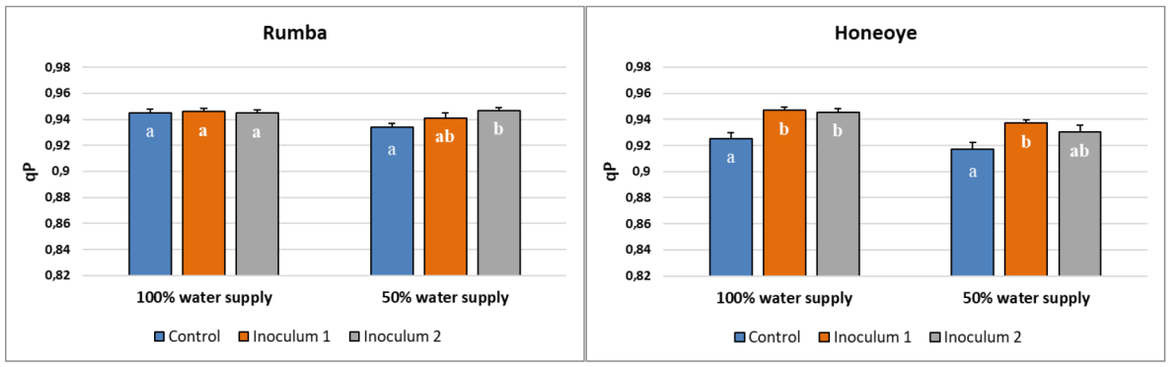
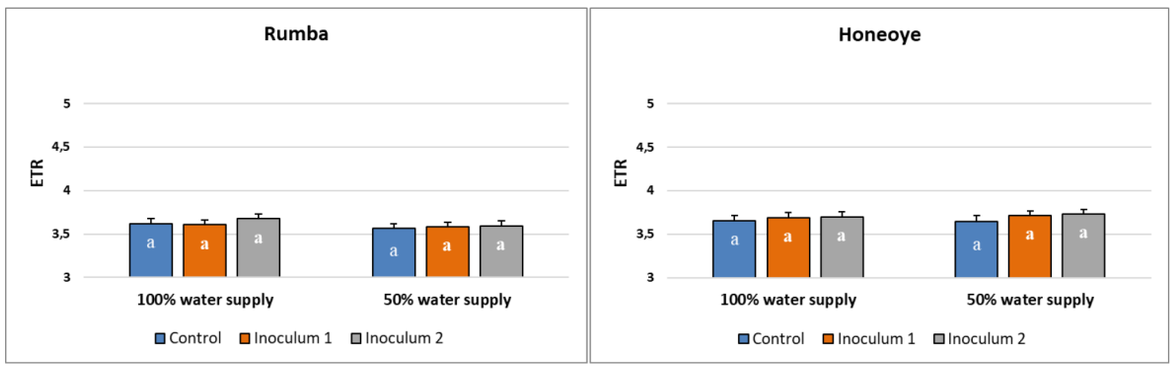
a ab b
a b b
a ab b
a b ab
100% water supply
50% water supply
Control
Inoculum 1
Inoculum 2


Rumba
Honeoye
qP
0,98
0,96
0,94
0,92
0,9
0,88
0,86
0,84
0,82
a a a
a ab b
a b b
a b ab
100% water supply
50% water supply
Control
Inoculum 1
Inoculum 2


Rumba
Honeoye
ETR
5
4,5
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a a a
100% water supply
50% water supply
Control
Inoculum 1
Inoculum 2

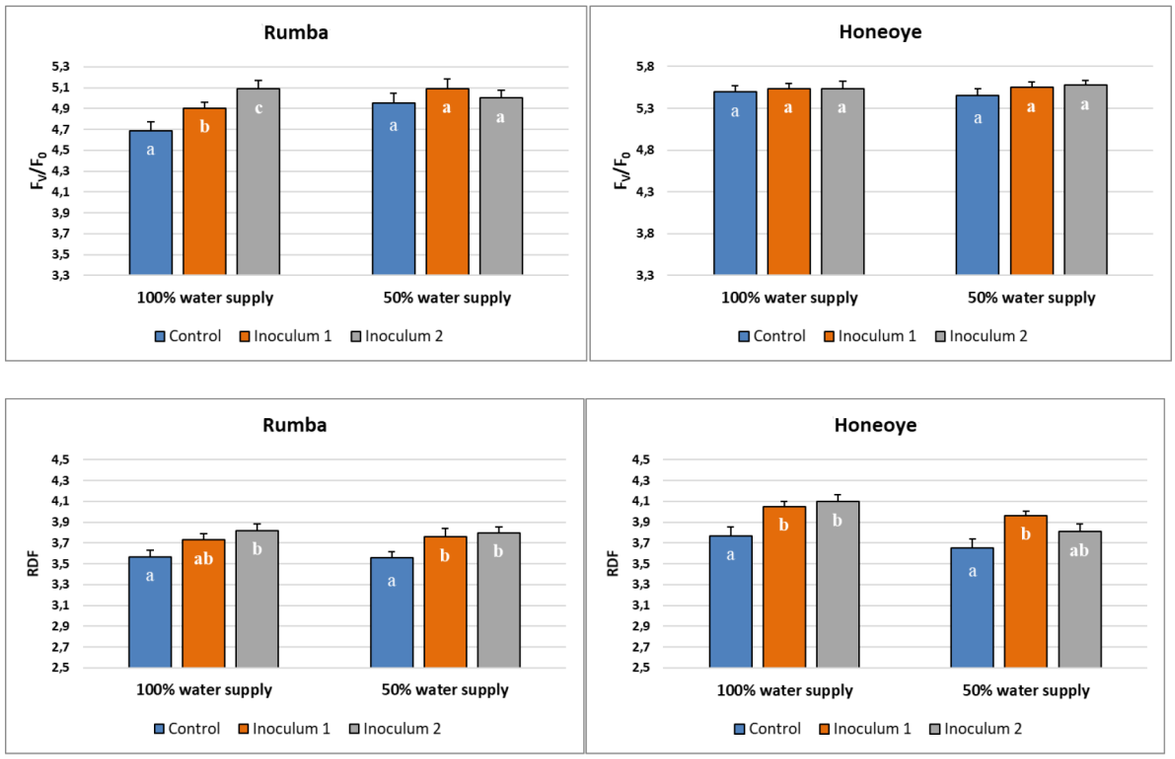

Fig. 15. The effect of microbiological inocula on photosynthesis efficiency on the basis of fluorescence activity in leaves of Honeoye cultivars in the pot experiment. Maximum efficiency of photochemical reaction (F_V/F_M), quantum efficiency of photochemical reaction in PSII (Φ_{PSII}), photochemical quenching (qP), non-photochemical quenching (qNP), electron flow rate through photosystems (ETR), maximum efficiency of water decomposition in PSII (F_V/F_0) and the vitality index (Rfd). The data within the variety and with the same letter do not differ significantly according to Tukey's test (5%). Values are the means of 7 replications, each comprising 3 plants

The study demonstrated the significant impact of microbial inocula on the root colonisation of Rumba and Honeoye strawberry plants. Inoculum 2, especially under reduced water supply, resulted in the highest levels of AMF colonisation in Rumba plants. This indicates that water stress might enhance mycorrhizal effectiveness. Inoculum 1 also increased AMF colonisation, though less effectively than Inoculum 2. Interestingly, no arbuscules were observed in the root segments of Rumba plants, suggesting that specific conditions may have resulted in their complete absence. Similar beneficial effects of the inoculants were noted in Honeoye plants, with Inoculum 2 showing the most pronounced impact on AMF colonisation regardless of water supply levels. The slight differences between the water regimes indicate that water availability had minimal effect on AMF colonisation in Honeoye plants. The pot experiment further supported these findings, with Inoculum 1 significantly increasing the abundance of arbuscules in Rumba roots. Both inocula improved mycorrhizal associations in Honeoye roots, with Inoculum 2 having a notable impact on mycorrhizal intensity and arbuscule abundance. These findings suggest that water stress may enhance AMF colonisation, particularly with Inoculum 2, offering strategies to improve strawberry resilience and adaptability under varying water availability. Chiomento et al. [2019, 2021] also reported that inoculation with

arbuscular mycorrhizal fungi improves strawberry growth and development, yield, fruit quality and overall performance, leading to more profuse root systems and increased fruit anthocyanin content. Similar to the findings on Rumba plants, it has been shown that water stress can enhance AMF colonisation. For instance, a study by Borowicz [2010] observed that AMF can improve water relations, thereby increasing the host plant's tolerance to drought stress. Hernández-Sebastià et al. [1999] demonstrated that AMF colonisation could improve water content in strawberry plants, leading to better water status and higher relative water content (RWC) under high humidity conditions. Hernández-Sebastià et al. [2000] found also that strawberry plantlets inoculated with AMF had distinct amino acid and starch concentration changes under water stress compared to non-mycorrhizal plants, suggesting specialised adaptation strategies. Research by Zhu et al. [2010] on maise showed that AMF symbiosis improves plant growth, water status, and photosynthetic capacity under stress conditions. The absence of arbuscules in Rumba plants under specific conditions suggests that environmental factors and the type of AMF can influence arbuscule formation. Moradtalab et al. [2019] demonstrated that both AMF and silicon could synergistically enhance strawberry plant growth under drought by increasing mycorrhizal intensity and nutrient uptake, indicating complex interactions affecting arbuscule formation. The observed enhancement of AMF colonisation in Rumba and Honeoye strawberries under different water regimes, particularly with Inoculum 2, is consistent with the broader scientific literature. Water stress appears to enhance AMF effectiveness, offering potential strategies for improving plant resilience and adaptability [Pérez-Moncada et al. 2024, Raturi et al. 2023, Silva et al. 2023].

The presented data indicated that reduced irrigation significantly impaired several parameters associated with photosynthetic efficiency in strawberry plants, especially in Honeoye cultivar. This negative impact is evident in the decreased maximum quantum yield of PSII (F_V/F_M) and quantum efficiency of photochemical reaction in PSII (Φ_{PSII}), as well as diminished photochemical quenching (qP) and vitality index (Rfd). In a study by El-Beltagi et al. [2022], drought stress (40% and 80%) led to decreased net photosynthetic rate, stomatal conductance and transpiration rate. The present findings suggest that Honeoye is particularly susceptible to water stress, which emphasises the importance of adequate irrigation for maintaining its photosynthetic performance. Conversely, the application of Inoculum 1 and Inoculum 2 showed positive effects on the photosynthetic efficiency of both Honeoye and Rumba cultivars under varying irrigation conditions. These inocula appear to mitigate the adverse effects of water deficit, as demonstrated by the improved F_V/F_M, ΦPSII, and F_V/F_0 values in treated plants. In particular, the positive impact of inocula was more evident in Honeoye plants exposed to 50% reduction in water supply, emphasising their potential to improve drought resistance. Valle-Romero et al. [2023] have found that biofertilisation with plant growth-promoting bacteria (PGPB) improves the photosynthetic efficiency of strawberry plants under water stress by increasing the net photosynthetic rate and intrinsic water use efficiency.

In the pot experiments, where irrigation regimes did not influence fluorescence parameters, the positive role of the inocula in enhancing photosynthetic efficiency was still evident. It suggests that the application of these inocula can stimulate photosynthetic efficiency regardless of irrigation conditions. Inoculum 1 consistently stimulated parameters such as F_V/F_M, ΦPSII, qP, electron flow rate (ETR), F_V/F_0, and the vitality index (Rfd) in leaves of both cultivars. The importance of arbuscular mycorrhizal fungi (AMF) in enhancing the photosynthetic efficiency of micropropagated strawberry plants under drought stress in greenhouse conditions has been emphasised [Borkowska 2002]. It was suggested that the main limitation under relatively severe conditions was stomatal closure due to photosynthetic limitations [Yokoyama et al. 2023].

Under reduced water supply conditions, Inoculum 2 appeared as especially effective in improving the photosynthetic parameters in Honeoye plants. It indicated that specific microbial inocula can offer benefits depending on the cultivar and environmental stressors. The improvement in quantum efficiency, photochemical quenching, and vitality index in Inoculum 2-treated plants suggests a potential strategy for maintaining high photosynthetic efficiency under suboptimal irrigation. This novelty of the present research includes

the identification of specific microbial inocula that improved strawberry fruit yield, AMF colonisation and photosynthetic efficiency, especially under water stress conditions, providing innovative strategies for improving crop resilience and productivity.

Further research is needed to explore the underlying mechanisms by which these inocula enhance the yield of strawberry fruit, the efficiency of photosynthesis in leaves, and arbuscular mycorrhizal fungi, especially for vulnerable varieties to water stress. The research also shows the potential of using microbial inocula to mitigate the adverse effects of reduced irrigation. Further research is needed to understand how these interventions enhance plant productivity under varying environmental conditions. These findings contribute to the knowledge of sustainable agriculture and the use of inocula to improve crop resilience to environmental stressors.

CONCLUSIONS

The presented study provides substantial evidence of the critical role that irrigation regimes and microbial inocula play in influencing the productivity, root mycorrhizal colonisation, and photosynthetic efficiency of strawberry plants. The results indicate that full irrigation (100% water supply) significantly increased fruit yield compared to reduced irrigation (50% water supply). Applying bacterial inoculants, specifically Inoculum 1 (*Pseudomonas* spp.) and Inoculum 2 (*Lysobacter* sp.), further enhances yield, with Inoculum 1 being most effective under full irrigation and Inoculum 2 under reduced irrigation. Root colonisation by arbuscular mycorrhizal fungi (AMF) was markedly improved by both inocula, particularly Inoculum 2, irrespective of the irrigation level, suggesting its potential role in enhancing plant resilience to water stress. Photosynthetic efficiency parameters, including the maximum quantum yield of PSII (F_V/F_M) and quantum efficiency of photochemical reaction in PSII (Φ_{PSII}), were significantly impaired by reduced irrigation, especially in the Honeoye cultivar. However, these adverse effects were substantially mitigated by applying microbial inocula, which improved photosynthetic performance under both irrigation regimes. These findings emphasise the potential of microbial inocula as a sustainable agricultural strategy to mitigate the adverse effects of water scarcity on strawberry plants. Future research should focus on elucidating the underlying mechanisms by which these inocula enhance plant productivity and resilience under various environmental conditions, thereby optimising their application for diverse agricultural practices.

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REFERENCES

Azam M., Ejaz S., Naveed Ur Rehman R., Khan M., Qadri R. (2019). Postharvest Quality Management of Strawberries. In: Asao T, Asaduzzaman M (eds), Strawberry. Pre-and post-harvest management techniques for higher fruit quality. IntechOpen. https://dx.doi.org/10.5772/intechopen.82341

Borkowska B. (2002). Growth and photosynthetic activity of micropropagated strawberry plants inoculated with endomycorrhizal fungi (AMF) and growing under drought stress. Acta Physiol. Plant. 24, 365–370. https://doi.org/10.1007/s11738-002-0031-7

Borowicz V.A. (2010). The impact of arbuscular mycorrhizal fungi on strawberry tolerance to root damage and drought stress. Pedobiologia 53(4), 265–270. https://doi.org/10.1016/j.pedobi.2010.01.001

Cecatto A.P., Ruiz F.M., Calvete E.O., Martínez J., Palencia P. (2016). Mycorrhizal inoculation affects the phytochemical content in strawberry fruits. Acta Sci. Agron. 38, 227–237. https://doi.org/10.4025/actasciagron.v38i2.27932

Chen S., Zhao H., Zou C., Li Y., Chen Y., Wang Z., Jiang Y., Liu A., Zhao P., Wang M., Ahammed G.J. (2017). Combined Inoculation with Multiple Arbuscular Mycorrhizal Fungi Improves Growth, Nutrient Uptake and Photosynthesis in Cucumber Seedlings. Front. Microbiol. 8, 2516. https://doi.org/10.3389/fmicb.2017.02516

Chiomento J.L.T., da Costa R.C., de Nardi F.S., Trentin N. dos S., Nienow A.A., Calvete E.O. (2019). Arbuscular mycorrhizal fungi communities improve the phytochemical quality of strawberry. J. Hortic. Sci. Biotechnol. 94(5), 653–663. https://doi.org/10.1080/14620316.2019.1599699

Chiomento J.L.T., Fracaro J., Görgen M., Fante R., Dal Pizzol E., Welter M., Klein A.P., Trentin T.S., Suzana-Milan C.S., Palencia P. (2024) Arbuscular Mycorrhizal Fungi, *Ascophyllum nodosum*, *Trichoderma harzianum*, and their combinations influence the phyllochron, phenology, and fruit quality of strawberry plants. Agronomy 14:860. doi:10.3390/agronomy14040860

Chiomento J.L.T., de Paula J.E.C., de Nardi F.S., Trentin T. dos S., Magro F.B., Dornelles A.G., Anzolin J., Fornari M., Trentin N. dos S., Rizzo L.H., Calvete E.O. (2021). Arbuscular mycorrhizal fungi influence the horticultural performance of strawberry cultivars. Res. Soc. Dev. 10(7), e45410716972. https://doi.org/10.33448/rsd-v10i7.16972

Derkowska E., Sas-Paszt L., Dyki B., Sumorok B. (2015). Assessment of mycorrhizal frequency in the roots of fruit plants using different dyes. Advances in Microbiology. 5, 54–64. http://creativecommons.org/licenses/by/4.0/

El-Beltagi H.S., Ismail S.A., Ibrahim N.M., Shehata W.F., Alkhateeb A.A., Ghazzawy H.S., El-Mogy M.M., Sayed E.G. (2022). Unravelling the effect of triacontanol in combating drought stress by improving growth, productivity, and physiological performance in strawberry plants. Plants 11, 1913. https://doi.org/10.3390/plants11151913

FAO 2023. Food and Agriculture Organization of the United Nations. Crops and livestock products. https://www.fao.org/faostat/en/#data/QCL.

Haghshenas M., van Delden S.H., Nazarideljou M.J. (2024). Effects of nutrient solution strength, PGPB, and mycorrhizal inoculation on growth, yield, and quality of strawberry. Turk. J. Agr. For. 48(3), 390–401. https://doi.org/10.55730/1300-011X.3189

Hernández-Sebastià C., Samson G., Bernier P-Y., Piché Y., Desjardins Y. (2000). Glomus intraradices causes differential changes in amino acid and starch concentrations of *in vitro* strawberry subjected to water stress. New Phytol. 148(1), 177–186. https://doi.org/10.1046/j.1469-8137.2000.00744.x

Hernández-Sebastià C., Piché Y., Desjardins Y. (1999). Water relations of whole strawberry plantlets in vitro inoculated with *Glomus intraradices* in a tripartite culture system. Plant Sci. 143(1), 81–91. https://doi.org/10.1016/S0168-9452(99)00014-X

Jamiołkowska A., Skwaryło-Bednarz B., Patkowska E., Buczkowska H., Gałązka A., Grządziel J., Kopacki M. (2020). Effect of Mycorrhizal Inoculation and Irrigation on Biological Properties of Sweet Pepper Rhizosphere in Organic Field Cultivation. Agronomy 10(11), 1693. https://doi.org/10.3390/agronomy10111693

Khan M.N. (2023). Melatonin regulates mitochondrial enzymes and ascorbate–glutathione system during plant responses to drought stress through involving endogenous calcium. S. Afr. J. Bot. 162, 622–632. https://doi.org/10.1016/j.sajb.2023.09.032

Lane D.J. (1991). 16S/23S rRNA sequencing, pp. 115–175. In: Stackebrandt E. and M. Goodfellow (eds). Nucleic acid techniques in bacterial systermatics. John Wiley & Sons Ltd, Chichester, United Kingdom.

Mazurek-Kusiak A., Sawicki B., Kobyłka A. (2021). Contemporary challenges to the organic farming: a Polish and Hungarian case study. Sustainability 13(14), 8005. https://doi.org/10.3390/su13148005

Mei C., Amaradasa B.S., Chretien R.L., Liu D., Snead G., Samtani J.B., Lowman S. (2021). A potential application of endophytic bacteria in strawberry production. Horticulturae 7(11), 504. https://doi.org/10.3390/horticulturae7110504

Moradtalab N., Hajiboland R., Aliasgharzad N., Hartmann T.E., Neumann. G. (2019). Silicon and the association with an arbuscular-mycorrhizal fungus (*Rhizophagus clarus*) mitigate the adverse effects of drought stress on strawberry. Agronomy 9(1), 41. https://doi.org/10.3390/agronomy9010041

Morais M.C., Mucha Â., Ferreira H., Gonçalves B., Bacelar E., Marques G. (2019). Comparative study of plant growth-promoting bacteria on the physiology, growth and fruit quality of strawberry. J. Sci. Food Agric. 99(12), 5341–5349. https://doi.org/10.1002/jsfa.9773

Oregel-Zamudio E., Angoa-Pérez M.V., Oyoque-Salcedo G., Aguilar-González C.N., Mena-Violante H.G. (2017). Effect of candelilla wax edible coatings combined with biocontrol bacteria on strawberry quality during the shelf-life. Sci. Hort. 214, 273–279. https://doi.org/10.1016/j.scienta.2016.11.038

Paliwoda D., Mikiciuk G., Mikiciuk M., Kisiel A., Sas-Paszt L., Miller T. (2022). Effects of rhizosphere bacteria on strawberry plants (*Fragaria* × *ananassa* duch.) under water deficit. Int. J. Mol. Sci. 23(18), 10449. https://doi.org/10.3390/ijms231810449

Pérez-Moncada U.A., Santander C., Ruiz A., Vidal C., Santos C., Cornejo P. (2024). Design of microbial consortia based on arbuscular mycorrhizal fungi, yeasts, and bacteria to improve the biochemical, nutritional, and physiological status of strawberry plants growing under water deficits. Plants 13(11), 1556. https://doi.org/10.3390/plants13111556

Raturi P., Rai R., Sharma A.K., Singh A.K., Dimri D.C., Bains G. (2023). Effects of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi

(AMF) on morpho-physiological parameters of strawberry cv. Chandler under different moisture levels. Int. J. Environ. Clim. Change. 13(9), 2707–2713. https://doi.org/10.9734/ijecc/2023/v13i92502

Redondo-Gómez S., García-López J.V., Mesa-Marín J., Pajuelo E., Rodriguez-Llorente I.D., Mateos-Naranjo E. (2022). Synergistic effect of plant-growth-promoting rhizobacteria improves strawberry growth and flowering with soil salinization and increased atmospheric CO_2 levels and temperature conditions. Agronomy 12(9), 2082. https://doi.org/10.3390/agronomy12092082

Sahana B.J., Madaiah D., Sridhara S., Pradeep S., Nithin K.M. (2020). Study on effect of organic manures on quality and biochemical traits of strawberry (*Fragaria× ananassa* Duch.) under naturally ventilated polyhouse. Int. J. Curr. Microbiol. Appl. Sci. 9 (10), 2692–2698. https://doi.org/10.20546/ijcmas.2020.910.325

Shahrajabian M.H., Petropoulos S.A., Sun W. (2023). Survey of the Influences of Microbial Biostimulants on Horticultural Crops: Case Studies and Successful Paradigms. Hortic. 9(2), 193. https://doi.org/10.3390/horticulturae9020193

Sharma K., Mirza A.A., Aarti. (2023). Micronutrient and Metabolic Profiling of Strawberry Cultivars Grown in Subtropical Conditions: A Review. Agric. Rev. 46(3), 437–443. https://doi.org/10.18805/ag.R-2642

Silva A.M.M., Feiler H.P., Qi X., Araújo V.L.V.P. de, Lacerda-Júnior G.V., Fernandes-Júnior P.I., Cardoso E.J.B.N. (2023). Impact of water shortage on soil and plant attributes in the presence of arbuscular mycorrhizal fungi from a harsh environment. Microorganisms 11(5), 1144. https://doi.org/10.3390/microorganisms11051144

Song W., Song R., Zhao Y., Zhao Y. (2023). Research on the characteristics of drought stress state based on plant stem water content. Sustain. Energy Technol. Assess. 56, 103080. https://doi.org/10.1016/j.seta.2023.103080

TIBCO Software Inc. (2017). Statistica (data analysis software system), version 13. http://statistica.io.

Todeschini V., AitLahmidi N., Mazzucco E., Marsano F., Gosetti F., Robotti E., Bona E., Massa N., Bonneau L., Marengo E., Wipf D., Berta G., Lingua G. (2018). Impact of Beneficial Microorganisms on Strawberry Growth, Fruit Production, Nutritional Quality, and Volatilome. Front. Plant Sci. 9, 1611. https://doi.org/10.3389/fpls.2018.01611

Trouvelot A., Kough J.L., Gianinazzi-Pearson V. (1986). Mesure du taux de mycorhization VA d'un systeme radi-culaire. Recherche de methods d'estimation ayant une signification fonctionnelle. In: Gianinazzi-Pearson V. and Gianinazzi, S., Eds, Physiological and Genetical Aspects of Mycorrhizae, INRA, Paris, 217–221.

Trzciński P., Frąc M., Lisek A., Przybył M., Frąc M., Sas-Paszt L. (2021). Growth promotion of raspberry and strawberry plants by bacterial inoculants. Acta Sci. Pol. Hortorum Cultus. 20(6), 71–82. https://doi.org/10.24326/asphc.2021.6.8

Valle-Romero P., García-López J.V., Redondo-Gómez S., Flores-Duarte N.J., Rodríguez-Llorente I.D., Idaszkin Y.L., Pajuelo E., Mateos-Naranjo E. (2023). Biofertilization with PGP bacteria improve strawberry plant performance under sub-optimum phosphorus fertilization. Agronomy 13(2), 335. https://doi.org/10.3390/agronomy13020335

Wang Q., Chu C., Zhao Z., Wu S., Zhou D. (2024). *Pseudomonas fluorescens* enriched by *Bacillus velezensis* containing agricultural waste promotes strawberry growth by microbial interaction in plant rhizosphere. Land Degrad. Dev. 35(7), 2476–2488. https://doi.org/10.1002/ldr.5074

Yokoyama G., Ono S., Yasutake D., Hidaka K., Hirota T. (2023). Diurnal changes in the stomatal, mesophyll, and biochemical limitations of photosynthesis in well-watered greenhouse-grown strawberries. Photosynthetica 61(1), 1–12. https://doi.org/10.32615/ps.2023.001

Zahedi S.M., Hosseini M.S., Fahadi Hoveizeh N., Kadkhodaei S., Vaculík M. (2023). Physiological and biochemical responses of commercial strawberry cultivars under optimal and drought stress conditions. Plants 12(3), 496. https://doi.org/10.3390/plants12030496

Zhu X.C., Song F.B., Xu H.W. (2010). Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil. 331, 129–137. https://doi.org/10.1007/s11104-009-0239-z

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INFLUENCE OF GENOTYPE AND CULTURE CONDITIONS ON *in vitro* GYNOGENESIS IN RED BEET (*Beta vulgaris* subsp. *vulgaris*)

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ABSTRACT

The process was examined or the effect of culture conditions on *in vitro* gynogenesis in red beet was analyzed, conditions were modified or optimized. A significant influence of the genotype on the gynogenesis process was demonstrated. Of the eight genotypes, 58.3% planted ovules regenerated embryo-like structures in breeding line 411, 2.1% in RA-10, RA-11, RA-12 breeding lines and 0.9% embryo-like structures in Opolski. For the gynogenesis induction, B5 medium containing 0.1 mg L^{-1} 2,4-dichlorophenoxyacetic acid was the most effective from all tested media. On this medium, the highest number of gynogenetic embryo-like structures was obtained. Most of the plants were regenerated on MS medium supplemented with 30 g L^{-1} sucrose, 0.2 mg L^{-1} 6-benzylaminopurine and 1 mg L^{-1} indole-3-acetic acid. Thirty nine percent of regenerated plants acclimatized. Cytometric evaluation of gynogenetic plants of four tested genotypes revealed that in three genotypes, 100% of tested plants were haploid. Plants showed diploid ploidy level in one genotype. Isoenzymatic analysis of gynogenetic plants demonstrated that 95% and 70% of examined populations were homozygotic for the phosphohexose isomerase isoenzyme and the aspartato aminotransferase isoenzyme, respectively. During the next generation sequencing, 93% of reads were successfully mapped, from which 83% to 85% were mapped in pairs. For 15% of pairs it was clear that obtained sequence was fully homozygous, the rest of the readings were not unambiguous, but similar to the sequence of a homozygous base pair system.

Keywords: gynogenesis, cytometry, isoenzymes, next generation sequencing

INTRODUCTION

Red beet is a common crop plant distributed throughout Asia Minor, the Mediterranean, and Europe. It is also known as an economically important plant. Due to the high content of biologically active substances, in particular betanin, red beet is classified as a nutraceutical food.

Currently, breeding of the new cultivar of crop plants is conducted with the use of traditional and biotechnological methods. Gynogenesis is one of the utilized methods, which allows researchers to obtain haploid plants and double haploid lines (DH) in a short period of time.

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Haploid plants became a valuable source for basic research such as genome mapping, genetic analyses, mutations, transformation, somatic hybridization, biochemical and physiological analyses, cytogenetic research, reference genome sequencing and genetic linkage analysis [Ferrie and Möllers 2011]. Most often, however, they are used in plant breeding programs. So far intensive research on production of haploid plants using gynogenesis were conducted mainly on sugar beet. First haploid plants of red beet were obtained by Hosemans and Bossoutrot in 1983 with the efficiency of 23 haploid plants produced from 10000 ovules [Hosemans and Bossoutrot 1983]. Subsequently, the successful induction of plant regeneration from unpollinated ovules was reported by Bossoutrot and Hosemans [1985]. Since then many researchers obtained embryos by gynogenesis in sugar beet, e.g. Gürel et al. [2000], Nagl et al. [2004], Tomaszewska-Sowa [2010], Aflaki et al. [2017], Pazuki et al. [2017]. For red beet, Barański [1996] obtained few haploid plants using gynogenesis. In 2021, two research teams confirmed the successful production of haploid red beet plants through *in vitro* gynogenesis [Zayachkovskaya et al. 2021, Kiszczak et al. 2021], and this was also confirmed by Kiszczak et al. [2023]. The process of induced gynogenesis is determined by numerous endogenous and exogenous factors such as genotype and the composition of induction and regeneration media. Genotypes vary greatly in their ability to form a gynogenetic embryo or plant regeneration [Gürel et al. 2000, Klimek-Chodacka and Barański 2013, Pazuki 2017]. Barański [1996] observed that ovules collected from donor plants with stable cultivar genotypes had a greater gynogenic ability than the ovules of hybrids or inbred lines. Other studies have confirmed genotypic differences in the efficiency of gynogenesis, but have not indicated that these differences are significant between stable varieties and inbred lines [Zayachkovskaya et al. 2021, Kiszczak et al. 2021]. In general, media based on N6 [Chu et al. 1975] and MS [Murashige and Skoog 1962] containing various growth regulator combinations were used to induce gynogenesis [Weich and Levall 2003, Aflaki et al. 2017, Pazuki et al. 2017]. Barański [1996] used N6 medium with the addition of IAA and 6-benzylaminopurine (BAP) to induce gynogenesis in red beet. However, after obtaining gynogenetic embryos Barański [1996] did not achieve direct conversion of sugar beet embryos into plants. Different authors obtained better results in androgenesis using IMB medium supplemented with thidiatabzuron (TDZ) [Zayachkovskaya et al. 2021] and B5 medium with the addition of IAA, BA and putrescine (Put.) [Kiszczak et al. 2021, Kiszczak et al. 2023].

Zayachkovskaya et al. [2021] obtained direct regeneration of callus in plants on MS medium containing BAP and GA_3, but the root system was weak, therefore passages of shoots were performed several times to medium without hormones. Kiszczak et al. [2021 and 2023] obtained plants with well-developed root system on MS medium supplemented with BA and IAA, however more shoots regenerated on medium with the addition of BA and Put. In the next stage, obtained shoots were rooted on ½ MS medium containing naphthylene-1-acetic acid (NAA) and Put.

Successful regeneration and adaptation are the most important stages in the whole procedure of deriving gynogenetic plants, but only ploidy level and homozygosity evaluation can confirm the obtaining of haploids or DH plants. The ploidy level of gynogenetic plants, can be confirmed by determination of the nuclear DNA content using flow cytometry [Bohanec 2013, de Oliveira et al. 2013, Keleş et al. 2016]. The above-mentioned authors have successfully used flow cytometry to determine the gametic origin of red beet [Zayachkovskaya et al. 2021, Kiszczak et al. 2021, 2023]. Another method for assessing the gynogenetic homozygosity of plants is the analysis of isoenzyme polymorphism. This approach enables the evaluation of differences in gene products at the protein level. Evaluation of isoenzyme polymorphism is commonly used to confirm the homozygosity of various plant species obtained in the gynogenesis process [Murovec and Bohanec 2012]. In case of red beet, authors have applied two isoenzymatic systems [Kiszczak et al. 2021, 2023].

According to Djedatin et al. [2017], next generation sequencing (NGS) is less expensive, more effective and quicker method for determining the homozygotic arrangement of alleles in the genome. NGS is known to be the most precise method that provides an immense amount of bioinformatic data. With advances of the NGS technology and DNA sequencing, it was

possible to use accurate genotyping as a tool for the genetic and evolutionary studies or in the process of accelerate the breeding processes [Song et al. 2016, Wang et al. 2016]. Polymorphism of the genome, including single nucleotide polymorphisms (SNPs), is determined by the NGS method [Kumar 2012, Gupta et al. 2017]. The spontaneous doubling of the genetic material often occurs in the gynogenesis process, which in case of the allelic forms of genes in tested isoenzymes, can cause difficulties for determination of the gametic origin of those plants. According to Djedatin et al. [2017], the most effective method for detection of the duplication of entire segments of the genome or even single genes is the NGS method. The above-mentioned method is very suitable for the isolation of homozygotic populations found in a transgenesis procedure [Passricha et al. 2016]. O'Malley et al. [2017] used results obtained from NGS for the isolation of homozygotic mutants from the population of *Arabidopsis thaliana*. Earlier in 2016, NGS sequencing, combined with Bulk Segregant Analysis, allowed researchers to accelerate the identification of causal mutations with a reference genome sequence in the sugar beet [Ries et al. 2016]. Szklarczyk et al. [2016] applied NGS as a supplementary method for the identification of mitochondrial DNA characteristics, which diversified the cytoplasmatic male sterile and male fertile forms of sugar beet. On the other hand, so far there is no information in the literature about the application of this method in the studies on the genome of red beet.

The aim of this study was to evaluate the influence of various factors on the gynogenesis process and haploid red beet plant regeneration. Different important factors for the gynogenesis process were under study, i.e. the induction medium, the genotype, media for gynogenesis induction and plant regeneration, acclimatization process. Ploidy of obtained plants was also evaluated and the usability of isoenzyme polymorphism analysis for the determination of homozygosity was tested. The correlation between the isoenzyme polymorphism and the analysis of the base pairs order in the genome of red beet on the basis of NGS were examined. The NGS analysis was also performed in order to obtain data that will be used in the databases. Thanks to the information included in the database, researchers will be able to design molecular markers and perform comparative transcriptomics. Knowing the nucleotide sequence of the genome or the transcriptome, it will be also possible to find single nucleotide mutations (SNPs) or simple sequence repeats (SSRs).

MATERIALS AND METHODS

Preparation of plant material

Roots of various red beet genotypes were provided by Breeding and Seed Company – Polan Sp. z o.o. in Cracow. The research was conducted in two vegetation seasons. In the first year, studies were conducted using breeding lines RA-10, RA-11, RA-12, RA-13, RA-14, 406, 411. In the second year of research, RA-5, 4/11, 5/11, 411 breeding lines were used. As a control, the roots of Opolski cultivar. Received roots of red beet plants with heterozygosity confirmed by breeding methods were placed in a substrate consisting of 1:3 (v/v) sand and soil and placed in a cold chamber at 4 °C for two-month vernalization. Then, roots were planted in plastic containers with a capacity of 20 L (two roots per container) in a growth chamber under controlled growth conditions at 18 °C during the day and 16 °C at night, with a 16/8 hour photoperiod.

General research plan

In the first stage of the study, the protocol for gynogenetic plant production was optimized for each red beet genotype. Initial research and then research on determining the composition of the medium that guarantees the formation of embryos were conducted on the Opolski cultivar (Fig. 1 A). In the following year, mainly optimization of the plant growth regulators (PGRs) composition was conducted on the 411 breeding line. At the stage of multiplication, the ploidy of obtained regenerated plants multiplication was analysed using flow cytometer. Shoots of breeding line No. 411 with cytometrically confirmed haploid number of chromosomes were placed on a solidified MS medium containing 5 g L^{-1} colchicine for 5 min [Pazuki et al. 2018] and then transferred onto MS media supplemented with 0.2 mg L^{-1} BAP and 1 mg L^{-1} IAA, on which roots have developed from shoots (Fig. 1 B, C). Plants with confirmed homozygosity were given to breeders, who included received plant material in their breeding programs (Fig. 1 D).

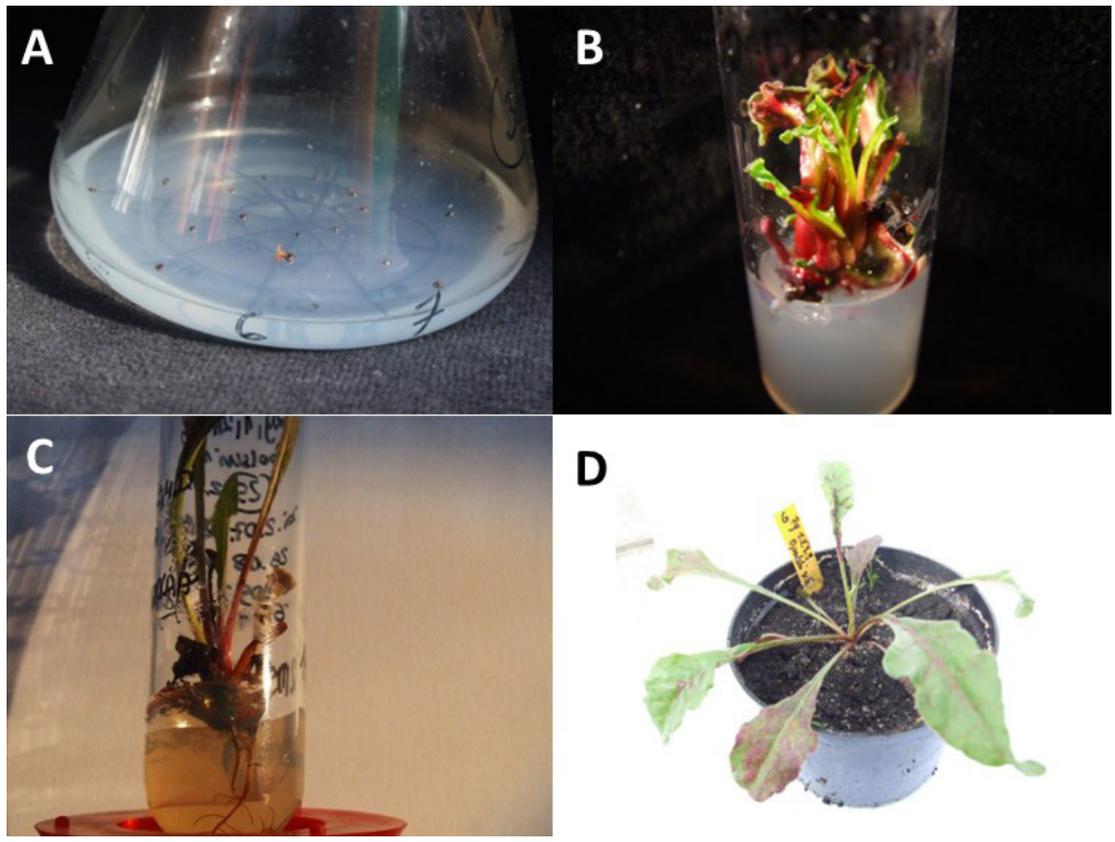

Fig. 1. The successive development stages of gynogenetic plants of red beet: A) gynogenetic embryo, B) regenerating plant, C) fully developed gynogenetic plant, D) acclimatized gynogenetic plants

Optimization of the protocol for gynogenetic plant production

Gynogenesis induction. Green, immature flower buds with unfolded petals of the cultivar and breeding lines were disinfected with 70% ethanol for 10 min and washed 2 times in sterile distilled water. Ovules were isolated from disinfected flower buds under a stereoscopic microscope. Using preparation needles, 24 ovules were placed in one Erlenmeyer flasks (100 mL) containing 30 mL of medium (media described below). All induction media were supplemented with 100 g L^{-1} sucrose and solidified with 6.5 g L^{-1} agar. The pH of all media was adjusted to 5.8 [Barański 1996]. The ovule cultures were kept at 27 °C at continuous light (24 hours a day) with photosynthetic photon flux density (PPFD) of 30 µmol m^{-2} s^{-1}. Formation of embryo-like structures (ELS) took place after 6–14 weeks. The efficiency of gynogenesis process was defined by the number of obtained ELS per 100 planted ovules (%).

Effect of genotype. In the first experiment, frequency of ELS formation was compared among all tested genotypes. Ovules were plated on the B5 [Gamborg et al. 1968] induction medium supplemented with 0.5 mg L^{-1} BAP and 0.2 mg L^{-1} IAA. This medium proved to be the most effective for inducing red beet gynogenesis in the preliminary studies conducted by the authors in the previous year.

Effect of medium composition. In the second experiment, the effect of medium composition on gynogenesis frequency was studied. Ovules of red beet Opolski were cultured on N6 media [Chu et al. 1975] or modified B5 (with the addition of 500 mg L^{-1} L-glutamine and 100 mg L^{-1} L-serine) supplemented with

0.1 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) [Górecka et al. 2017] in the first variant or 0.2 mg L^{-1} BAP and 0.5 mg L^{-1} IAA in the second variant [Barański 1996, Górecka et al. 2017].

Plant regeneration. *Effect of the sucrose concentration*. The ELS were transferred to the media selected on the base of the preliminary studies, consisting of the N6 medium containing 0.2 BAP mg L^{-1}, B5 medium without hormones and MS medium supplemented with 1 mg L^{-1} TDZ with the addition of sucrose at concentrations of 10, 20, or 30 g L^{-1}.

All tested media were solidified with 6.5 g L^{-1} agar, pH adjusted to 5.6 [Ghosh et al. 2013]. ELS were cultured in a 30 mL tube containing 10 mL of medium, placed in a growth room and exposed to continuous light with Photosynthetic Photon Flux Density (PPFD) of 30 µmol m^{-2} s^{-1} [16 hours a day] at a temperature of 20 °C. Observations were made after six weeks of culture. One ELS was placed in each of the 10 tubes containing the tested media.

Effect of the PGR. In the next experiment, ELSs were transferred onto the MS regeneration medium containing 1 mg L^{-1} BAP with the addition of 30 g L^{-1} sucrose. Six weeks later, regenerating plants were placed on MS medium with BAP at a lower concentration of 0.2 mg L^{-1}, supplemented with various auxins, IAA or NAA each at the concentration of 1 mg L^{-1}. Observation of frequency and quality of regenerated plants was conducted after four weeks. At this stage, ploidy analysis was performed using a flow cytometer.

Acclimatization. Plants underwent the acclimatization process in order to conduct further studies on methods of chromosome doubling. Fully developed plants of red beet breeding line No. 411 were rinsed in distilled water after removing from tubes, dipped for a second in 2% Kaptan solution. Next seedlings in *ex vitro* conditions planted in multipots containing peat and sand medium (1:3, v/v), in high humidity conditions in a plastic tunnel localized in a growth chamber at a temperature of 20 °C during the day and 18 °C at night and the light intensity of 30 µmol m^{-2} s^{-1} for 16 hours. After 3–4 weeks, the plastic tunnel was gradually ventilated to reduce the humidity. In the fifth week, an observation of adapted plants was made. Plants that survived the acclimatization process were counted. In the final stage, adapted plants were transplanted to pots and cultured in the same growth chamber.

Ploidy evaluation. Flow cytometric analysis (FCM-DAPI) was performed on leaf samples taken from plant material. Samples of ca. 0.5 cm^2 of leaf blade were taken from the reference plants (donor diploid red beet cultivars) and regenerants. Plant tissue was ground in a Petri dish containing 0.5 mL of Partec buffer for nucleus isolation [Śliwińska 2008], with 1% polyvinylpyrrolidone (PVP-40), to which the fluorescent dye 4′,6-diamidino-2-phenylindole (DAPI) was added (2 µg mL^{-1}). After adding 1 mL of isolation buffer, samples were filtered through a 30 µm filter and incubated at room temperature for 30–45 min in the dark. Fluorescence of nuclei was measured using CyFlow ploidy analyser (Partec, Germany) with software CyView (CyFlow PA, Partec), with UV-LED 365 nm. Sample measurements were performed for at least 1000 nuclei. The ploidy level was read on the histograms, expressed as the value of the position of the nuclear DNA fluorescence peak on the X axis. The external standard for determining the position of the DNA fluorescence peak on the X axis were the leaf samples of diploid reference plants. The position of the fluorescence peak for haploid plants should be within half of the value determined for diploid reference plants.

Homozygosity evaluation. Homozygosity was evaluated using isoenzymes and NGS for the selected obtained from 399, 426 and 521 plants of breeding line No. 411, which was produced using the optimized protocol developed in the first research stage.

Isoenzyme system. To assess homozygosity of 26 plants obtained in ovule cultures (genotype o. 411), two isoenzymes were analyzed: phosphoglucose isomerase (EC:5.3.1.9, PGI) and aspartato aminotransferase (EC 2.6.1.1, AAT) [Westphal and Wricke 1989, Kiszczak et al. 2011]. Electrophoresis was conducted on a 10% starch gel according to the Gottlieb [1973] method. Separation of enzymes was performed according to the Selander et al. [1971] protocol. Weeden and Gottlieb's [1980] method was used for visualization of polymorphism of tested isoenzymes.

RNA isolation. Three plants No. 399, 426 and 521 of breeding line No. 411, maintained in *in vitro* conditions have been used for this analysis. Total RNA used for the preparation of complementary DNA (cDNA) libraries (and further transcriptome analysis) was obtained from red beetroot plants grown *in vitro*. About 400 mg of leaves and stems were used for RNA iso-

lation from each one of three plant lines. Plant/fungi total RNA Purification kit (Norgen Biotek #25800) was used according to the procedure recommended by a manufacturer with a minor modification – added two extra washes of the column before elution of purified RNA. Eluted RNA was precipitated overnight at –20 °C after addition of 1/10 volume of 3 M sodium acetate, pH 5.2, and 2.5 volumes of cold (–20 °C) 99% ethanol. Purified RNA was pelleted by centrifugation (30 minutes at 14,000 rpm; 4 °C), washed twice with cold (–20 °C) 80% ethanol, dried at room temperature, resuspended in water. DNA was deigested using Turbo DNAse (Life Technologies kit #AM 1907) according to a standard procedure. RNA was precipitated, washed and resuspended finally in autoclaved MilliQ water (18.2 MΩ). Verification of the quality and concentration of the preparation was based on UV absorbance measurements in the range 140–220 nm (NanoDrop) and electrophoretic profile in a non-denaturing 2% agarose gel. Purified RNA was aliquoted and stored at –70 °C.

Sequencing of cDNA and construction of cDNA libraries. Preparations of total RNA have been sent to a commercial company Genomed S.A., (Warsaw, Poland). RiboZero cDNA libraries have been constructed there and sequenced on Illumina HiSeq platform d.

Bioinformatic analysis. Bioinformatic analysis was performed using CLC Bio Genomics Workbench software (QIAGEN (n.d.) https://digitalinsights.qiagen.com) and services like BLAST provided by NCBI. The raw reads have been trimmed and filtered for quality, then mapped to a reference transcriptome of sugar beet (*Beta vulgaris*) published by Dohm et al. [2014]. The reference consisted of 29,088 contigs of average length 1,526 nts.

Statistical analyses. A flask containing 48 ovules was treated as a repetition in conducted experiments. The number of repetitions varied in a particular experiment and was dependent on the availability of plant material. Obtained data were analyzed using ANOVA/MANOVA multivariate models and non-parametric analyses such as the Kruskal and Wallis [1952], at an adopted level of significance of $\alpha = 0.05$. Statistical analyses were performed using Statistica 8.0 software package for Windows (StatSoft Inc. Tulsa, USA).

RESULTS

Gynogensis induction

The highest percentage of gynogenetic ELS/100 ovules (58.3) was obtained in red beet breeding line No. 411 and the lowest in Opolski (0.9 ELS/100) (Table 1). No embryos were obtained in three breeding lines (RA-13, RA-14 and 406). The most effective medium for gynogenesis induction in red beet was B5 medium supplemented with 0.1 mg L^{-1} 2,4-D (Table 2). On this medium, 2.5 out of 100 planted ovules formed ELSs. More than twice less of these

Table 1. The influence of the genotype on the gynogenesis induction in ovule *in vitro* culture of red beet

Genotype	Number of cultured ovules	Number of obtained ELS	Number of responding ovules	Number of ELS per 100 ovules
RA-10	48	1	1	2.1b*
RA-11	48	1	1	2.1b
RA-12	48	1	1	2.1b
RA-13	24	0	0	0
RA-14	47	0	0	0
406	96	0	0	0
411	24	14	1	58.3a
Opolski	216	2	2	0.9b

* Combinations located in the same homogeneous group (with the same letter) do not differ statistically at a significance level of $\alpha = 0.05$. Kruskal-Wallis test.

Table 2. Effect of the medium on the gynogenesis efficiency in ovule cultures of red beet Opolski cultivar

Medium	Number		
	cultured ovules	ELS	ELS per 100 plated ovules
B_5 + 2,4D	80	2	2.5a*
B_5 + BA, IAA	216	2	0.9a
N_6 + 2,4D	128	2	1.6a
N_6 + BA, IAA	54	0	0.0a

* Combinations located in the same homogeneous group (with the same letter) do not differ statistically at a significance level of $\alpha = 0.05$. Kruskal-Wallis test.

structures were obtained on the same medium containing BA and IAA. Whereas, on N6 medium, in the presence of 2,4-D, 1.6 ELSs per 100 ovules were produced. No ELSs were formed on N6 medium containing BAP and IAA.

Plant regeneration

Regenerated shoots of various quality and/or callus formation were obtained after transferring gynogenetic ELSs with a different frequency depending on regeneration media (MS, N6 and B5) and sucrose concentration (10, 20 and 30 g L^{-1}) – Table 3. The highest number of shoots was obtained on MS medium containing 30 g L^{-1} sucrose, that is 2,88 average per 1 ELSs, also the highest number of callus (2,89 per 1 ELSs) was observed on MS medium. When this medium contained lower amount of sugar (10 and 20 g L^{-1}), approximately twice less shoots were obtained. No shoots developed from gynogenetic embryos on B5 medium; however, a small amount of callus formation was observed.

On the most effective regeneration medium (MS supplemented with 30 g L^{-1} sucrose) the effect of growth regulators (BAP in combination with IAA or NAA) on shoot development of red beet breeding line No. 411 was examined. Obtained results indicate that whole plants with a well-developed root system can be obtained on media supplemented with both types of auxin combined with BAP (Table 4). However, the higher number of well-developed plants was obtained on medium containing 0.2 mg L^{-1} BA and 1 mg L^{-1} IAA. All regenerated plants (18) of this cultivar were planted *ex vitro* and 39% survived the acclimatization process.

Ploidy evaluation

All tested plants of the Opolski red beet, as well as the 411 and 5/11 breeding lines contained the amount of DNA in the cell nuclei corresponding to the haploid number of chromosomes (Table 5, Fig. 2). Plants RA-5 breeding line consisted of DNA equivalent to a diploid number of chromosomes.

Homozygosity – isoenzyme analysis

Homozygosity analysis of gynogenetic plants from breeding line No. 411 showed that in case of PGI isoenzyme, 95% of examined plants were homozygotes and 5% were heterozygotes. Regarding the indole-3-acetic acid (AAT) isoenzyme, 70% of these plants were homozygous, 23% heterozygous and for the remaining 7%, due to the illegible bands polymorphism, we were not able to confirm their homozygosity.

Homozygosity – NGS

Ninety three percent of reads were successfully mapped for each from three tested genotypes, from which 83% to 85% was mapped in pairs. For the set of 29,088 reference transcripts with a total length of 44,686,800 nucleotides, the following fragments were mapped respectively: 16,530,673 fragments (total length of mapped fragments: 1,645,771,149 nts) for sample No. 399, 56,121,204 fragments (5,398,960,791 nts) for sample No. 426, 39,111,596 fragments (3,901,891,001 nts) for sample No. 521. The number of sugar beet transcripts, to which reads obtained for the samples of red beet were mapped (with the applied mapping parameters: 60%, 80%), is

Table 3. The effect of three sucrose concentrations (10, 20, 30 g L^{-3}) in three media (MS, N6, B5) on the regeneration of shoots from ELS formed by gynogenesis in red beet Opolski cultivar

Medium/sucrose concentration g L^{-3}	Number of cultures ELS	Multiplication – the average per 1 embryo			
		shoots without root			callus
		long more than 0.5 cm	long less than 0.5 cm	total number	
MS-10	62	0.40b*	0.83b*	1.23b*	2.78ab*
MS-20	58	0.30b	0.88b	1.18b	2.89a
MS-30	61	0.77a	2.11a	2.88a	2.56ab
N6-10	60	0.22b	0.11bc	0.33cd	1.11b
N6-20	63	0.11b	0.78b	0.89bc	1.44b
N6-30	59	0.00b	0.00c	0.00d	2.00ab
B5-10	61	0.00b	0.00c	0.00d	1.00b
B5-20	58	0.00b	0.00c	0.00d	1.00b
B5-30	58	0.00b	0.00c	0.00d	1.00b

* Combinations located in the same homogeneous group (with the same letter) do not differ statistically at a significance level of α = 0.05. Kruskal-Wallis test.

Table 4. The effect of PGR (BA 0.2 mg L^{-1}, IAA 1 mg L^{-1}, NAA 1 mg L^{-1}) on the number of obtained regenerants from gynogenetic embryos of red beet on MS medium (breading line 411) – the average per 1 embryo

Medium	Number of cultures ELS	Frequency of plant regeneration		
		shoots		without regeneration
		with root	without root	
BA, IAA	63	0.25a*	1.31a	0.02a
BA, NAA	51	0.04b	1.68a	0.06a

* Combinations located in the same homogeneous group (with the same letter) do not differ statistically at a significance level of α = 0.05. Kruskal-Wallis test.

presented in Table 6. A list of observed variants was made for every tested plant (in a simplified form: the differences in sequence in comparison with the reference transcripts).

Bioinformatic analysis

The total number of 100 nt paired reads obtained for three analyzed plants: No. 399, 426 and 521, was 17,755,074, 57,828,394 and 41,841,448, respectively.

The percentage of reads mapped successfully was 93.10%, 93.59%, and 93.48% of their total number for three samples. From 83.03% to 84.98% reads were mapped in pairs, with the observed distance in pairs from 83–334 nt, which increased their effective length. Library reads were mapped (separately for every sample) based on the sequence of 29,088 transcripts read for sugar beet [Dohm et al. 2014].

During analysis, the possibility of the occurrence of sequencing errors was taken into consideration, therefore an advanced software with complex algorithms was used for the elimination or reduction of those errors. The possibility of the presence of several copies of the same genes was also considered. In the course of analysis, 172,710 potential variants diversifying transcriptome of sugar beet and tested breeding lines of red beet were identified, which in conclusion

Table 5. Ploidy evaluation of gynogenetic plant material conducted during the multiplication of red beet plants

Genotype	Number of rosettes	Ploidy			
		1x		2x	
		number	%	number	%
Opolski	18	18	100	0	0
411	24	24	100	0	0
RA 5	18	0	0	18	100
5/11	2	2	100	0	0

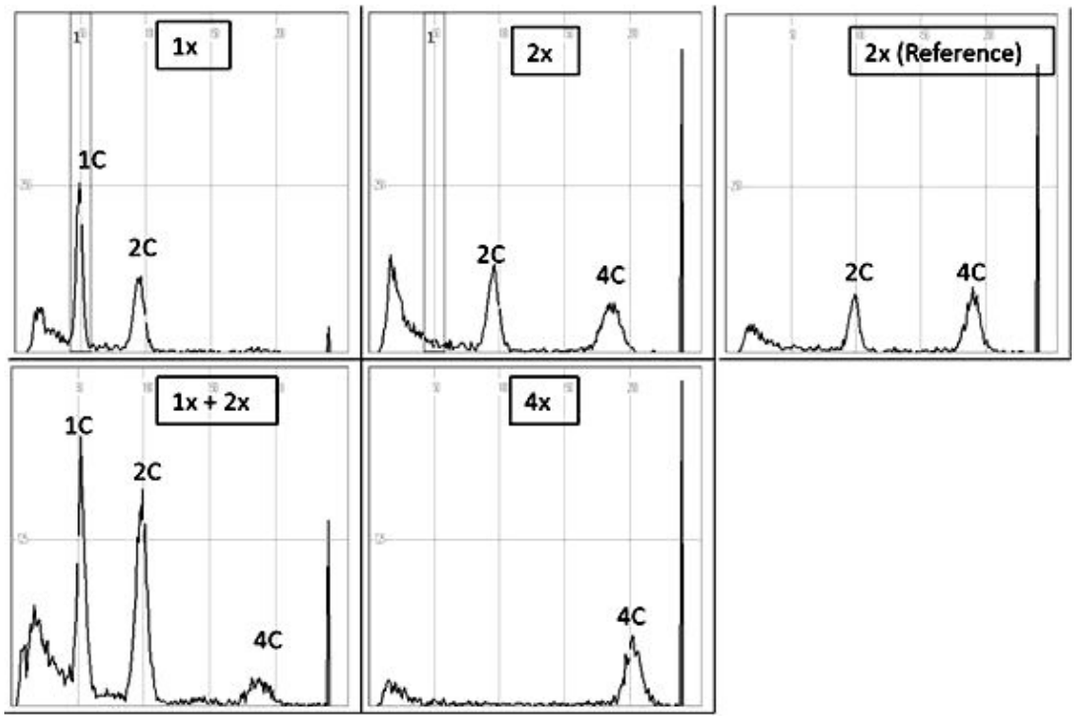

Fig. 2. Sample histograms of cytometric analysis of haploid (1x), diploid (2x), mixoploid plants with a haploid and diploid genomes (1x + 2x), tetraploid (4x) plant of the red beet line 3/2010 and diploid reference plant (donor plant Czerwona Kula)

gave 86,355 potential sites of difference. For more than 95% of those spots, the heterozygosity of tested plants was not specified (399, 426 and 521). The remainder of the sequence was fully homozygotic. Approximately 20,000 identical single positions were examined within the reference transcriptome. During the analysis, all chromosomes nine were identified by at least 400 transcriptomes (genes or their fragments). For each chromosome of tested breeding line of red beet at least 4,000 variants (SNV, MSV or ins/del) were analyzed for the homozygosity (Table 7).

DISSCUSION

In red beet, a significant influence of genotype on the efficiency of gametic embryogenesis was confirmed. Barański [1996] observed gynogenesis in all tested red beet cultivars, but the frequency of embryo

Table 6. The number of transcripts, on which the sequence reads were mapped for the tested red beet breeding line no 411. The total number of reference transcripts: 29,088

Plant individuals	The number of reads mapped on transcripts		
	>0	>10	>100
399	23,310	19,644	13,291
426	24,930	22,532	17,703
521	25,128	22,853	18,148

Table 7. The number and the character (heterozygosity/homozygosity) of the discovered sequence variants observed in the transcriptome of three plants line no 411 of red beet at various limits of the number of single sequence reads in the place of the occurrence of tested variant

Plant individuals	The number of all nucleotide/nucleotides reads including the variant		
	>0	>20	>200
	hetero*/in total	hetero*/in total	hetero*/in total
399	8,392/88,306	3,747/45,483	330/4,377
426	18,333 /163,238	12,870/111,444	701/17,318
521	18,750 /172,710	12,446/113,927	985/13,836

* The numbers presented in the table as a „hetero" are referring to the number of possible variants. The amount of places of their occurrence in the analyzed transcripts of red beet was at least two times lower.

formation was dependent on genotype and ranged from 0-2.86%.

In 2021, Zayachkovskaya et al. obtained a higher induction factor dependent on the genotype, up to 25% of induced ovules. The highest gynogenesis efficiency of 33% was obtained by Kiszczak et al. [2023]. In their studies, the number of obtained gynogenetic embryos was dependent on the genotype. In presented studies, we also confirmed that the efficiency of gynogenesis depends on the genotype. We obtained embryos in several genotypes. In the breeding line, we found the presence of embryos in over 58.3% ovules, but e.g. in the RA-13 line, no gynogenetic sources were observed.

Medium composition is one of the most important factors in the induction of haploids either in the process of androgenesis or gynogenesis. Barański [1996] noted that the use of N6 medium supplemented with 0.5 mg L^{-1} IAA and 0.2 mg L^{-1} BA was the most effective in red beet embryo formation from ovules. We did not receive any gynogenetic embryos on this medium, while the highest number of embryos was received on B5 medium with the addition of 2,4-D. The above-mentioned auxin has the best ability to induce cell divisions and callus differentiation [Zheng et al. 1999] and its usability for inducing gynogenesis process in plants was confirmed by various authors [Rekha et al. 2013, Alan et al. 2016]. In the studies presented by Kiszczak et al. 2023, this auxin added to B5 medium did not cause a significant increase in the number of gynogenetic embryos, but increased on the N6 medium. However, in these experiments, authors obtained most of embryos on cultures conducted on the media with the addition of polyamines. In our study, in the presence of 2,4-D, considerably fewer embryos were obtained on N6 medium compared to B5 medium.

Plant regeneration is the next very important stage in the process of obtaining DH plants via gametic embryogenesis [Górecka et al. 2009, Kiszczak et al. 2015]. Medium is one of the main factors affecting the efficiency of plant regeneration in this process [Se-

gui-Simarro and Nuez 2008, Wędzony et al. 2009]. In 2017, Pazuki carried out the regeneration process in one stage using MS medium, but with the addition of BAP, that resulted in 18.98% of plants. Direct germination and formation of microrosettes occurred when the embryoid was placed on regenerating MS medium with the addition of 1 mg L^{-1} BAP and 0.1 mg L^{-1} GA_3 [Zayachkovskaya et al. 2021]. However, in these experiments, the shoots did not develop or developed roots poorly, therefore additional passages on the hormone-free MS medium were performed. Other researchers obtained direct regeneration into plants red beet on MS medium supplemented with 0.2 mg L^{-1} BA and 1 mg L^{-1} IAA, but this method was inefficient [Kiszczak et al. 2023]. Therefore, regeneration from callus was conducted in two stages. Authors regenerated shoots on MS medium supplemented with BA and 0.5 mg L^{-1} putrescine, afterwards rhizogenesis was conducted on MS medium containing ½ MS macronutrients and supplemented with NAA at the concentrations of 1 or 3 mg L^{-1} and Put at 0.5 or 160 mg L^{-1}. In our studies, higher numbers of fully developed plants (reaching 10%) were obtained on MS medium, compared to N6 and B5 media. This confirms that MS-based media are the most suitable for regeneration of plants from gynogenetic embryos in red beet. The authors applied the standard of 30 g L^{-1} of sucrose. The increased presence of callus in our studies was due to the application of 0.2 mg L^{-1} BAP and 1 mg L^{-1} NAA for the regeneration of red beet embryos. Similar results were demonstrated earlier by Gürel et al. [2000]. Authors applied the same combination of two growth regulators in the concentration of 1 mg L^{-1} BAP and 0.5 mg L^{-1} NAA. They observed a higher amount of formed callus comparing to other media used for the regeneration in *in vitro* cultures of sugar beet.

In presented studies, only haploids underwent the acclimatization process, which in general are characterized by lower vigor [Murovec and Bohanec 2012]. In case of sugar beet, Gośka et al. [2004] selected only diploid gynogenetic plants for the acclimatization, which allowed approximately 95% of plants to adapt to *ex vitro* conditions. In 2010, Tomaszewska-Sowa acclimatized almost 80% of gynogenetic plants of sugar beet. Some authors are emphasizing the special significance of the root system for the efficiency of acclimatization [Salvi et al. 2002]. Our observations of the acclimatization process of carrot androgenetic plants [Kiszczak et al. 2018] and current studies on the gynogenetic red beet regenerants confirm this thesis. It is most likely that one of the reasons a low percentage of plants in our experiments adapted was the very poor root system of haploid plants.

Our research has shown that the tendency to spontaneously double the chromosome number was strongly dependent on the genotype. All the gynogenetic plants (18 pcs.) of Opolski red beet and two breeding lines RA 5, 5/11 were haploids, whereas in one breeding line 4/11 and 411 all 24 pcs. gynogenetic plants were diploids. The emergence of breeding line with a doubled set of chromosomes is probably related to the occurrence of the phenomenon of endoreduplication [Joubes and Chevalier 2000]. Strong DNA endoreduplication was also observed during flow cytometry analysis in our study. Lukaszewska et al. [2011] observed this phenomenon in *in vitro* cultures of sugar beet. Authors showed that the application of medium with NAA at a concentration of 1 mg L^{-1}, the same concentration as used in our experiments, intensified the process of endoreduplication. These observations indicate that doubling the chromosome number may be associated with tendency for a given genotype to endoreduplication.

During the homozygosity analysis with the use of two isoenzymatic systems, PGI and AAT, the polymorphism that allowed recognition of homozygote from heterozygote was obtained for the PGI isoenzymatic system. Sabir et al. [1992] showed the usability of this isoenzymatic system for the analysis of the somaclonal variation frequency in plant material of sugar beet and chard, propagated *in vitro*. Authors also observed polymorphism in the PGI system, whereas the AAT system did not generate any variations in the bands. Ludina and Levites [2003] assigned the absence of the polymorphism for the malate dehydrogenase isoenzyme in the studies on the population of sugar beet to the not-allelic character of isoenzymes located in various cellular organelles. This finding indicates that genes of an isoenzyme, such as AAT, can be inherited with deviation from standard Mendel's law. The appearance of a heterozygotic pattern of bands for both isoenzymes in tested population, may be due to the reasons explained above, also described by Levites et al. [2005]. In conducted studies, authors demonstrated

that spontaneous polyploidization caused by their prolonged culturing occurs in the haploid tissues of sugar beet under *in vitro* conditions. According to their results, the emergence of heterozygotes in polymorphic populations regarding the isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase isoenzymes in combination with the simultaneous homozygotic profile for the other isoenzyme in the same plants indicate the occurrence of spontaneous polyploidization.

Evaluation of the homozygosity of three red beet plants [399, 426 and 521 breeding lines] was performed on the basis of the transcriptome analysis [read with the use of the high-throughput sequencing and NGS] in terms of the occurrence of different variants of nucleotide sequences [SNV, MNV, ins/del]. Results presented in Table 1 indicate that even in consideration of only reference transcripts, for which at least 100 mapped reads were obtained [approximately 100 nt each], conducted analysis included from 45% to 62% of potential red beet genes. High percentage [93%] of mapped reads, when adding the reads mapped in pairs with the distance in line with the expectations, indicates the high reliability of obtained results. Lower percentage of mapped reads was obtained by various researchers in other plant species, for example Wang et al. [2016] achieved 70% of mapped reads in corn. Obtained results are considered to be significant only when 95% of genes are mapped in comparison with cDNA databases [Claros et al. 2012]. Analysis was performed on the transcripts originated from all red beet chromosomes, which allowed for the detection of potential aberrations during the chromosome duplication. The occurrence of well documented (over 200 single reads) cases of simultaneous presence of two variants (heterozygosity) was discovered on every tested chromosome. However, the overwhelming part of the genome had a homozygotic character (Table 2). The appearance of false segmental duplications in the assemblies, which occurs when heterozygous sequences from two haplotypes are assembled into separate contigs and are scaffolded adjacent to each other rather than being merged, this is the main problem during the analysis of the material derived from a heterozygotic plant [Kelley and Salzberg 2010]. This can also be referred to as the process of spontaneous doubling of chromosomes that occur while obtaining plants through gynogenesis. Therefore, part of the 200 single reads may be incorrectly categorized, which in reality leads to the appearance of a greater number of homozygotic variants. It should be emphasized that applied method was considerably more sensitive to the detection of differentiation variants (heterozygosity) in the tested genomes in comparison to the classic methods.

CONCLUSION

The influence of individual factors on the gynogenesis process was determined in the presented studies and their optimal range for obtaining the highest number of doubled haploids of red beet. A significant influence of genotype on the efficiency of red beet ovule cultures was confirmed. In conducted experiment the most effective medium for gynogenesis induction proved to be the B5 medium containing 0.1 mg L^{-1} 2,4-D. Based on results obtained after analyzing the influence of various media base it was shown that MS containing 30 g L^{-1} sucrose was the best medium. It was also proven that the gynogenesis process was the most effective on the media with the addition of 0.2 mg L^{-1} BA and 1 mg L^{-1} IAA. The study of the nuclear DNA content at the stage of multiplication of the genotype 411 and after acclimatization of the genotype 5/11 showed that they are haploids. The analysis of polymorphism of two isozymes PGI and AAT demonstrated that the majority of the regenerants displayed the homozygous band pattern. To confirm obtained results, next generation sequencing was performed, within which 3 tested genotypes were mapped using sugar beet transcripts. Subsequently, bioinformatic analyzes were performed based on the obtained transcripts. During the analysis, all chromosomes (nine) were identified by at least 400 transcriptomes (genes or their fragments). For each chromosome of tested breeding line of red beet at least 4,000 variants (SNV, MSV or ins/del) were analyzed for the homozygosity.

Significance statement

Optimal parameters of each factor were defined for obtaining haploid plants of red beet in gynogenesis process. Ploidy analysis confirmed the presence of haploid variants among the obtained multiplications.

The gametic origin of these plants was confirmed after obtaining homozygotic polymorphism systems

of PGI and AAT isoenzymes during the study of regenerants. For the first time, mapping of regenerants of the three studied genotypes to sugar beet transcripts was carried out and a picture was obtained confirming that the predominant part of the genome was homozygous. Application of different evaluation methods confirmed the gametic origin of regenerants obtained by gynogenesis in beet ovule cultures.

AUTHORS' CONTRIBUTIONS

Waldemar Kiszczak, Maria Burian, Tadeusz Malinowski, Małgorzata Podwyszyńska, Krystyna Górecka contributed to the study conception and design. Material preparation, data collection and analysis were performed by Waldemar Kiszczak, Krystyna Górecka and Maria Burian. The first draft of the manuscript was written by Waldemar Kiszczak, Krystyna Górecka and Małgorzata Podwyszyńska and they authors and Marcin Domaciuk read and approved the final manuscript.

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REFERENCES

Aflaki, F., Pazuki, A., Gurel, S., Stevanato, P., Biancardi, E., Gurel, E. (2017). Doubled haploid sugar beet: an integrated view of factors influencing the processes of gynogenesis and chromosome doubling. Int. Sugar J., 119, 884–895. https://doi.org/10.1007/978-1-0716-1331-3_21

Alan, A.R., Celebi, T.F., Kaska, A. (2016). Production and evaluation of gynogenic leek (*Allium ampeloprasum* L.) plants. Plant Cell. Tiss. Organ Cult., 125, 249–259. https://doi.org/10.1007/s11240-016-0944-2

Andersen, S.B., Christiansen, I., Farestveit, B. (1990). Carrot (*Daucus carota* L.). In vitro production of haploids and field trials. In: Bajaj, Y.P.S. (ed). Biotechnol. Agric. For. 12, 393–402.

Barański, R. (1996). In vitro gynogenesis efficiency in red beet (*Beta vulgaris* L). Effect of ovule culture conditions. Acta Soc. Bot. Pol., 65(1–2), 57–60. https://doi.org/10.5586/asbp.1996.010

Bohanec, B. (2013). Ploidy determination using flow cytometry. In: Maluszynski, M., Kasha, K.J, Forster, B.P., Szarejko, I. (eds). Doubled Haploid Production in Crop Plants IV, Springer, Dordrecht, 397–403. https://doi.org/10.1007/978-94-017-1293-4_52

Bossoutrot, D., Hosemans, D. (1985). Gynogenesis in *Beta vulgaris* L. From in vitro culture to the production of doubled haploids plants in soil. Plant Cell Rep., 4(6), 300–303. https://doi.org/10.1007/bf00269883

Claros, M.G., Bautista, R., Guerrero-Fernández, D., Benzerki. H., Seoane, P., Fernández-Pozo, N. (2012). Why assembling plant genome sequences is so challenging. Biology, 1, 439–459. https://doi.org/10.3390/biology1020439

Chu, C. C., Wang, C. C., Sun, C. S., Hsu, C., Yin, K. C., Chu, C. Y., Bi, F. Y. (1975). Establishment of an efficient medium for another culture of rice through comparative experiments on the nitrogen sources. Sci. Sin., 18(5), 659–668.

Djedatin, G., Monat, C., Engelen, S., Sabot, F. (2017). Duplication detector, a light weight tool for duplication detection using NGS data. Curr. Plant. Biol., 9(10), 23–28. https://doi.org/10.1016/j.cpb.2017.07.001

Dohm, J.C., Minoche, A.E., Holtgrawe, D., Capella-Gutierrez, S., Zakrzewski, F., Tafer, H., Rupp, O., Sorensen, T., Stracke, R., Reinhardt, R., Goesmann, A., Kraft, T., Schulz, B., Stadler, P.F., Schmidt, T., Gabaldon, T., Lehrach, H., Weisshaar, B., Himmelbauer, H. (2014). The genome of the recently domesticated crop plant sugar beet (*Beta vulgaris*). Nature, 505, 546–549. https://doi.org/10.1038/nature12817

Ferrie, A.M.R., Möllers, C. (2011). Haploids and doubled haploids in *Brassica* spp. for genetic and genomic research. Plant Cell Tiss. Organ Cult., 104(3), 375–386. http://dx.doi.org/10.1007/s11240-010-9831-4

Gamborg, O.L., Miller, R.A., Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res., 50(1), 151–158. https://doi.org/10.1016/0014-4827(68)90403-5

Ghosh, N., Caraway, E., Das, A. B., Dani, R. G. (2013). In vitro regeneration of Sugar Beet (*Beta vulgaris* L.) via leaf explants and callusing. Ann. Plant Sci., 2(10), 405–411.

Gośka, M., Krysińska, T., Strycharczuk, K. (2004). The use of in vitro gynogenesis for obtaining sugar beet dihaploids. IHAR Bulletin, 234, 27–34. https://doi.org/10.37317/biul-2004-0004

Gottlieb, L.D. (1973). Enzyme differentiation and phylogeny in *Clarkia franciscana*, *C. rubicunda* and *C. amoena*. Evolution, 27(2), 205–214. https://doi.org/10.1111/j.1558-5646.1973.tb00666.x

Górecka, K., Dorota, K., Urszula, K. (2007). Regeneration and evaluation of androgenetic plants of head cabbage

(*Brassica Oleracea* var. capitata L.) Veg. Crop. Res. Bull., 67, 5–15. https://doi.org/10.2478/v10032-007-0025-5

Górecka, K., Krzyżanowska, D., Kiszczak, W., Kowalska, U. (2009). Plant regeneration from carrot (*Daucus carota* L.) anther culture derived embryos. Acta Physiol. Plant., 31(6), 1139–1145.

Górecka, K., Krzyżanowska, D., Kiszczak, W., Kowalska, U., Podwyszyńska, M. (2017). Development of embryoids by microspore and anther cultures of red beet (*Beta vulgaris* L. subsp. vulgaris). J. Central Eur. Aagric. 18(1), 185–195. https://doi.org/10.5513/JCEA01/18.1.1877

Gupta, P., Reddaiah, B., Salava, H., Upadhyaya, P., Tyagi, K., Sarma, S., Datta, S., Malhotra, B., Thomas, S., Sunkum, A., Devulapalli S., Till, B.J., Sreelakshmi, Y., Sharma, R. (2017). Next-generation sequencing (NGS)-based identification of induced mutations in a doubly mutagenized tomato *(Solanum lycopersicum*) population. Plant J., 92(3), 495–508. https://doi.org/10.1111/tpj.13654

Gürel, S., Gürel, E., Kaya, Z. (2000). Doubled haploid production from unpollinated ovules of sugar beet (*Beta vulgaris* L.). Plant Cell Reprod., 19, 1151–1159. https://doi.org/10.1007/s002990000248

Hansen, A.L., Plever, C., Pedersen, H.C., Keimer, B., Andersen, S.B. (1994). Efficient *in vitro* chromosome doubling during *Beta vulgaris* ovule culture. Plant Breed., 112(2), 89–95. https://doi.org/10.1111/j.1439-0523.1994.tb00655.x

Hosemans, D., Bossoutrot, D. (1983). Induction of haploid plants from in vitro culture of unpollinated beet ovules (*Beta vulgaris* L.). Z. Pflanzenziichtg, 91, 74–77.

Joubès, J., Chevalier, C. (2000). Endoreduplication in higher plants. Plant Mol. Biol., 43, 735–745. https://doi.org/10.1023/A:1006446417196

Keleş, D., Özcan, C., Pınar, H., Ata, A., Denli, N., Yücel, N.K., Taşkin, H., Buyukalaca, S. (2016). First report of obtaining haploid plants using tissue culture techniques in spinach. HortSci., 51(6), 742–749. https://doi.org/10.21273/HORTSCI.51.6.742

Kelley, D.R., Salzberg, S.L. (2010). Detection and correction of false segmental duplications caused by genome mis-assembly. Genom. Biol., 11, R28. https://doi.org/10.1186%2Fgb-2010-11-3-r28

Kirikovich, S.S., Svirshchevskaya, A.M., Levites, E.V. (2003). Variation at isozyme loci in seed offspring of sugar beet gynogenetic lines. Sugar Tech., 5, 289–292. http://dx.doi.org/10.1007/BF02942487

Klimek-Chodacka, M., Baranski, R. (2013). Comparison of haploid and doubled haploid sugar beet clones in their ability to micropropagate and regenerate. Electron. J. Biotechnol., 16(2). http://dx.doi.org/10.2225/vol16-issue2-fulltext-3

Kiszczak, W., Krzyżanowska, D., Strycharczuk, K., Kowalska, U., Wolko, B., Górecka, K. (2011). Determination of ploidy and homozygosity of carrot plants obtained from anther cultures. Acta Physiol. Plant, 33(2), 401–407. https://doi.org/10.1007/s11738-010-0559-x

Kiszczak, W., Kowalska, U., Kapuścińska, A., Burian, M., Górecka, K. (2015). Effect of low temperature on in vitro androgenesis of carrot (*Daucus carota* L.). In Vitro Cell Dev. Biol.-Plant, 51(2),135–142. https://doi.org/10.1007/s11627-015-9665-1

Kiszczak, W., Kowalska, U., Burian, M., Górecka, K. (2018). Induced androgenesis as a biotechnology method for obtaining DH plants in *Daucus carota* L. J. Hortic. Sci. Biotechnol., 93(6), 625–633.

Kiszczak, W., Burian, M., Kowalska, U., Górecka, K., Podwyszyńska M. (2021). Production of homozygous red beet (*Beta vulgaris* L. subsp. vulgaris) plants by ovule culture. Methods Mol. Biol., 2289, 301–312. https://doi.org/10.1007/978-1-0716-1331-3_20.

Kiszczak, W., Kowalska, U., Burian, M., Podwyszyńska, M., Górecka, K. (2023). Influence of polyamines on red beet (*Beta vulgaris* L. ssp. *vulgaris*) gynogenesis. Agronomy, 13(2), 537. https://doi.org/10.3390/agronomy13020537

Kumar, S., Banks, T.W., Cloutier, S. (2012). SNP Discovery through Next-Generation Sequencing and its applications. Int. J. Plant. Genom., 1–15. http://dx.doi.org/10.1155/2012/831460

Kruskal, W.H., Wallis, W.A. (1952). Use of ranks in one-criterion variance analysis. J. Am. Stat. Assoc. 47(260), 583–621. https://doi.org/10.2307/2280779

Levites, E.V., Svirshchevskaya, A.M., Kirikovichi, S.S., Mil'ko, L.V. (2005). Variation at isozyme loci in cultured in vitro sugar beet regenerants of gynogenetic origin. Sug. Tech., 7(1), 71–75. https://doi.org/10.1007/BF02942421

Linsmaier, E.M., Skoog, F. (1965). Organic growth factor requirements of tobacco tissue cultures. Physiol. Plant., 18, 100–128. https://doi.org/10.1111/j.1399-3054.1965.tb06874.x

Ludina, R.S., Levites, E.V. (2003). [Subcellular localization of isozymes of NAD-dependent malate dehydrogenase in sugar beet *Beta vulgaris* L.]. Genetika, 44(12), 1638–1643 [in Russian].

Lukaszewska, E., Virden, R., Sliwinska, E. (2011). Hormonal control of endoreduplication in sugar beet (*Beta vulgaris* L.) seedlings growing *in vitro*. Plant Biol., 14(1), 216–222. http://dx.doi.org/10.1111/j.1438-8677.2011.00477.x

de Oliveira, C.E.G., Chamma, D.L.M., Oliveira, B.F., Von, P.R.G., Nayara, S.T. (2013). Identification of haploid maize by flow cytometry, morphological and molecular

markers. Ciência Agrotec., 37(1), 25–11. https://dx.doi.org/10.1590/S1413-70542013000100003

O'Malley, R.C., Barragan, C.C., Ecker, J.R. (2017). A user's guide to the arabidopsis T-DNA insertional mutant collections. In: Alonso, J., Stepanova, A. (eds). Plant Functional Genomics. Methods in Molecular Biology, vol. 1284. Humana Press, New York, NY, 323–342. https://doi.org/10.1007%2F978-1-4939-2444-8_16

Maraschin, S.F., de Priester, W., Spaink, H.P., Wang, M. (2005). Androgenic switch: an example of plant embryogenesis from the male gametophyte perspective. J. Exp. Bot., 56(417), 1711–1726. https://doi.org/10.1093/jxb/eri190

Metwally, E.I., Moustafa, S.A., El-Sawy, B.I., Haroun, S.A., Shalaby, T.A. (1998). Production of haploid plants from in vitro culture of unpollinated ovules of *Cucurbita* pepo. Plant Cell Tiss. Org. Cult., 52(3), 117–121. http://dx.doi.org/10.1023/A:1005948809825

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioasseys with tobacco tissue cultures. Physiol. Plant., 15(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Murovec, J., Bohanec, B. (2012). Haploids and doubled haploids in plant breeding biochemistry, genetics and molecular biology. In: Ibrokhim, Y. (ed). Plant Breed., 5, 1–21. http://doi.org/10.5772/29982

Nagl, N., Mezei, S., Kovačev, L., Vasić, D., Čačić, N. (2004). Induction and micropropagation potential of sugar beet haploids. Genetik, 36(3), 187–194. http://dx.doi.org/10.2298/GENSR0403187N

Nielsen, R., Paul, J.S., Albrechtsen, A., Song, Y.S. (2011). Genotype and SNP calling from next-generation sequencing data. Nat. Rev. Genet. 12(6), 443–451. http://dx.doi.org/10.1038/nrg2986

Nitsch, J.P., Nitsch, C. (1969). Haploid plants from pollen grains. Sci., 163, 85–87. http://dx.doi.org/10.1126/science.163.3862.85

Passricha, N., Saifi, S., Khatodia, S., Tuteja, N. (2016). Assessing zygosity in progeny of transgenic plants: current methods and perspectives. J. Biol. Methods., 3(3), e46. https://doi.org/10.14440/jbm.2016.114

Pazuki, A., Aflaki, F., Gürel, E., Ergül, A. (2017). Gynogenesis induction in sugar beet (*Beta vulgaris*) improved by 6-benzylaminopurine (BAP) and synergized with cold pretreatment. Sugar Tech., 20, 69–77. http://dx.doi.org/10.1007/s12355-017-0522-x

Ries, D., Holtgräwe, D., Viehöver, P., Weisshaar, B. (2016). Rapid gene identification in sugar beet using deep sequencing of DNA from phenotypic pools selected from breeding panels. BMC Genomics, 17, 236. http://dx.doi.org/10.1186/s12864-016-2566-9

Rekha, H.R., Rakhi, C. (2013). Establishment of dedifferentiated callus of haploid origin from unfertilized ovaries of tea (*Camellia sinensis* (L.) O. Kuntze) as a potential source of total phenolics and antioxidant activity. In Vitro Cell Dev. Biol-Plant., 49, 960–969. https://doi.org/10.1007/s11627-013-9490-3

Rogozińska, J.H., Gośka, M. (1982). Attempts to induce haploids in anther cultures of sugar, fodder and wild-species of beet. Acta Soc. Bot. Pol., 51(1), 91–105. https://doi.org/10.5586/asbp.1982.009

Sabir, A., Newbury, H.J., Todd, G., Catty, J., Ford-Lloyd, B.V. (1992). Determination of genetic stability using isozymes and RFLPs in beet plants regenerated in vitro. Theor. Appl. Genet., 84, 113–117. https://doi.org/10.1007/bf00223989

Segui-Simarro, J.M., Nuez, F. (2008). How microspores transform into haploid embryos: changes associated with embryogenesis induction and microspore derived embryogenesis. Physiol Plant., 134(1), 1–12. https://doi.org/10.1111/j.1399-3054.2008.01113.x

Salvi, N.D., George, L.Y., Eapen, S. (2002). Micropropagation and field evaluation of micropropagated plants of tumeric. Plant Cell Tiss. Oorg. Cult., 68, 143–151. http://dx.doi.org/10.1023/A:1013889119887

Selander, R.K., Smith, M.H., Yang, S.Y., Johnson, W.E., Gentry, J.B. (1971). Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-tield mouse (*Peromyscus polionotus*). Univ. Texas Publ., 7103, 49–90.

Song, H.J., Lee, J.M., Graf, L., Rho, M., Qiu, H., Bhattacharya, D., Yoon, H.S. (2016). A novice's guide to analyzing NGS-derived organelle and metagenome data. Algae 31(2), 137–154. https://doi.org/10.4490/algae.2016.31.6.5

Svirshchevskaya, A., Dolezel, J. (2001). Karyological characterization of sugar beet gynogenetic lines cultured in vitro. J. Appl. Genet., 42(1), 21–32.

Szklarczyk, M. (2016). The search for mitochondrial polymorphisms differentiating cytoplasmic male-sterile and male-fertile beets. Scien. J. Agr. Univ. Hugo Kołłątaj Krakow, 408, 1–108.

Szkutnik, T. (2010). Apomixis in the sugar beet reproduction system. Acta Biol. Cracov Ser. Bot., 52(2), 87–96. https://doi.org/10.2478/v10182-010-0011-y

Śliwińska, E. (2008). [Estimation of DNA content in plants using flow cytometry]. Adv. Cell Biol. Suppl., 35(24), 165–176 [in Polish].

Tomaszewska-Sowa, M. (2010). Cytometric analyses of sugar beet (*Beta vulgaris* l.) Plants regenerated from unfertilized ovules cultured *in vitro.* Electron. J. Pol. Agric. Univ., 13(4).

Tyukavin, G.B., Shmykova, N.A., Mankhova, M.A. (1999). Cytological study of embryogenesis in cultured carrot anthers. Russ. J. Plant. Physl., 46(6), 876–884.

Wang, B., Tseng, E., Regulski, M., Clark, T.A., Hon, T., Jiao, Y., Lu, Z., Olson, A., Stein, J.C., Ware, D. (2016). Unveiling the complexity of the maize transcriptome by single-molecule long-read sequencing. Nat. Commun., 7, 11708. https://doi.org/10.1038/ncomms11708

Weeden, F.N., Gottlieb, L.D. (1980). Isolation of cytoplasmic enzymes from pollen. Plant Physiol., 66(3), 400–403. https://doi.org/10.1104/pp.66.3.400

Weich, E.W., Levall, M.W. (2003). Doubled haploid production of sugar beet (*Beta vulgaris* L.). In: Maluszynski, M., Kasha, K.J., Forster, B.P., Szarejko, I. (eds). Doubled haploid production in crop plants. Berlin, Springer, Dordrecht, 255–263. https://doi.org/10.1007/978-94-017-1293-4_38

Westphal, L., Wricke, G. (1989). Genetic analysis of DIA, GOT and PGI isozyme loci in *Daucus carota* L. ssp. *sativas*. Plant Breed, 102(1), 51–57. https://doi.org/10.1111/j.1439-0523.1989.tb00314.x

Wędzony, M., Żur, I., Golemiec, E., Szechyńska-Hebda, M., Dubas, E., Gołębiowska, G. (2009). Progress in doubled haploid technology in higher plants. In: Touraev, A., Forster, B.P., Jain, S.M. (eds). Advances in Haploid Production in Higher Plants, Springer, Dordrecht, 1–14.

Wremerth-Weich, E., Leval, M. (2003). Doubled haploid production of sugar beet (*Beta vulgaris* L.). In: Maluszynski, M., Kasha, K.J., Forster, B.P., Szarejko, I. (eds). Doubled Haploid Production in Crop Plants – A Manual. Kluwer, Dordrecht, Boston, London, 255–265.

Van Geyt, J., Speckmann, G.J. Jr, D'Halluin, K., Jacobs, M. (1987). *In vitro* induction of haploid plants from unpollinated ovules and ovaries of the sugarbeet (*Beta vulgaris* L). Theor. Appl. Genet., 73, 920–925. https://doi.org/10.1007/bf00289399

Zayachkovskaya, T., Domblides, E., Zayachkovsky, V., Kan, L., Domblides, A., Soldatenko, A. (2021). Production of gynogenic plants of red beet (*Beta vulgaris* L.) in unpollinated ovule culture in vitro. Plants, 10(12), 2703. https://doi.org/10.3390/plants10122703

Zheng, K., Konzak C.F. (1999). Effect of 2,4D-dichlorofenoxyacetic acid on callus induction and plant regeneration in another culture of wheat (*Triticum aestivum* L.). Plant Cell Rep., 19(1), 69–73. https://doi.org/10.1007/s002990050712

Zhuzhzhalova, T.P., Podvigina, O.A., Znamenskaya, V.V., Vasil'chenko, E.N., Karpechenko, N.A., Zemlyanukhina, O.A. (2016). Sugar beet (*Beta vulgaris* L.) haploid parthenogenesis in vitro: factors and diagnostic characters. Agric. Biol., 51(5), 636–644. http://dx.doi.org/10.15389/agrobiology.2016.5.636eng

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EFFECT OF SALINITY ON THE GROWTH AND DEVELOPMENT OF ORNAMENTAL EVERGREENS

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ABSTRACT

Salt stress is the main problem facing evergreen plants in cities. To a large extent, these plants have stunted growth, lose their ornamental qualities and finally die. The aim of this study was to investigate the response of a selected three ornamental evergreen plants: *Pachysandra terminalis*, *Buxus sempervirens* and *Hedera helix*, to the effects of three different concentrations of sodium chloride (NaCl) – 100, 200 and 300 mM. As a result of a number of experiments, it was found that increased NaCl concentrations resulted in inhibition of plant growth – even more than 90% shorter growth, as in the case of ivy. In addition, the analyses made it possible to conclude that NaCl influences biochemical changes in plant tissues, in particular chlorophyll, soluble proteins or stress parameters such as MDA or free proline. The results obtained allow the validity of the use of selected species in urban greenery in temperate climates to be established.

Keywords: salt stress, sodium chloride, ornamental plants, morphological changes, biochemical changes

INTRODUCTION

Salinity is a significant global issue, with saline soils covering approximately 400 million hectares (mln ha), or nearly 3% of the Earth's total land area, as of 2011. The fraction of land lost to cultivation due to soil salinity, according to researchers, will increase in the coming years as a result of the effects of global warming or inadequate irrigation [Janz et al. 2012]. Taking into account new reports in the literature, the degree of salinity has already reached approximately 950 mln ha [Dustnazarova et al. 2021, Thaker et al. 2021]. According to the FAO (Food and Agriculture Organisation of the United Nations), 3% of the world's soils are salinised in the upper layers, while 6% are in the lower layers [FAO 2023]. In December 2024, the same organisation issued a report on the problem of soil salinisation. According to analysis by FAO experts, as much as 1.4 billion hectares (bn ha) of land in the world is salinised, representing 10.7% of the global land area. In addition, a further 1 bn ha are believed to be at risk. According to the FAO, if current trends continue, saline soils could account for 24 to 32% of the Earth's land surface by the end of the 21st century [FAO 2024].

Plants are the organisms most exposed to stress factors, including salinity. High salt concentrations cause stunted growth and development in the first stage, yellowing of individual organs in the later stage and finally even death of the whole plant [Gupta and Huang 2014, Safdar et al. 2019, Toscano et al. 2020]. The occurrence of long-term substrate salinisation leads to a reduction in the size of the root system in plants, due to partial dieback. With regard to the aboveground part of the plant, a strong shortening of the shoots can be observed. Salinity also affects the reduction of leaf blades in plants [Jameel et al. 2024]. In the case of flowering plants, there may be a reduction in

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inflorescences/flowers, but instead there will be significantly more of them than in plants not exposed to salt stress [Li and Li 2017, Cerrato et al. 2024]. A key factor influencing plant growth and appearance is the EC (electrical conductivity) value. An increase in EC in both the soil and irrigation water alters the plant's water potential, leading to reduced water absorption or even complete inhibition [Ahmadi and Souri 2020, Corwin and Yemoto 2020].

Pivotal to proper plant growth and development is what happens in their tissues. This relates primarily to the effect of high concentrations of salt on biochemical changes. One of the most important changes that occur is a reduction in the content of the plant pigment – chlorophyll. A reduction in its content in plant cells leads to a disruption in the proper functioning of photosynthesis in plants [Kibria and Hoque 2019, Jameel et al. 2024, Boorboori and Li 2025]. Furthermore, rising salinity levels lead to a reduction in the concentration of soluble proteins in certain plants [Hakim et al. 2014]. The malondialdehyde (MDA) level in plant tissues is a key indicator of oxidative stress and can also reflect the extent of cellular damage [Biczak et al. 2016]. Elevated salt levels lead to a significant accumulation of free proline, which helps alleviate the effects of osmotic stress [Cirillo et al. 2016, Rahneshan et al. 2018]. Additionally, the production of hydrogen peroxide (H_2O_2) in plant tissues increases, which is highly toxic and can cause chlorophyll degradation, often resulting in dieback [Shahid et al. 2020, Lu et al. 2021]. The strongly increasing H_2O_2 content is correlated with the activity of antioxidant enzymes, primarily catalases and peroxidases, whose function is to neutralise the threat of increased H_2O_2 by degrading it [Kim et al. 2018, Lu et al. 2021, Cerrato et al. 2024].

The highest levels of salinity are observed during the winter period along traffic routes. This problem is due to the use of salt by road services to reduce icing on roads. However, what is beneficial for humans is not necessarily so for plants [Devecchi and Remotti 2004, Marosz 2004, Marosz 2011]. Evergreen plants, including deciduous ornamental shrubs as well as climbers and perennials, are largely exposed. In addition to being characteristically evergreen, these plants are characterised by higher resistance to weather conditions such as low temperatures. However, they cannot always cope with high salt concentrations [Sedaghathoor and Zare 2019]. Common evergreen species along pathways include common ivy (*Hedera helix* L.) [Roeder and Meyer 2022], European boxwood (*Buxus sempervirens* L.) [De Jong et al. 2012] or Japanese spurge (*Pachysandra terminalis* Siebold & Zucc) [Ju et al. 2016].

The aim of the experiments was to investigate the changes resulting from the effects of salinity on three selected species of ornamental evergreen plants: Japanese spurge, European boxwood and common ivy, using three different concentrations of sodium chloride (NaCl) (100, 200 and 300 mM).

MATERIAL AND METHODS

Plants material and growing condition

The plant material consisted of three species of ornamental evergreen plants: Japanese spurge, a perennial, European boxwood, a shrub, and common ivy, a climber. All three species came from the resources of the Department of Ornamental Plants, the Warsaw University of Life Sciences. They were biennial plants planted in P9 pots in peat substrate with pH of 6.5–7. The whole experiment was conducted under greenhouse conditions, where the plants had the same temperature conditions: 15 °C during the day and 6–8 °C at night. In addition, all plants had equal access to natural sunlight. Watering was carried out at a three-day interval.

Experiment design

The experiment was set up on 1 December 2022. 120 plants were used for each of the selected species, resulting in 30 plants per treatment (3 replicates of 10 plants each). In order to introduce the plants to salt stress, watering with an aqueous NaCl solution was applied at three concentrations, chosen on the basis of preliminary test studies and available literature, with 100 mL applied to each pot (Fig. 1). Watering with the salt solution was carried out cyclically every fortnight, giving a total of four treatments. The experiment was completed on 19 January 2023.

Measurements of electrical conductivity (EC)

During the experiment, EC measurements were taken using a METER ProCheck handheld reader to which a TEROS 12 soil moisture, temperature, and

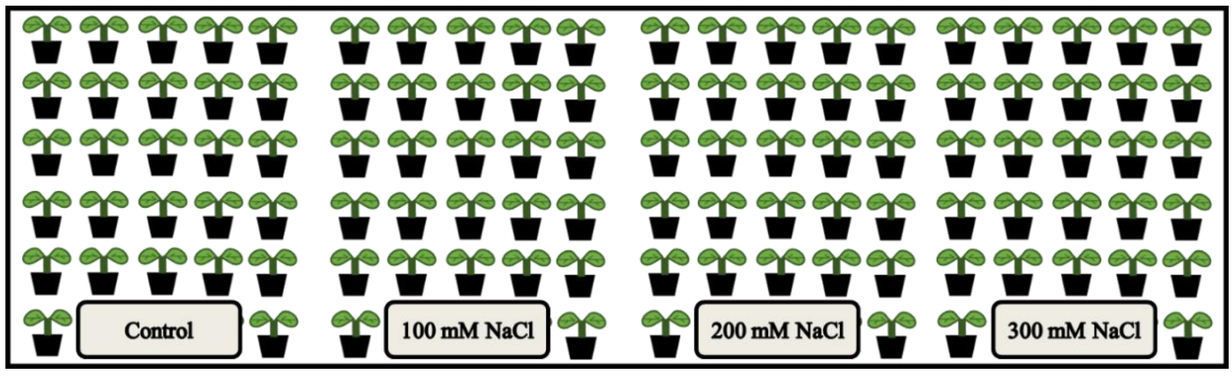

Fig. 1. Scheme of applied concentrations of NaCl

electrical conductivity sensor was connected. These measurements were taken twice, in the middle of the experiment (21.12.2022) and at the end (19.01.2023).

Measurements of growth

Approximately two months after the start of the experiment, shoot length measurements were taken to record differences in the length of new growth in the different combinations. In addition, the necessary photographic documentation was taken during the measurements.

Biochemical analyses

At the end of the experiment, plant material in the form of leaves − four well-developed and free of necrotic and chlorotic damage from each plant − was collected to understand the biochemical changes occurring in plants as a result of salt stress. Until the analyses, the plant material was stored in a deep freezer at –86 °C.

To determine the effect of a high NaCl concentration on the content of the basic plant pigments, chlorophyll and carotenoids, the method of Lichtenthaler and Wellburn [1983] was used. Plant material (0.5 g) was ground in a mortar in the presence of a little quartz sand and 5 mL of cold acetone at a percentage concentration of 80%. The extracts obtained were then filtered through filter paper into 50 mL volumetric flasks and made up to the mark with acetone. After obtaining clear filtrates, the absorbance was measured at four wavelengths − 470 nm, 646 nm, 652 nm and 663 nm.

Soluble proteins were analysed according to the method of Bradford [1976]. A 0.5 g of sample was ground with a mortar in hot 80% ethanol. This was then centrifuged for 20 minutes (min) at 20,000 rpm. After centrifugation, the clear solution had to be transferred into resealable test tubes and made up to 25 mL with ethanol (80%). A 0.1 mL each of the supernatant was taken into the tubes and 5 mL of Bradford reagent was added to it. After thorough mixing, samples were incubated for 5 minutes. After the time had elapsed, the absorbance was measured at 595 nm.

In addition, analyses were carried out to determine the degree of plant stress, i.e. the free proline content was examined according to the method of Bates et al. [1973]. A 0.5 g of plant material was ground with a mortar in 10 mL of a 0.3% aqueous sulphosalicylic acid solution, centrifuged for 20 min at 4 °C at 18,000 rpm. The supernatant was then used in a volume of 2 mL for further analysis. To the supernatant was added 2 mL of reagent A (ninhydrin acid; dissolve 1.25 g of ninhydrin in 30 mL of glacial acetic acid, then add 20 mL of 6 M phosphoric acid − 150 mL of H_3PO_4 per 500 mL of H_2O; this reagent should be kept at 4 °C, its shelf life is 24 h) and 2 mL of glacial acetic acid and incubated for one hour in a water bath at 100 °C. After this time, a cold bath was used to cool the analysed samples. Then 4 mL of toluene was added and mixed to obtain 2 phases. For measurement with a spectrophotometer, the top layer (toluene) was extracted and measured against the standard curve at 520 nm. Pure toluene was used as a blank test.

Malondialdehyde (MDA) content was tested according to the method of Hodges et al. [1999]. A 0.5 g of plant material was ground in 3 mL of 0.1% TCA and then centrifuged for 15 min at 20,000 rpm at 4 °C. After

centrifugation, the supernatant was collected in empty glass tubes. Sample mixtures consisted of 1.5 mL of 0.5% TBA dissolved in 20% TCA, 0.75 mL of supernatant and 0.75 mL of 0.1 M phosphate buffer, pH 7.6. For the blank, 0.1% TCA in a volume of 0.75 mL was used instead of supernatant. The prepared samples were incubated in a water bath for 30 min at 95 °C, with the samples covered with aluminium foil. After the time had elapsed, the samples were centrifuged for 15 min at 20,000 rpm at 4 °C to obtain the clear supernatant needed for spectrophotometric determination at two wavelengths: 532 and 600 nm. The MDA content was calculated according to the formula:

$$\text{MDA content (nmol·mL}^{-1}) = \\ =[(A_{532} - A_{600}) / 155000]\cdot 10^6$$

The hydrogen peroxide content of the plant material was analysed using the method of Siedlecka [2010]. Plant material samples (0.5 g) were ground in K-phosphate buffer using a mortar. After grinding and transferring the samples to plastic tubes, they were centrifuged for 20 min at 20,000 rpm. The obtained supernatants were decanted into glass tubes used for further analysis. A 0.1 mL of extract was transferred to the glass tubes and then made up to 0.5 mL with K-phosphate buffer. To the resulting solution, 0.5 mL of 0.1 M K-phosphate buffer and 1 mL of 1 M potassium iodide (KI) were added. The mixture was mixed and incubated for one hour in the dark. After incubation, the absorbance was measured at 390 nm.

In addition, the activities of the basic oxidative stress enzymes, i.e. catalases [Goth 1991] and peroxidases [Toczko and Grzelińska 2001], were determined.

Catalase activity: The same supernatant used for the hydrogen peroxide assay was employed to determine catalase activity. The samples were divided into two groups. A volume of 0.05 mL of plant extract was transferred into each test tube, followed by the addition of 0.45 mL of potassium phosphate buffer (0.1 M, pH 6.8). Group A samples were supplemented with 1.0 mL of potassium phosphate buffer (0.1 M), while group B samples received 1.0 mL of hydrogen peroxide (H_2O_2) solution (65 µM) prepared in the same buffer. To establish appropriate reaction backgrounds, two control samples were also prepared: sample K (buffer control), containing 1.5 mL of potassium phosphate buffer (0.1 M), and sample C (H_2O_2 control), consisting of 0.5 mL of potassium phosphate buffer (0.1 M) and 1.0 mL of H_2O_2 solution (65 µM), also prepared in the buffer. All tubes were incubated in the dark for 10 min. After this period, 1 mL of 32.5 mM ammonium molybdate was added to each tube and mixed thoroughly. Absorbance was then measured at 405 nm [Goth 1991].

Peroxidase activity: A 0.5 g portion of plant material was homogenised using a Polytron PT 3000 homogeniser in 10 mL of chilled phosphate buffer (50 µM, pH 7.0). The homogenate was centrifuged at 20,000 rpm for 20 min at 4 °C. The resulting supernatant was collected, and the volume was adjusted to 10 mL with the same buffer. For further analysis, 0.5 mL aliquots of the extracts were transferred to test tubes and brought to a final volume of 1.0 mL with 50 µM phosphate buffer. The samples were then divided into two groups: material samples (group A) and complete reaction samples (group B). To each tube, 0.3 mL of 0.2 M phosphate buffer (pH 7.0) was added. Subsequently, 1.4 mL of distilled water was added to group A samples, and 0.4 mL of distilled water to group B samples. The mixtures were vortexed and incubated in a water bath at 25 °C for 5 min. Following incubation, 0.3 mL of 0.2 M pyrogallol was added to each sample. Additionally, group B samples received 1.0 mL of 0.01 M H_2O_2. The reaction mixtures were vortexed again and incubated in a water bath at 25 °C for 10 min. After this step, all tubes were tightly wrapped in black foil. Then, 1.0 mL of 10% H_2SO_4 and 1.0 mL of 10% Na_2SO_3 were added to each sample. The contents were mixed thoroughly, and absorbance was measured at 430 nm using a spectrophotometer. A control sample was prepared in parallel by combining the following reagents: 0.3 mL of 0.2 M phosphate buffer (pH 7.0), 1.4 mL of distilled water, 1.0 mL of 0.01 M H_2O_2, 0.3 mL of 0.2 M pyrogallol, 1.0 mL of 10% H_2SO_4, and 1.0 mL of 10% Na_2SO_3 [Toczko and Grzelińska 2001].

All analyses were carried out for each species in 5 replicates for each combination. Absorbance was measured using a UV-1601 PC spectrophotometer (Shimadzu, Columbia, MD, USA).

Statistical analyses

All collected results were analysed using one-way ANOVA in Statistica software (TIBCO Statistica, TIB-

CO Software Inc., Santa Clara, CA, USA). After performing several tests, including the Shapiro-Wilk test and the Tukey test at a significance level of $p \leq 0.05$, specific homogeneous groups were obtained for each parameter tested.

RESULTS

Changes in electrical conductivity (EC) in tested plants

On both measurement dates, it was observed in all tested species that the electrical conductivity of the soil was highest in the combination with 300 mM NaCl added to the substrate. In this variant, on the final day of the experiment, the values were 89% higher in spurge and more than 85% higher in both boxwood and ivy compared to the measurements taken from the substrate of the control plants (Table 1).

Morphological changes in tested plants

The first changes were observed after just three NaCl treatments, when the plants stopped growing uniformly. In the case of spurge, it was observed that the addition of 200 and 300 mM NaCl to the substrate caused the leaves to lose their vivid green colour, showing signs of yellowing or partial necrosis and drooping (Fig. 2).

With regard to the other two species tested, in combinations with increased NaCl content in the substrate, the plants were characterised by a more stocky abo-

Table 1. Effect of different concentration of NaCl on EC ($dS \cdot m^{-1}$) in tested species in two terms under salinity stress

Species	Term	0 mM NaCl	100 mM NaCl	200 mM NaCl	300 mM NaCl
Pachysandra terminalis	21.12.2022	2.4 ±0.3 a*	11.6 ±3.1 b	13.3 ±2.7 b	25.6 ±1.8 c
	19.01.2023	3.1 ±0.3 a	7.7 ±0.5 b	11.2 ±0.3 c	28.3 ±2.4 d
Buxus sempervirens	21.12.2022	2.1 ±0.4 a	6.2 ±0.3 b	16.8 ±6.4 c	26.1 ±3.0 d
	19.01.2023	3.4 ±0.9 a	9.6 ±1.9 b	14.9 ±1.9 c	22.1 ±1.8 d
Hedera helix	21.12.2022	2.2 ±0.1 a	8.6 ±0.3 b	11.9 ±0.6 c	21.2 ±0.3 d
	19.01.2023	2.8 ±0.3 a	7.8 ±0.5 b	11.5 ±0.6 c	19.2 ±0.4 d

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation

Fig. 2. Effect of salinity on the length of growth of *Pachysandra terminalis* plants. From left: 1 – Control, 2 – 100 mM NaCl, 3 – 200 mM NaCl, 4 – 300 mM NaCl

Fig. 3. Effect of salinity on the length of growth of *Buxus sempervirens* plants. From left: 1 – Control, 2 – 100 mM NaCl, 3 – 200 mM NaCl, 4 – 300 mM NaCl

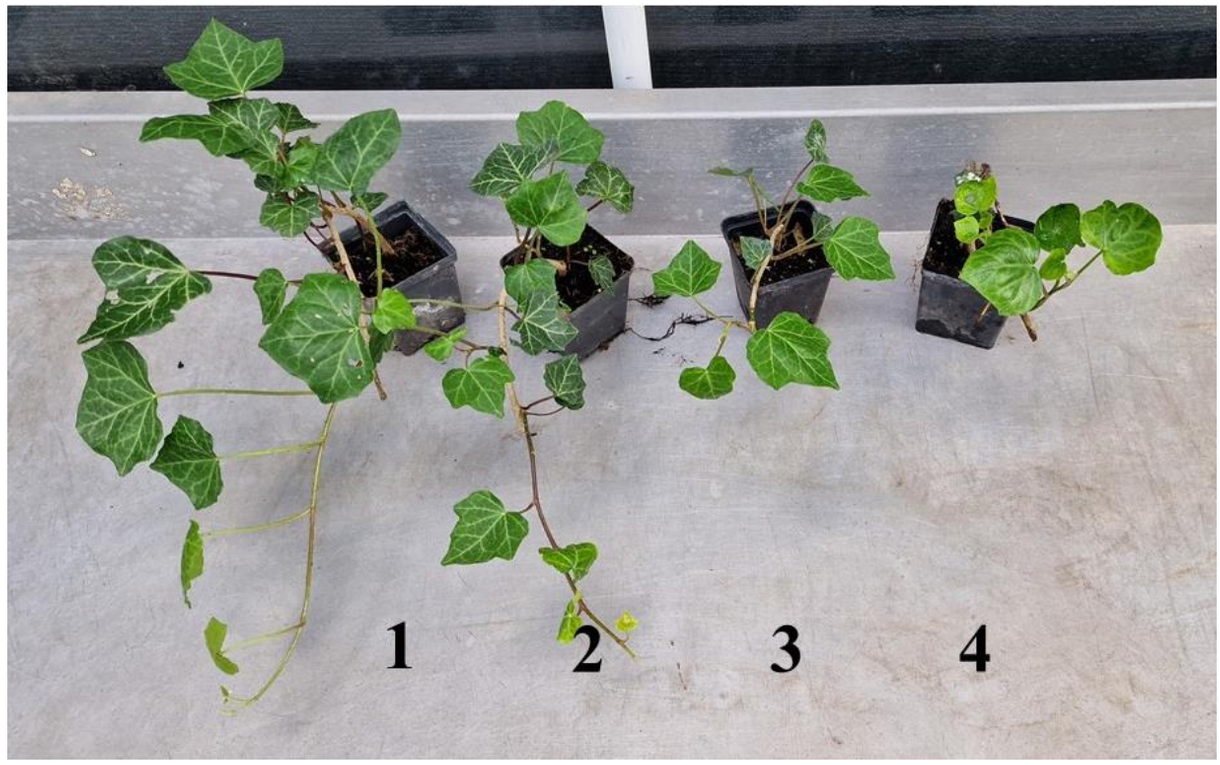

Fig. 4. Effect of salinity on the length of growth of *Hedera helix* plants. From left: 1 – Control, 2 – 100 mM NaCl, 3 – 200 mM NaCl, 4 – 300 mM NaCl

veground structure in boxwood and an inhibition of shoot growth in ivy (Figs 3 and 4).

Measurements taken on the final day of the experiment, after the 4-fold NaCl treatment, showed that in the combination with 300 mM NaCl added to the substrate plant growth was reduced compared to the control − approx. 77% in spurge, approx. 86% in boxwood and even by 94% in ivy.

Biochemical changes

An important aspect is the changes occurring in the plant itself, i.e. biochemical changes, including changes in the content of basic plant compounds, such as chlorophyll, carotenoids or soluble proteins during salinity stress.

In tested plants, it was observed that a high salt concentration in the substrate (300 mM NaCl) resulted in significantly lower chlorophyll content in the plant tissues, by up to 66% compared to the control in spurge, 41% in boxwood and 17% in ivy. During the analysis performed, the changes in carotenoid content were not recorded for all tested species; however, a slight effect of salinity on carotenoid content was observed only in *Buxus sempervirens* (Table 3).

The results of the analysis of soluble protein content show that the effect of a given stressor depends on the species in which it occurs. In spurge, lower protein content was observed in plants from the combination where NaCl was added to the substrate compared to the control plants, while the opposite trend was noted in the other two species. In both boxwood and ivy, the highest protein content was recorded in the plant material from the combination watered with 300 mM NaCl – in boxwood, it was about 28% higher than in the control, and in ivy about 32% higher (Table 3).

A second important aspect of the biochemical changes occurring in the plant as a result of salt stress is the increase in MDA, free proline, and hydrogen peroxide content. In the plants tested, it was observed that the highest MDA content was in the plant material taken from the combination where 300 mM NaCl was added to the substrate, differing from the control by 5.36 μmol kg^{-1} in spurge, 1.91 μmol kg^{-1} in boxwood, and 3.38 μmol kg^{-1} in ivy (Table 4).

In the case of the second parameter indicating the occurrence of stress, i.e. the content of free proline, the highest values were recorded in the plants grown in the substrate treated with 300 mM NaCl. Compared to the control, the levels were approximately 60% higher in spurge and boxwood, and approximately 67% higher in ivy (Table 4).

Regarding the hydrogen peroxide parameter, an increase in its concentration was observed in plant material from all tested species. In common ivy, the value was three times higher than in the control in the combination where 300 mM NaCl was added to the substrate (Table 4).

The natural responses of plants to an increase in hydrogen peroxide are changes in the activities of oxidative stress enzymes, i.e. catalases and peroxidases. In all tested species, catalase activity was highest in plant material taken from combinations where 300 mM NaCl was added to the substrate. In spurge, the value was more than 3-fold higher than in the control, in boxwood more than 4-fold, and in ivy even 5-fold. Turning to the second enzyme analysed, peroxidase, its activity was highly species-dependent. In both spurge and boxwood, the lowest values were obtained for the combinations where 200 and 300 mM NaCl were added to the substrate, where in the case

Table 2. Effect of different concentration of NaCl on the length of growth of *Pachysandra terminalis*, *Buxus sempervirens* and *Hedera helix* (cm)

Species	0 mM NaCl	100 mM NaCl	200 mM NaCl	300 mM NaCl
Pachysandra terminalis	11.0 ±1.8 c*	5.6 ±1.5 b	2.9 ±1.1 a	2.5 ±0.9 a
Buxus sempervirens	13.3 ±1.4 c	13.0 ±1.6 c	3.4 ±1.0 b	1.8 ±0.8 a
Hedera helix	36.5 ±1.9 d	21.1 ±1.8 c	14.0 ±1.3 b	2.2 ±1.3 a

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation

Table 3. Changes in the content of basic compounds in plant material from tested species under salinity stress

Species	NaCl [mM]	Chlorophyll [mg g^{-1} DW]	Carotenoids [mg g^{-1} DW]	Soluble protein [mg g^{-1} DW]
Pachysandra terminalis	0	6.57 ±0.06 d*	0.83 ±0.02 a	4.25 ±0.01 d
	100	5.34 ±0.04 c	0.78 ±0.11 a	3.86 ±0.09 c
	200	5.04 ±0.03 b	0.68 ±0.03 a	3.25 ±0.04 a
	300	2.23 ±0.02 a	0.77 ±0.01 a	3.56 ±0.02 b
Buxus sempervirens	0	8.14 ±0.03 d	0.98 ±0.02 ab	4.54 ±0.03 a
	100	6.63 ±0.02 c	0.94 ±0.05 a	5.23 ±0.18 b
	200	5.86 ±0.06 b	0.91 ±0.03 a	5.23 ±0.04 b
	300	4.80 ±0.03 a	1.04 ±0.02 b	6.27 ±0.02 c
Hedera helix	0	8.85 ±0.01 c	1.42 ±0.04 a	4.87 ±0.04 a
	100	8.26 ±0.05 b	1.44 ±0.07 a	5.87 ±0.08 b
	200	8.34 ±0.05 b	1.43 ±0.03 a	6.09 ±0.03 c
	300	7.33 ±0.05 a	1.34 ±0.05 a	7.15 ±0.03 d

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation
DW – dry weight

Table 4. Changes in the content and activity of important stress parameters in tested species under salinity stress

Species	NaCl [mM]	MDA [µmol kg^{-1}]	Free proline [µmol g^{-1} DW]	H_2O_2 [µg g^{-1} DW]	catalase [mcat g^{-1} DW]	peroxidase [µmol min^{-1} g^{-1} DW]
Pachysandra terminalis	0	2.03 ±0.02 a*	3.32 ±0.04 a	15.41 ±1.05 a	180.99 ±3.69 a	0.084 ±0.003 d
	100	2.14 ±0.11 a	3.94 ±0.11 ab	22.27 ±0.84 b	294.11 ±3.69 b	0.064 ±0,003 c
	200	4.59 ±0.03 b	4.12 ±0.05 b	24.23 ±0.21 b	371.08 ±3.57 c	0.023 ±0.003 b
	300	7.39 ±0.04 c	8.34 ±0.03 c	23.59 ±0.29 b	551.52 ±7.97 d	0.012 ±0.001 a
Buxus sempervirens	0	0.95 ±0.03 a	0.93 ±0.09 a	7.21 ±0.56 a	156.35 ±9.59 a	0.050 ±0.001 c
	100	1.04 ±0.02 a	1.13 ±0.01 a	8.32 ±0.10 ab	298.78 ±18.98 b	0.036 ±0.003 b
	200	2.57 ±0.11 b	1.23 ±0.02 a	9.57 ±0.24 bc	421.11 ±11.11 c	0.026 ±0.002 a
	300	2.86 ±0.31 b	2.31 ±0.02 b	11.29 ±1.15 c	677.02 ±24.96 d	0.034 ±0.002 b
Hedera helix	0	1.94 ±0.05 a	1.42 ±0.02 a	8.75 ±0.64 a	117.29 ±10.14 a	0.037 ±0.001 ab
	100	2.11 ±0.01 a	1.73 ±0.11 ab	10.78 ±0.17 b	426.86 ±28.05 b	0.041 ±0.001 b
	200	3.67 ±0.03 b	1.94 ±0.08 b	18.37 ±0.52 c	479.02 ±17.18 b	0.032 ±0.001 a
	300	5.32 ±0.14 c	4.35 ±0.02 c	25.08 ±0.18 d	629.18 ±16.99 c	0.056 ±0.003 c

* the same letter in the lines indicates no difference between the means at a significance level of α = 0.05 ± means standard deviation
DW – dry weight

of spurge, the difference compared to the control was up to 7-fold. The only species that stood out in terms of peroxidase activity was ivy, as its highest value was recorded in the combination where the highest concentration of NaCl was used for substrate supplementation − about 34% higher than in the control plants (Table 4).

DISCUSSION

Strong stress factors are contributing to the degradation of numerous species sensitive to adverse condi-tions, resulting in a decline in the biodiversity of both natural and urbanised habitats [Razzaq et al. 2020]. Researchers are therefore focusing on understanding how plants respond to abiotic stress conditions (i.e. salinity, drought or high concentrations of heavy metals).

The main site of excessive salt ion accumulation, which is harmful to plants, is the soil in which they grow. It is the pillar of good plant growth and development, and any disturbance is due to inappropriate soil physico-chemical parameters, including the electrical conductivity (EC) value [Passioura 1991, Khalil et al. 2015]. n the experiment carried out by the authors of this paper, it was observed that the higher the NaCl concentration in the substrate, the higher the electrical conductivity, reaching around 20 $dS \cdot m^{-1}$ in all the species tested at a concentration of 300 mM NaCl. A similar trend was obtained by Bekmirzaev et al. [2020], who treated *Tetragonia tetragonioides* Pall. plants with three NaCl concentrations (50, 100 and 200 mM NaCl), as the substrate with the highest NaCl concentration exhibited the highest EC value. Also Wu et al. [2001] confirm that in both soil and container cultivation, the application of NaCl increases the electrical conductivity of the substrate, which was also noted by the authors of the present study.

A plant's longevity largely depends on its structural integrity, i.e. shoot growth, absence of damage to leaf blades, and absence of damage caused by external factors [Kumar et al. 2021]. The research conducted in this study showed that plants grown in a substrate with increasing concentrations of NaCl exhibited reduced growth – in some cases, such as ivy, shoot growth was more than 16 times lower compared to the control. In addition, leaf yellowing, chlorotic changes, and, in the case of spurge, complete leaf loss were observed. Similar visual symptoms of growth reduction in two ornamental shrub species (*Hibiscus rosa-sinensis* L. and *Mandevilla splendens* Hook.f.) in response to high salt concentrations in the substrate were observed by Yu et al. [2021]. Studies conducted on *Rosa chinensis* var. *minima* Rouletii confirm that an increase in EC results in reduced plant growth [Asgari and Diyanat 2020].

An important aspect of proper plant growth and development is an adequate content of biologically active compounds, such as plant pigments or proteins. These are responsible for the most important processes in the plant, such as photosynthesis and the stress response [Simkin et al. 2022, Zhang et al. 2022]. In the course of experiments and analyses, it was found that high concentrations of NaCl in the substrate resulted in a decrease in the content of the most important of plant pigments − chlorophyll, with the value in spurge in the 300 mM NaCl combination differing from the control by approximately 66%. A study by Alam et al. [2020] on *Fortunella japonica* Thunb., *Citrus reshni* Hort. ex Tan and *Citrus maxima* Merr., confirms that the higher the EC the lower the chlorophyll content in plant tissues, where the highest difference in concentration of this pigment was 60%. A reduction in chlorophyll content under salinity stress was also observed in two species of beardtongues (*Penstemon barbatus* Cav. and *Penstemon strictus* Benth.), where, in one of them, the value decreased by approximately threefold compared to the control [Paudel and Sun 2024].

A second important parameter in the structure of plant tissues is the concentration of proteins that accumulate when salinity stress occurs, in order to subsequently store nitrogen that could be reused by the plant in the future. In addition, they can play a role in regulating the osmotic potential in plant cells [Parvaiz and Satyawati 2008]. Experiments and analyses performed on three species of ornamental evergreens showed that these values could vary depending on the tested species, as for two of them, boxwood and ivy, the concentration of soluble proteins was higher in plant material from salt-stressed combinations, while for spurge the values were lower than in the control. A study by Xu et al. [2020] showed that the addition of NaCl to the substrate increases soluble protein content, as *Ginkgo biloba* L. seedlings grown in a substrate with 300 mM NaCl resulted in a 3-fold higher protein concentration in plant tissues. Goharrizi et al. [2020a], in their study conducted on pistachio (*Pistacia* L.) plants subjected to salt stress, confirmed that the addition of NaCl to the substrate can result in a reduction of soluble protein content in plant tissues, which in the present study was obtained by the authors for plant material taken from spurge plants grown in substrate with NaCl.

The contents of malondialdehyde (MDA) [Hamani et al. 2020] and free proline [Hussein and Alshammari 2022] are important indicators of stress occurrence. In a study conducted on three evergreen species, it was found that increasing concentrations of NaCl added to the substrate increased the content of both MDA and free proline. An experiment conducted on little walnut (*Juglans microcarpa* Berlandier) seedlings showed that the concentration of MDA in the plant material was highest at a 300 mM NaCl treatment [Ji et al. 2022]. A more than twofold increase in the content of this parameter was also observed for *Reaumuria songarica* (Pall.) Maxim. seedlings [Yan et al. 2022] and *Sorghum bicolor* (L.) Moench seedlings [Yilmaz et al. 2020]. For the second parameter mentioned above, i.e. the free proline content, the experiment conducted by the authors of this publication showed that higher NaCl concentrations increased the concentration of this compound in the plant material. Similar results were obtained in studies on *Rosmarinus officinalis* Spenn. plants [Hassanpouraghdam et al. 2020], *Portulaca oleracea* L. plants [Hnilickova et al. 2021], and *Linum usitatissimum* L. seedlings [Hussein and Alshammari 2022].

When a stress factor, such as salinity, is intensified, there is a large accumulation of reactive oxygen species (ROS) in plant cells, which are highly toxic to plants [Akyol et al. 2020]. Among the main ones is hydrogen peroxide (H_2O_2). Its elevated content in plant tissues can lead to disruption of the plant metabolism through autophagy of chloroplasts and peroxisomes, ultimately leading to activation of the programmed cell death process [Smirnoff and Arnaud 2019]. In the plant material analysed by the authors, it was observed that higher concentrations of NaCl in the substrate resulted in a strong increase in hydrogen peroxide content in plant tissues. A similar result was obtained by Hassanpouraghdam et al. [2019], who used different NaCl concentrations in *Rosmarinus officinalis* L., and at the highest concentration, 225 mM, a fourfold higher concentration of this compound was recorded than in the control. Also, exposure to high salt concentrations in *Lepidium draba* L. plants results in more than a twofold accumulation of the compound H_2O_2 in plant tissues [Goharrizi et al. 2020b].

Important parameters in the plant response to stress, including the salinity as studied in the experiment, are the activities of antioxidant enzymes. These enzymes are responsible for the breakdown of harmful H_2O_2 into oxygen and water [Berwal et al. 2021], or for reducing its levels through the oxidation of phenolic compounds, during which phenolic polymers are ultimately formed. These polymers contribute to strengthening the cell wall and inhibiting the penetration of harmful compounds into the cells [Kidwai et al. 2020]. Analysis of the plant material collected from the experimental plants revealed that catalase activity also increased with increasing H_2O_2 levels. The effect of sodium chloride on pistachio plants confirmed this relationship, as the activity of this enzyme in stressed plants relative to control plants was significantly higher [Goharrizi et al. 2020a]. The study conducted on *Antigonon leptopus* Hook. & Arn. also confirms the results obtained in the authors' experiment, indicating that the increase in catalase activity in the plant is correlated with the rise in NaCl concentration applied to the substrate [El-Zaiat et al. 2020]. For the second oxidative stress enzyme tested, peroxidase, results varied depending on whether the species produced a defence system or not, as the activity of this enzyme was higher only in ivy. A similar relationship to that observed in ivy was found in the study by Jha and Subramanian [2013] on *Oryza sativa* L. or in the study by Cai and Gao [2020] on *Chenopodium quinoa* Willd. The opposite situation was observed by the authors of this paper in spurge and boxwood, where the peroxidase activity in stressed plants was comparable to or lower than in the control. Xu et al. [2020], in an experiment where *Ginkgo biloba* L. plants were subjected to salinity stress, obtained a similar result, as the application of 200 and 300 mM NaCl caused a decrease in the activity of this antioxidant enzyme.

CONCLUSION

The experiments and analyses carried out suggest that the response of ornamental evergreen plants to salinity is species-specific. The use of Japanese spurge in areas exposed to stress factors such as salinity was found to be inappropriate, as the plants may die back or lose their ornamental value. In contrast, in the other two species tested, the changes were not as drastic, as periodic increases in salt concentrations in the substrate led only to stunted growth, without dieback. As

far as biochemical changes are concerned, the analyses performed show that salt stress can cause severe disturbances in the content of essential compounds in the plant. The analyses revealed that the activation of defence mechanisms, indicated by increased activity of specific oxidative stress enzymes, was species-dependent.

This research provides a foundation for further investigation into the mechanisms by which plants respond to stress conditions. Given the widespread use of evergreen species, it is also important to deepen our understanding of these mechanisms within this group and to develop strategies to mitigate the effects of stress factors.

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REFERENCES

Ahmadi, M., Souri, M.K. (2020). Growth characteristics and fruit quality of chili pepper under higher electrical conductivity of nutrient solution induced by various salts. AGRIVITA J. Agric. Sci., 42(1), 143–152. http://doi.org/10.17503/agrivita.v42i1.2225

Akyol, T.Y., Yilmaz, O., Uzilday, B., Uzilday, R.Ö., Türkan, İ. (2020). Plant response to salinity. An analysis of ROS formation, signaling, and antioxidant defense. Turk. J. Bot., 44(1), 1–13. https://doi.org/10.3906/bot-1911-15

Alam, A., Ulla, H., Attia, A., Datta, A. (2020). Effects of salinity stress on growth, mineral nutrient accumulation and biochemical parameters of seedlings of three citrus rootstocks. Int. J. Fruit Sci., 20(4), 786–804. https://doi.org/10.1080/15538362.2019.1674762

Asgari, F., Diyanat, M. (2020). Effects of silicon on some morphological and physiological traits of rose (*Rosachinensis* var. *minima*) plants grown under salinity stress. J. Plant Nutr., 44(4), 536–549. https://doi.org/10.1080/01904167.2020.1845367

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

Bekmirzaev, G., Ouddane, B., Beltrão, J., Fujii, Y. (2020). The impact of salt concentration on the mineral nutrition of *Tetragonia tetragonioides*. Agriculture, 10(6), 238. https://doi.org/10.3390/agriculture10060238

Berwal, M.K., Kumar, R., Prakash, K., Rai, G.K., Hebbar, K.B. (2021). Abiotic stress tolerance mechanisms in plants. 1st ed. CRC Press, Boca Raton, FL, USA, 175–202.

Biczak, R., Pawłowska, B., Feder-Kubis, J. (2016). [Growth inhibition and oxidative stress in plants under the influence of chiral imidazolium ionic liquid with tetrafluoroborate anion]. Chem. Environ. Biotechnol., 19, 35–45 [in Polish].

Boorboori, M.R., Li, J. (2025). The effect of salinity stress on tomato defense mechanisms and exogenous application of salicylic acid, abscisic acid, and melatonin to reduce salinity stress. Soil Sci. Plant Nutr., 71(1), 93–110. https://doi.org/10.1080/00380768.2024.2405834

Bradford, M.M. (1976). A rapid and sensitive metod for the quantification of microgram quantities of protein utilizng the principle of protein dye binding. Anal. Biochem., 72(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cai, Z.Q., Gao, Q. (2020). Comparative physiological and biochemical mechanisms of salt tolerance in five contrasting highland quinoa cultivars. BMC Plant Biol., 20(1), 1–15. https://doi.org/10.1186/s12870-020-2279-8

Cerrato, M.D., Mir-Rosselló, P.M., Cortés-Fernández, I., Ribas-Serra, A., Douthe, C., Cardona, C., Sureda A., Flexas J., Gil Vives, L. (2024). Insights on physiological, antioxidant and flowering response to salinity stress of two candidate ornamental species: the native coastal geophytes *Pancratium maritimum* L. and *Eryngium maritimum* L. Physiol. Mol. Biol. Plants, 30(9), 1533–1549. https://doi.org/10.1007/s12298-024-01502-0

Cirillo, C., Rouphael, Y., Caputo, R., Raimondi, G., Sifola, M., De Pascale, S. (2016). Effects of high salinity and the exogenous application of an osmolyte on growth, photosynthesis, and mineral composition in two ornamental shrubs. J. Hortic. Sci. Biotechnol., 91, 14–22. https://doi.org/10.1080/14620316.2015.1110988

Corwin, D.L., Yemoto, K, 2020. Salinity. Electrical conductivity and total dissolved solids. Soil Sci. Soc. Am. J., 84(5), 1442–1461. https://doi.org/10.1002/saj2.20154

De Jong, S., Addink, E., Hoogenboom, P., Nijland, W. (2012). The spectral response of *Buxus sempervirens* to different types of environmental stress. A laboratory experiment. ISPRS J. Photogramm. Remote Sens., 74, 56–65. https://doi.org/10.1016/j.isprsjprs.2012.08.005

Devecchi, M., Remotti, D. (2004). Effect of salts on ornamental ground covers for green urban areas. Acta Hortic., 643, 153–156. https://doi.org/10.17660/ActaHortic.2004.643.18

Dustnazarova, S., Khasanov, A., Khafizova, Z., Davronov, K. (2021). The threat of saline lands, for example, in the Republic of Uzbekistan. E3S Web of Conf. 284, 02002.

El-Zaiat, R.A., El-Sayed, I.M., Taha, L.S., Abrahim, E.A. (2020). Enzyme activity of micropropagated *Antigonon leptopus* plant under effect of salinity stress. Plant Arch., 20, 3599–3605.

FAO (2023). GSASmap. Global Soil Partnership. Food and Agriculture Organization of the United Nations. Available: https://www.fao.org/global-soil-partnership/gsasmap/en [date of access: 26.05.2025].

FAO (2024). FAO launches first major global assessment of salt-affected soils in 50 years. Food and Agriculture Organization of the United Nations. Available: https://www.fao.org/newsroom/detail/fao-launches-first-major-global-assessment-of-salt-affected-soils-in-50-years/en [date of access: 26.05.2025].

Goharrizi, K.J., Baghizadeh, A., Kalantar, M., Fatehi, F. (2020a). Combined effects of salinity and drought on physiological and biochemical characteristics of pistachio rootstocks. Sci. Hortic., 261, 108970. https://doi.org/10.1016/j.scienta.2019.108970

Goharrizi, K.J., Riahi-Madvar, A., Rezaee, F., Pakzad, R., Bonyad, F.J., Ahsaei, M.G. (2020b). Effect of salinity stress on enzymes' activity, ions concentration, oxidative stress parameters, biochemical traits, content of sulforaphane, and CYP79F1 gene expression level in *Lepidium draba* plant. J. Plant Growth Regul., 39, 1075–1094. https://doi.org/10.1007/s00344-019-10047-6

Goth, L. (1991). A simple method for determination of serum catalase activity and revision range. Clin. Chim. Acta., 196, 143–151.

Gupta, B., Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. Int. J. Genom., 2014, 701596. https://doi.org/10.1155/2014/701596

Hakim, M.A., Juraimi, A.S., Hanafi, M.M., Ismail, M.R., Selamat, A., Rafii, M.Y., Latif, M.A. (2014). Biochemical and anatomical changes and yield reduction in rice (*Oryza sativa* L.) under varied salinity regimes, Biomed Res. Int., 2014, 208584. https://doi.org/10.1155/2014/208584

Hamani, A.K.M., Wang, G., Soothar, M.K., Shen, X., Gao, Y. Qiu, R., Mehmood, F. (2020). Responses of leaf gas exchange attributes, photosynthetic pigments and antioxidant enzymes in NaCl-stressed cotton (*Gossypium hirsutum* L.) seedlings to exogenous glycine betaine and salicylic acid. BMC Plant Biol., 20, 434. https://doi.org/10.1186/s12870-020-02624-9

Hanin, M., Ebel, C., Ngom, M., Laplaze, L., Masmoudi, K. (2016). New insights on plant salt tolerance mechanisms and their potential use for breeding. Front. Plant Sci., 7, 1787. https://doi.org/10.3389/fpls.2016.01787

Hassanpouraghdam, M.B., Mehrabani, L.V., Tzortzakis, N. (2020). Foliar application of nano-zinc and iron affects physiological attributes of *Rosmarinus officinalis* and quietens NaCl salinity depression. J. Soil Sci. Plant Nutr., 20, 335–345. https://doi.org/10.1007/s42729-019-00111-1

Hnilickova, H., Kraus, K., Vachova, P., Hnilicka, F. (2021). Salinity stress affects photosynthesis, malondialdehyde ofrmation, and proline content in *Portulaca oleracea* L. Plants., 10, 845. https://doi.org/10.3390/plants10050845

Hodges, D.M., Delong, J.M., Forney, C.F, Prange, R.K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta., 207, 604–611. https://doi.org/10.1007/s004250050524

Hussein, H.A.A., Alshammari, S.O. (2022). Cysteine mitigates the effect of NaCl salt toxicity in flax (*Linum usitatissimum* L.) plants by modulating antioxidant systems. Sci. Rep., 12(1), 11359. https://doi.org/10.1038/s41598-022-14689-7

Jameel, J., Anwar, T., Majeed, S., Qureshi, H., Siddiqi, E.H., Sana, S., Zaman, W., Ali, H.M. (2024). Effect of salinity on growth and biochemical responses of brinjal varieties: implications for salt tolerance and antioxidant mechanisms. BMC Plant Biol., 24(1), 128. https://doi.org/10.1186/s12870-024-04836-9

Janz, D., Lautner, S., Wildhagen, H., Behnke, K., Schnitzler, J., Rennenberg, H., Fromm, J., Polle, A. (2012). Salt stress induces the formation of a novel type of 'pressure wood' in two *Populus* species. New Phytol., 194(1), 129–141. https://doi.org/10.1111/j.1469-8137.2011.03975.x

Jha, Y., Subramanian, R.B. (2013). Paddy plants inoculated with PGPR show better growth physiology and nutrient content under saline conditions. Chil. J. Agr. Res., 73(3), 213–219. https://doi.org/10.4067/s0718-58392013000300002

Ji, X., Tang, J., Zhang, J. (2022). Effects of salt stress on the morphology, growth and physiological parameters of *Juglans microcarpa* L. seedlings. Plants., 11, 2381. https://doi.org/10.3390/plants11182381

Ju, J.H., Choi, E.Y., Yoon, Y.H. (2016). A pilot study to determine the substrate threshold for heavy metal toxicity in groundcover plants used in urban landscapes. Appl. Ecol. Environ. Res., 14, 59–70. http://dx.doi.org/10.15666/aeer/1404_059070

Khalil, H.A., Hossain, M.S., Rosamah, E., Azli, N.A., Saddon, N., Davoudpoura, Y., Islam, M.N., Dungani, R. (2015). The role of soil properties and it's interaction towards quality plant fiber. A review. Renew. Sustain. Energy Rev., 43, 1006–1015. https://doi.org/10.1016/j.rser.2014.11.099

Kibria, M.G., Hoque, M.A. (2019). A review on plant responses to soil salinity and amelioration strategies. Open J. Soil Sci., 9, 219–231. https://doi.org/10.4236/ojss.2019.911013

Kidwai, M., Ahmad, I.Z., Chakrabarty, D. (2020). Class III peroxidase. An indispensable enzyme for biotic/abiotic stress tolerance and a potent candidate for crop improvement. Plant Cell Rep., 39, 1381–1393. https://doi.org/10.1007/s00299-020-02588-y

Kim, Y., Mun, B.G., Khan, A.L., Waqas, M., Kim, H.H., Shahzad, R., Imram, M., Yun, B.W., Lee, I.J. (2018). Regulation of reactive oxygen and nitrogen species by salicylic acid in rice plants under salinity stress conditions. PLoS One, 13(3), e0192650. https://doi.org/10.1371/journal.pone.0192650

Kumar, S., Li, G., Yang, J., Huang, X., Ji, Q., Liu, Z., Ke, W., Hou, H. (2021). Effect of salt stress on growth, physiological parameters, and ionic concentration of water dropwort (*Oenanthe javanica*) cultivars. Front. Plant Sci., 12, 660409. https://doi.org/10.3389/fpls.2021.660409

Li, W., Li, Q. (2017). Effect of environmental salt stress on plants and the molecular mechanism of salt stress tolerance. Int. J. Environ. Sci. Nat. Res., 7(3), 555714. https://doi.org/10.19080/IJESNR.2017.07.555714

Lichtenthaler, H.K., Wellburn, A.R. (1983). Determinations of total carotenoids and chlorophylls a and b leaf extracts in different solvents. Biochem. Soc. Trans., 603, 591–592.

Lu, Y., Zeng, F., Li, X., Zhang, B. (2021). Physiological changes of three woody plants exposed to progressive salt stress. Photosynthetica, 59, 171–184. https://doi.org/10.32615/ps.2021.007

Marosz, A. (2004). Effect of soil salinity on nutrient uptake, growth, and decorative value of four ground cover shrubs. J. Plant Nutr., 27(6), 977–989. https://doi.org/10.1081/PLN-120037531

Marosz, A. (2011). Effect of green waste compost and mycorrhizal fungi on calcium, potassium, and sodium uptake of woody plants grown under salt stress. Water Air Soil Pollut., 223, 787–800. https://doi.org/10.1007/s11270-011-0902-x

Parvaiz, A., Satyawati, S. (2008). Salt stress and phyto-biochemical responses of plants – a review. Plant Soil Environ., 54(3), 89–99. https://doi.org/10.17221/2774-PSE

Passioura, J.B. (1991). Soil structure and plant growth. Aust. J. Soil Res., 29(6), 717–728. https://doi.org/10.1071/SR9910717

Paudel, A., Sun, Y. (2024). Effect of salt stress on the growth, physiology, and mineral nutrients of two penstemon species. HortSci., 59(2), 209–219. https://doi.org/10.21273/HORTSCI17409-23

Rahneshan, Z., Nasibi, F., Moghadam, A. (2018). Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. J. Plant Interact., 13, 73–82. https://doi.org/10.1080/17429145.2018.1424355

Razzaq, A., Ali, A., Safdar, L.B., Zafar, M.M., Rui, Y., Shakeel, A., Shaukat, A., Ashraf, M., Gong, W., Yuan, Y. (2020). Salt stress induces physiochemical alterations in rice grain composition and quality. J Food Sci. Jan., 85(1), 14–20. https://doi.org/10.1111/1750-3841.14983

Roeder, M., Meyer, K. (2022). English Ivy (*Hedera helix*) is fast, but Ash (*Fraxinus excelsio*r) too. Decomposition of English Ivy litter compared to four common host trees. A multisite citizen sciences project. Acta Oecol., 115, 103832. https://doi.org/10.1016/j.actao.2022.103832

Safdar, H., Amin, A., Shafiq, Y., Ali, A., Yasin, R., Sarwar, M.I. (2019). A review. Impact of salinity on plant growth. Nat. Sci., 1, 34–40. https://doi.org/10.7537/marsnsj170119.06

Sedaghathoor, S., Zare, S.K.A. (2019). Interactive effects of salinity and drought stresses on the growth parameters and nitrogen content of three hedge shrubs. Cogent. Environ. Sci., 5(1). https://doi.org/10.1080/23311843.2019.1682106

Shahid, M., Sarkhosh, A., Khan, N., Balal, R., Ali, S., Rossi, L., Gómez, C., Mattson, N., Jatoi, W., García-Sánchez, F. (2020). Insights into the physiological and biochemical impacts of salt stress on plant growth and development. Agronomy, 10, 938. https://doi.org/10.3390/agronomy10070938

Siedlecka, M. (2010). Skrypt do ćwiczeń z fizjologii roślin [Script for plant physiology exercises]. Uniwersytet Warszawski [Warsaw University], Zakład Molekularnej Fizjologii Roślin [Department of Molecular Plant Physiology], Warszawa, 28–29 [in Polish].

Simkin, A.J., Kapoor, L., Doss, C.G.P., Hofmann, T.A., Lawson, T., Ramamoorthy, S. (2022). The role of photosynthesis related pigments in light harvesting, photoprotection and enhancement of photosynthetic yield

in planta. Photosynth. Res., 152(1), 23–42. https://doi.org/10.1007/s11120-021-00892-6

Smirnoff, N., Arnaud, D. (2019). Hydrogen peroxide metabolism and functions in plants. New Phytol., 221(3), 1197–1214. https://doi.org/10.1111/nph.15488

Thaker, P., Brahmbhatt, N., Shah, K. (2021). A review: impact of soil salinity on ecological, agricultural and socio-economic concerns. Int. J. Adv. Res., 9, 979–986. http://dx.doi.org/10.21474/IJAR01/13200

Toczko, M., Grzelińska, A. (2001). Materiały do ćwiczeń z biochemii [Biochemistry exercise materials]. Wydawnictwo SGGW, Warszawa, 99–101 [in Polish].

Toscano, S., Branca, F., Romano, D., Ferrante A. (2020). An evaluation of different parameters to screen ornamental shrubs for salt spray tolerance. Biology, 9, 250. https://doi.org/10.3390/biology9090250

Wu, L., Guo, X., Hunter, K., Zagory, E.M., Waters, R., Brown, J. (2001). Studies of salt tolerance of landscape plant species and california native grasses for recycled water irrigation. Slosson Report, 1–14. Available: http://slosson.ucdavis.edu/newsletters/Wu_200129031.pdf [date of access: 8.06.2024].

Xu, N., Liu, S., Lu, Z., Pang, S., Wang, L., Wang, L., Li, W. (2020). Gene expression profiles and flavonoid accumulation during salt stress in *Ginkgo biloba* seedlings. Plants, 9, 1162. https://doi.org/10.3390/plants9091162

Yan, S., Chong, P., Zhao, M. (2022). Effect of salt stress on the photosynthetic characteristics and endogenous hormones, and: A comprehensive evaluation of salt tolerance in *Reaumuria soongorica* seedlings, Plant Signal. Behav., 17(1), 2031782. https://doi.org/10.1080/15592324.2022.2031782

Yilmaz, S., Temizgül, R., Yürürdurmaz, C., Kaplan, M. (2020). Oxidant and antioxidant enzyme response of redbine sweet sorghum under NaCl salinity stress. Bioagro, 32(1), 31–38.

Yu, X., Her, Y., Chang, A., Song, J.H., Campoverde, E.V., Schaffer, B. (2021). Assessing the effects of irrigation water salinity on two ornamental crops by remote spectral imaging. Agronomy, 11, 375. https://doi.org/10.3390/agronomy11020375

Zhang, H., Zhu, J., Gong, Z., Zhu, J.K. (2022). Abiotic stress responses in plants. Nat. Rev. Genet., 23(2), 104–119. https://doi.org/10.1038/s41576-021-00413-0

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PLANT-PARASITIC NEMATODES ASSOCIATED WITH *Citrus aurantiifolia* (Christm.) SWINGLE AND THEIR RELATIONSHIP WITH SOIL TYPE

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ABSTRACT

Plant-parasitic nematodes (PPNs) pose a significant challenge to citrus farming worldwide, but their distribution and impact in the Al-Ahsa Oasis, Saudi Arabia (KSA), remain poorly understood. This study investigates the prevalence, diversity, and ecological dynamics of PPNs associated with Hasawi Lumi (*Citrus aurantiifolia*) trees, a key crop in the region. During the summer of 2024, a survey was performed in ten major Hasawi Lumi-growing areas, with 250 soil and root samples collected. Four genera of PPNs were identified, with *Tylenchulus semipenetrans* (52%), *Helicotylenchus* (44.8%), *Pratylenchus* (42.8%), and *Xiphinema* (22%) being the most prevalent. The physicochemical properties of the soil (e.g., texture, pH, and minerals) were determined to assess their impact on nematode populations. Our results revealed that soil characteristics significantly affect the distribution of PPNs, with sandy soils and moderate organic matter favoring nematode diversity, while high salinity suppresses it. The current research constitutes the initial attempt to assess PPNs in Hasawi Lumi orchards and offers important recommendations that can be implemented to improve citrus fruit yield in Al-Ahsa Oasis. These results indicate that soil factors must be considered in any attempt to manage nematode infection, reflecting the necessity for adopting strategies to improve the productivity of citrus crops in the concerned area.

Keywords: citrus crops, diversity, Hasawi Lumi, plant-parasitic nematodes, soil properties, *Tylenchulus semipenetrans*

INTRODUCTION

Citrus is among the frequently produced fruit crops, especially in regions with tropical and subtropical climates [Maqbool et al. 2023]. Two-thirds of the global citrus production is concentrated in the US, China, Brazil, Mexico, India, Spain, and Egypt [Abd-Elgawad et al. 2016, Khan et al. 2021], with annual global output surpassing 100 million tons [Zou et al. 2016]. In Saudi Arabia (KSA), Hasawi Lumi (*Citrus aurantiifolia*) is a major fruit crop of Al-Ahsa Oasis. However, the Hasawi Lumi sector faces significant production constraints. Generally, the spread of plant-parasitic nematodes (PPNs) reduces citrus quality and yield in KSA [Al-Yahya 1988, Al-Hazmi 1997]. Citrus rhizospheres host several PPNs, including *Belonolaimus longicaudatus*,

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Helicotylenchus, *Hoplolaimus*, *Longidorus*, *Tylenchulus semipenetrans*, *Pratylenchus coffee*, *Radopholus similis*, and *Meloidogyne* spp., that inflict substantial economic damage on a global scale [Hammam et al. 2021]. Other nematodes, such as *Pratylenchus brachyurus*, *Pratylenchus vulnus*, *Hemicycliophora arenaria*, *Hemicycliophora nudata*, *Paratrichodorus lobatus*, *Paratrichodorus minor*, *Xiphinema brevicolle*, and *Xiphinema index*, are considered minor pests due to their limited impact or regional distribution [Badii et al. 2015]. The nematode *T. semipenetrans*, which infects citrus plants, is linked to many rootstocks in many citrus cultivation areas in KSA [Eissa 1979]. Its prevalence varies globally, with Egypt reporting 99.1%, northern Iran at 89%, California and Florida (USA) at 26%, Morocco at 88%, and Spain showing a range of 70% to 90% [Sorribas et al. 2008, Mokrini et al. 2018, Zoubi et al. 2022]. Drought conditions exacerbate the impact of nematode infestations, impairing the root system's capacity to uptake water and minerals [Duncan 2005, Maafi and Damadzadeh 2008].

The occurrence, density, and distribution of plant-parasitic nematodes in Hasawi Lumi trees in Al-Ahsa Oasis, along with their correlation with soil physicochemical characteristics, remain largely unexplored. This study aims to address this gap by:

1. Conducting an extensive survey of PPNs in the main Hasawi Lumi-growing zones of Al-Ahsa Oasis, where data is currently lacking.
2. Evaluating the PPNs' incidence, distribution, and diversity in the rhizosphere of Hasawi Lumi trees.
3. Investigating how soil variables influence PPN populations.

Additionally, this study aims to characterize the PPN communities infecting Hasawi Lumi trees in Al-Ahsa to improve management strategies.

MATERIALS AND METHODS

Survey and sample acquisition

Sampling was carried out during the summer of the 2024 growing season in Al-Ahsa Oasis, Saudi Arabia. The prevailing environmental conditions included high temperature (average: 38–45 °C), low humidity (20–30%), and minimal rainfall. A survey was performed in the ten primary Hasawi Lumi (*Citrus aurantiifolia*) cultivation regions, including Al-Bataliyah, Al-Bustan, Al-Halila, Al-Hofuf, Al-Jubail, Al-Omran, Al-Shaharin, Al-Shiraa, Al-Tarabil, and Briqa. From the rhizosphere of several Hasawi Lumi trees, 250 soil and root samples were collected at a depth of 20–40 cm using a stainless steel auger, as illustrated in Figure 1.

A 1 kg composite sample of soil and roots was gathered from each sampling location along a zigzag pattern. To avoid moisture evaporation, the samples were sealed in plastic bags and kept at 5 °C until further examination. Nematode-related investigations were conducted at the Nematology Laboratory within the Arid Land Agriculture Department, King Faisal University's College of Agricultural and Food Sciences (https://maps.app.goo.gl/4arHC1ZGM1Q2etcp9).

Extraction and identification of nematodes

The modified Baermann method was employed to isolate mobile nematodes from soil and root samples [Hooper et al. 2005]. Each sample's roots were rinsed under tap water and sectioned into 1 cm slices, using 25 g for nematode extraction. Nematodes were extracted from 250 g soil samples utilizing an adapted version of the Baermann technique. After extraction, nematodes were retained for processing. The number of individuals per 250 g of soil and 25 g of root fragments was counted. Nematodes were classified by genus based on morphological characteristics using stereomicroscopes, supplemented by light microscopy for detailed observations according to Mai et al. [1996]. For preservation, nematode specimens were soaked in 4% hot formalin–formaldehyde [Ryss 2017]. They were then put in a 7-centimeter square watch glass containing liquid I (99 parts 4% formaldehyde and 1 part pure glycerol). The latter was desiccated in one-tenth of its volume of 96% ethanol for 12 hours at 40 °C. The watch glass with nematodes was then detached from the desiccator and transferred to a 37 °C incubator. Nematodes were prepared using a dehydration solution of 95% ethanol and 5% pure glycerol, referred to as Dehydration Liquid II. Three milliliters of liquid II were added to the watch glass, which was partially covered to facilitate evaporation. Next, 2 mL of liquid III (a 1:1 mixture of pure glycerol and 96% ethanol) was introduced, and the watch glass was incubated overnight at 37 °C. Nematodes were generated on microscope slides for observation and identification using light microscopy.

Fig. 1. Survey Map of the ten regions of Hasawi Lumi-growing in Al-Ahsa Oasis, KSA (Ministry of Environment, Water and Agriculture, MEWA)

Nematode community assessment

The incidence and diversity of nematodes were calculated using prevalence, mean intensity, and maximum density according to Bello et al. [2020]:

$$\% \text{ Prevalence} = (\text{Number of Infected Samples} / \text{Total Samples}) \times 100$$

$$\text{Mean intensity} = (\text{Total Nematode Species in Infected Samples} / \text{Number of Infected Samples}) \times 100$$

$$\text{Maximum density} = \text{the Maximum Number of Certain Nematode Species Recovered}$$

DNA extraction, PCR amplification, sequencing, and phylogenetic analysis

DNA was isolated from four individual worms for molecular identification, as previously outlined by Holterman et al. [2006]. The D2–D3 segment was amplified utilizing forward primer D2a (5′-ACA AGTACC GTG AGG GAA AGT TG-3′) and reverse primer D3b (5′-TCG GAA GGA ACCAGC TAC TA-3′) following De Ley et al. [1999]. One microliter (µL) of DNA was included in the PCR reaction mixture, which consisted of 22 µL of ddH2O, 25 µL of 2 × OnePCR™ (GeneDireX, Germany, Cat. No. MB203-0100), and 1 µM of each of the two primers. PCR was performed on the C1000 Touch PCR thermal cycler (Bio-Rad) using the following conditions: 5 minutes of denaturation at 95 °C, followed by 35 cycles of 1 minute at 94 °C, 45 seconds at 49 °C, and 1 minute at 72 °C. The final elongation was 8 minutes at 72 °C. Five µL of each PCR product with 1 µL of 6 × loading buffer (Fermentas Life Sciences, Germany) were electrophoresed on 1% Tris-Borate-EDTA (TBE) buffer.

After electrophoresis at 100 V for 40 minutes, the gel was stained with ethidium bromide (0.1 µg mL^{-1}) for 20 minutes and then examined and photographed under ultraviolet light. PCR product purification and sequencing were carried out at Macrogen, South Korea, in both directions.

The new sequences were blasted against the NCBI database for sequence identity, and three representative sequences were downloaded for each new sequence. Phylogenetic analysis was executed using MEGA11, with the Maximum Likelihood approach and the Kimura 2-parameter model. The tree was assessed using 1000 bootstrap replications.

Soil assessment

Surface and sub-surface soil samples were collected in triplicate using a stainless steel auger at two depths (20 cm and 40 cm) to account for variations in soil properties throughout the study region. Sampling locations were selected randomly within predefined areas to represent diverse soil types. The specimens underwent air drying, followed by pulverization and filtration through a 2 mm sieve to ensure they were ready for physicochemical evaluation. To determine the particle size distribution (including sand, silt, and clay amounts), we employed the hydrometer technique [Gee and Bauder 2018], which adheres to sedimentation principles. A calibrated pH meter was used to estimate the pH of the soil on a saturated soil paste, following the USDA Handbook [Sparks et al. 2020]. Electrical conductivity (EC) was assessed in a 1 : 5 soil-to-water extract utilizing an EC meter, providing a reliable estimate of soil salinity. The sodium adsorption ratio (SAR) was evaluated following Page et al. [1982] using the equation:

$$SAR = \frac{Na}{\sqrt{\frac{Ca + Mg}{2}}}$$

where: Ca, Na, and Mg refer to calcium, sodium, and magnesium concentrations in mmol L^{-1}.

Cation concentrations were measured using flame photometry or atomic absorption spectroscopy for accuracy and precision in chemical analysis. All physicochemical analyses were conducted at the Soil Laboratory, Environment and Natural Agricultural Resources Department, King Faisal University's College of Agricultural and Food Sciences.

Taxonomic diversity

The taxonomic diversity of PPNs was evaluated through two nematode indices. The Shannon-Wiener and Evenness indices are the two indices referenced (https://www.omnicalculator.com/ecology/shannon-index).

Data processing

Data were compared using Duncan's Multiple Range Test (DMRT) at a 5% level of significance, as determined by SPSS software version 23 [SPSS 2016]. One-way ANOVA was employed to statistically evaluate the obtained data and determine whether there were any significant differences between the means of the analyzed parameters. A principal component analysis (PCA) was conducted to delineate the distribution of nematode taxa and soil properties based on their sampling locations. The FactoMineR package [Lê et al. 2008] was utilized for this purpose, while the factoextra package [Kassambara and Mundt 2020] was employed to generate the corresponding biplot.

RESULTS

Distribution and diversity of PPN populations

Four genera of PPNs were recognized through morphological analysis in soil and root samples obtained from the Hasawi Lumi orchards. Table 1 presents the prevalence, maximum density, and mean intensity for each analyzed region. Mean intensity and maximum density serve as quantitative metrics derived to assess the relative abundance of PPNs in soil and root systems and to gauge the potential distribution of nematodes within the plant environment. Citrus, spiral, lesion, and dagger nematodes were identified in all assessed Hasawi Lumi cultivation regions. The predominant PPNs identified in root and soil samples were citrus, spiral, and lesion nematodes, exhibiting prevalence rates reaching as high as 52%, 44.8%, and 42.8%, respectively. Dagger nematodes constituted 22% of the overall nematode population. Citrus nematode had significant prevalence in Briqa (72%), Al-Jubail and Al-Omran (64%), Al-Shiraa (60%), Al-Bataliyah (56%), Al-Bustan (52%), Al-Halila and Al-Shaharin (48%); however, it was the least widespread in Al-Hofuf (28%) and Al-Tarabil (24%). The citrus nematode exhibited the most significant overall density in Al-Jubail, reaching 533 individuals/soil sample (Figure 2). Soil and root analyses revealed the presence of several genera, including *Helicotylenchus*, *Pratylenchus*, *Tylenchulus*, and *Xiphinema*, with densities of 5 to 150 individuals per 250 g of soil.

The molecular identification results, as illustrated in Figure 3, confirmed the morphological classification of PPNs in Hasawi Lumi orchards. Phylogenetic analysis of the D2–D3 segment of the 28S rDNA revealed a high genetic similarity (≥99%) between the identified nematode species and their corresponding GenBank references. The predominant species, *Tylenchulus semipenetrans*, exhibited strong phylogenetic clustering, supporting its widespread prevalence across the surveyed regions. *Helicotylenchus*, *Pratylenchus*, and *Xiphinema* species were also confirmed through molecular sequencing, with discrete clades reflecting their genetic diversity. These findings validate the morphological identifications and highlight the reliability of combining molecular and morphological approaches for accurate nematode species charac-

Table 1. Plant-parasitic nematodes prevalence, intensity, and density from soil (250 g) and root (25 g) of Hasawi Lumi in the ten surveyed growing regions in Al-Ahsa Oasis, KSA

Nematode genera	*Helicotylenchus* (*He*)					*Pratylenchus* (*Pr*)					*Tylenchulus* (*Ty*)					*Xiphinema* (*Xi*)				
	Pr	In		De		Pr	In		De		Pr	In		De		Pr	In		De	
		Ro	So	Ro	So		Ro	So	Ro	So		Ro	So	Ro	So		Ro	So	Ro	So
Al-Bataliyah	36	6	18	14	28	44	8	20	20	68	56	31	123	73	423	20	6	20	–	33
Al-Bustan	36	4	15	14	25	40	5	17	20	65	52	28	120	73	420	20	4	17	–	30
Al-Halila	4	2	3	9	9	40	3	4	14	24	48	20	44	53	162	16	2	4	–	10
Al-Hofuf	76	1	8	3	13	48	3	8	4	20	28	3	10	6	43	32	–	5	–	5
Al-Jubail	48	5	15	14	35	44	8	8	21	20	64	18	390	34	533	16	–	13	–	28
Al-Omran	60	4	13	11	150	52	4	10	14	125	64	14	85	45	155	32	–	18	–	75
Al-Shaharin	32	6	16	12	25	36	7	18	18	62	52	28	113	64	389	16	6	18	–	30
Al-Shiraa	44	4	14	12	32	40	7	7	19	18	60	15	359	30	490	12	–	12	–	25
Al-Tarabil	72	1	7	2	12	44	2	7	3	18	24	2	9	6	39	28	–	5	–	5
Briqa	40	3	10	3	18	40	4	10	5	15	72	18	225	43	508	28	4	5	4	8

Pr: Prevalence (considering the nematodes' juvenile and adult stages) = % Prevalence = (Number of Infected Samples / Total Samples) × 100; In: Intensity = (Nematode Species in Infected Samples / Total Infected Samples) × 100; De: Density = the Maximum No. of Certain Nematode Species Recovered; Ro: Root; So: Soil

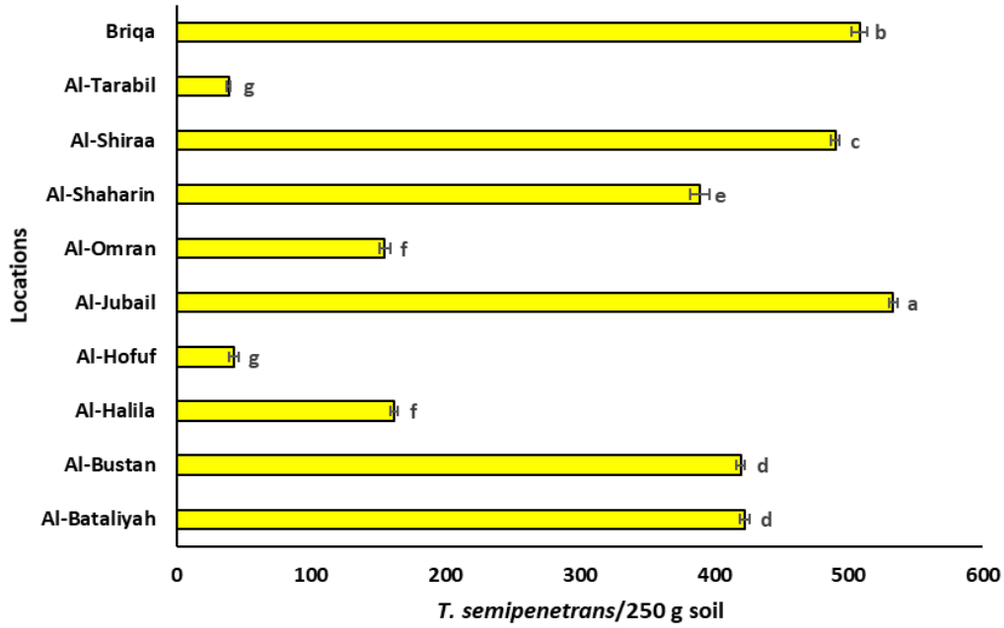

Fig. 2. Tylenchulus semipenetrans population densities per 250 g of soil in ten regions surveyed of Hasawi Lumi-growing in Al-Ahsa Oasis, KSA

terization. The sequences were submitted to GenBank with the accession numbers PQ843101, PQ843102, PQ843103, and PQ843104 for *Helicotylenchus digonicus*, *Tylenchulus semipenetrans*, *Pratylenchus loosi*, and *Xiphinema pachtaicum*, respectively. The phylogenetic tree further underscores the genetic relationships among the identified PPNs, providing a robust framework for understanding their distribution and ecological roles in Hasawi Lumi orchards.

The evolutionary history was reconstructed using the Maximum Likelihood approach with the Kimura 2-parameter model. The bootstrap values supporting the clustering were shown. The accession of newly sequenced data is shown in bold.

Interaction between physicochemical properties and nematode communities

The soil physicochemical properties across the Hasawi Lumi cultivation regions in Al-Ahsa Oasis are presented in Table 2. The data reveal significant variability in soil characteristics, likely influencing the distribution and abundance of PPNs in these regions.

Particle size distribution and soil texture. The distribution of the particle size varied considerably across the regions. Al-Halila had the highest sand content (90%), followed by Al-Bustan (88%) and Al-Jubail (84%). In contrast, Al-Omran and Briqa exhibited higher clay content (16% and 15%, respectively). Soil texture ranged from sandy in Al-Halila to sandy loam in Al-Bataliyah, Al-Hofuf, Al-Omran, and Al-Tarabil, and loamy sand in Al-Bustan and Al-Shaharin. These variations in texture likely affect water retention, aeration, and root penetration, which are critical factors for nematode survival and movement.

Bulk density and porosity. Bulk density values ranged from 1.44 g cm^{-3} in Al-Omran to 1.53 g cm^{-3} in Al-Halila, indicating relatively compact soils. Porosity, which reflects the soil's capability for holding water and air, ranged from 42.5% in Al-Omran to 47% in Al-Halila. Higher porosity in sandy soils, such as Al-Halila, may facilitate nematode movement, while lower porosity in clay-rich soils, like Al-Omran, could restrict nematode activity.

Cation exchange capacity (CEC) and pH. CEC values varied from 13.5 mmol 100 g^{-1} in Al-Halila to 19 mmol 100 g^{-1} in Al-Omran, indicating differences

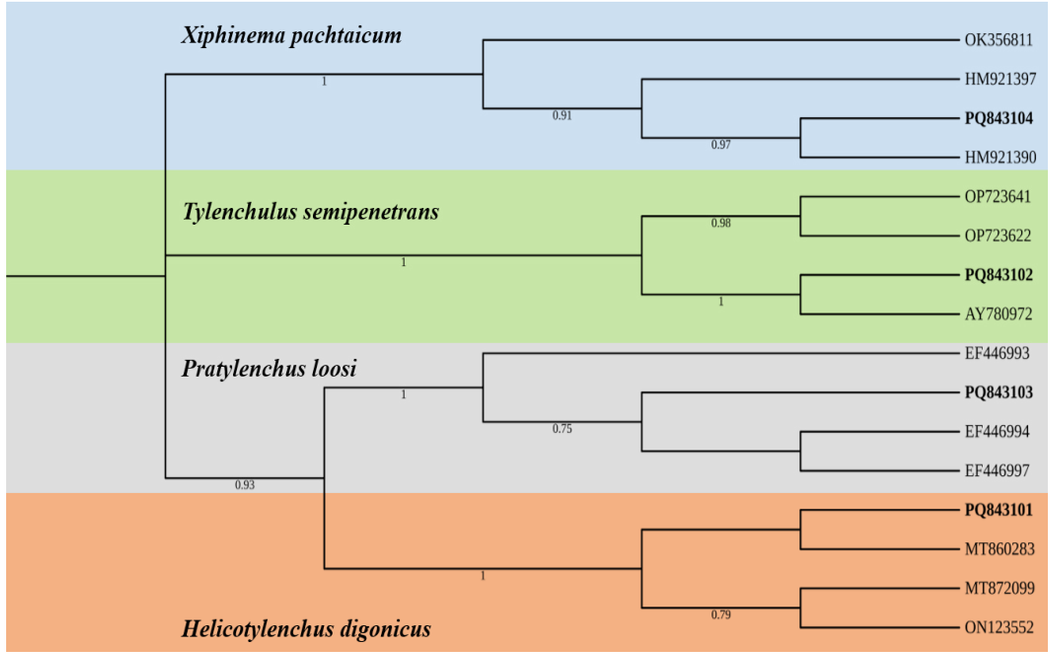

Fig. 3. Phylogenetic relationship of nematodes based on the D2D3 region of the 28S rDNA

in soil fertility and nutrient-holding capacity. The pH values were consistently alkaline, ranging from 8.1 in Al-Shaharin to 8.8 in Al-Tarabil. Specific nematodes, such as *Tylenchulus semipenetrans*, prefer alkaline soils and can tolerate an alkalinity range from neutral to slightly alkaline.

Electrical conductivity (EC) and total dissolved solids (TDS). EC values fluctuated considerably, with Al-Omran having the highest salinity (30 dS m^{-1}), followed by Briqa (25 dS m^{-1}). In contrast, Al-Hofuf had the lowest salinity (4.5 dS m^{-1}). High salinity in Al-Omran and Briqa may reduce nematode diversification, as many species are susceptible to salt content. TDS values range between 890 mg L^{-1} in Al-Hofuf and 970 mg L^{-1} in Al-Omran, which supports the salinity distributions inferred from EC values.

Organic matter (OM) and calcium carbonate ($CaCO_3$). Organic matter content ranged from 4.8% in Al-Halila and Al-Omran to 6.6% in Al-Bataliyah. Higher organic matter in Al-Bataliyah and Al-Jubail may support a more hospitable habitat and food resource, and induce more diverse nematode communities. The calcium carbonate content varied widely, ranging from 11.7% in Briqa to 24.8% in Al-Bustan. Nematodes like Xiphinema, which prefer calcareous soils, may develop well in soils with a moderate $CaCO_3$ content (e.g., Al-Bustan).

Sodium adsorption ratio (SAR) and major ions. SAR values ranged from 4.6 in Al-Hofuf to 5.1 in Al-Omran, indicating moderate sodium levels. Sodium (Na^+) concentrations were relatively consistent across regions, ranging from 11.2 mmol L^{-1} in Al-Hofuf to 12.5 mmol L^{-1} in Al-Omran. Potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) levels also showed minor variations, with Al-Omran having the highest Ca^{2+} (9.8 mmol L^{-1}) and Mg^{2+} (5.5 mmol L^{-1}) concentrations. These ions are involved in soil structure and nutrient availability, which can potentially influence nematode populations.

Anions (Cl^-, SO_4^{2-}, HCO_3^-). Chloride (Cl^-) and sulfate (SO_4^{2-}) concentrations were relatively uniform across regions, with Al-Omran having the highest Cl^- (13.0 mmol L^{-1}) and SO_4^{2-} (11.8 mmol L^{-1}) levels. Bicarbonate (HCO_3^-) levels ranged from 4.3 mmol L^{-1} in Al-Halila to 4.6 mmol L^{-1} in Al-Omran and Briqa. These anions are involved in soil salinity and may also affect nematode existence, particularly in areas of high salinity, such as Al-Omran.

Table 2. Hasawi Lumi samples soil type and physicochemical properties across locations

	Unit	Al-Bataliyah	Al-Bustan	Al-Halila	Al-Hofuf	Al-Jubail	Al-Omran	Al-Shaharin	Al-Shiraa	Al-Tarabil	Briqa
Particle size distribution	% sand	75.0	88.0	90.0	85.5	84.0	72.0	86.0	80.5	78.0	74.0
	% silt	10.0	7.0	5.0	8.0	9.0	12.0	8.5	10.5	12.0	11.0
	% clay	15.0	5.0	5.0	6.5	7.0	16.0	5.5	9.0	10.0	15.0
Texture		sandy loam	loamy sand	sandy	sandy loam	sandy	sandy loam	loamy sand	loamy	sandy loam	loamy
Bulk density	g cm^{-3}	1.45	1.5	1.53	1.48	1.49	1.44	1.47	1.46	1.45	1.48
Porosity	%	43.0	45.5	47.0	44.5	43.5	42.5	44.0	43.5	44.0	43.0
CEC	$cmol_{(+)}/kg$	17.0	15.0	13.5	15.5	16.0	19.0	15.5	17.5	18.0	17.5
pH		8.5	8.6	8.4	8.7	8.45	8.52	8.1	8.75	8.8	8.55
$EC_{1:5}$	dS m^{-1}	10.0	9.5	7.5	4.5	5.0	30.0	7.0	5.0	6.0	25.0
TDS	mg L^{-1}	940.0	950.0	920.0	890.0	910.0	970.0	900.0	940.0	930.0	960.0
Na^+	mmol L^{-1}	12.0	11.8	11.5	11.2	11.7	12.5	11.6	12.0	11.8	12.3
K^+	mmol L^{-1}	1.5	1.6	1.7	1.55	1.65	1.5	1.6	1.55	1.6	1.65
Ca^{2+}	mmol L^{-1}	9.5	9.4	9.3	9.6	9.7	9.8	9.5	9.5	9.6	9.8
Mg^{2+}	mmol L^{-1}	5.1	5.2	5.0	5.1	5.3	5.5	5.1	5.0	5.2	5.4
Cl^-	mmol L^{-1}	12.4	12.5	12.3	12.2	12.6	13.0	12.5	12.4	12.6	12.8
SO_4^{2-}	mmol L^{-1}	11.4	11.5	11.3	11.2	11.6	11.8	11.5	11.4	11.6	11.7
HCO_3^-	mmol L^{-1}	4.4	4.5	4.3	4.4	4.5	4.6	4.5	4.4	4.5	4.6
OM	%	6.6	5.2	4.8	5.0	6.5	4.8	6.2	5.5	6.4	5.7
SAR		4.8	4.9	4.7	4.6	4.9	5.1	4.8	4.7	4.9	5.0
$CaCO_3$	%	15.3	24.8	16.4	14.5	14.8	21.6	14.7	14.9	15.3	11.7

CEC: cation exchange capacity; EC: electrical conductivity; TDS: total dissolved solids (TDS); OM: organic matter; SAR: sodium adsorption ratio

Relationship between soil properties, nematode abundances, and location preference

The association between soil physicochemical characteristics and nematode genera was analyzed using PCA, which revealed that PC1 and PC2 explained 37% and 23.46% of the variability, respectively (Figure 4). The results showed that electrical conductivity (EC), CaCO3, and *Tylenchulus* are positively correlated. The nematode *Helicotylenchus* showed a positive correlation with pH, and a negative correlation with EC.

Organic matter (OM) was negatively correlated with EC, indicating that soils high in salinity may have lower organic matter content due to reduced fertility and microbial activity. Both nematodes, *Pratylenchus* and *Xiphinema*, were positively correlated with soil properties like $CaCO_3$ and, to a lesser extent, EC.

The variance explained percentage attained 37% and 23.46% for PC1 and PC2, respectively. Each location is represented by a unique color in the legend, with labels displayed near its corresponding point. Variables (red arrows) represent the contribution and direction of soil properties and nematode preferences. Variables aligned in the same direction demonstrate a positive correlation, whereas those pointing in opposite directions signify a negative relationship.

Variability in nematode diversity and abundance linked to soil physicochemical properties

The Shannon-Wiener diversity index (H′) and Evenness (E) values for plant-parasitic nematodes (PPNs) in the Hasawi Lumi cultivation regions of Al-Ahsa Oasis are presented in Table 3. The findings reveal significant variations in nematode diversity and community structure, which can be linked to the soil physicochemical properties of each region.

Nematode diversity and soil properties. The highest Shannon-Wiener diversity index (H′ = 1.33)

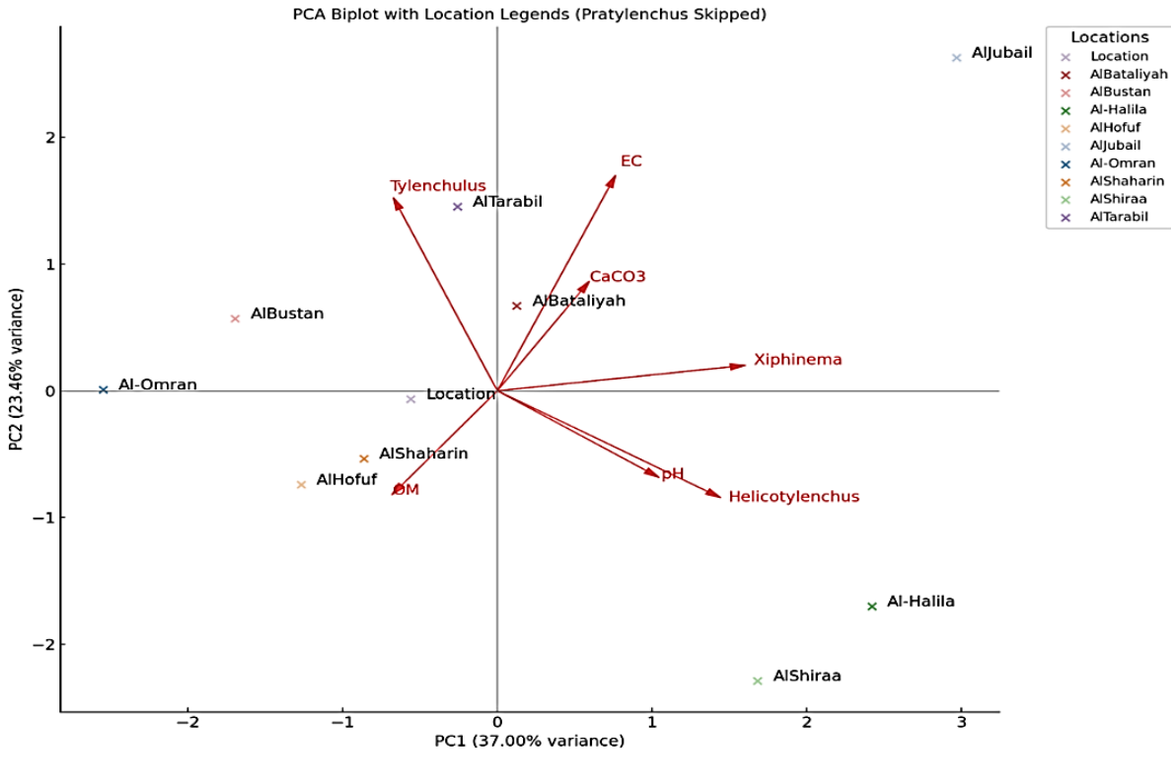

Fig. 4. Principal Component Analysis (PCA) of the association between soil physicochemical characteristics and nematode abundances in the ten regions surveyed in Al-Ahsa Oasis, KSA. EC: electrical conductivity; OM: organic matter

Table 3. The average number of nematode genera, Shannon-Wiener diversity (H′), and Evenness (E) in each of the ten locations surveyed in Hasawi Lumi

No.	Location	Average of PPNs*	Shannon Diversity Index (H′)*	Evenness (E)*
1	Al-Bataliyah	165 ab	0.81 c	0.53 d
2	Al-Bustan	162 ab	0.79 c	0.57 cd
3	Al-Halila	70 e	0.77 c	0.56 cd
4	Al-Hofuf	24 f	1.15 b	0.83 b
5	Al-Jubail	171 a	0.64 d	0.47 e
6	Al-Omran	144 d	1.33 a	0.96 a
7	Al-Shaharin	150 cd	0.80 c	0.58 c
8	Al-Shiraa	157 bc	0.64 d	0.46 e
9	Al-Tarabil	21 f	1.15 b	0.83 b
10	Briqa	151 cd	0.39 e	0.28 f
×	*P*	<0.05	<0.05	<0.05

Means followed by the same letter(s) within a column are not significantly different ($p \leq 0.05$) according to Duncan's multiple range test.
* https://www.omnicalculator.com/ecology/shannon-index

was observed in Al-Omran, followed by Al-Hofuf and Al-Tarabil (H′ = 1.15). These regions also exhibited higher organic matter (OM) content (4.8% in Al-Omran, 5.0% in Al-Hofuf, and 6.4% in Al-Tarabil) and moderate calcium carbonate ($CaCO_3$) levels (21.6% in Al-Omran, 14.5% in Al-Hofuf, and 15.3% in Al-Tarabil). Higher OM and moderate $CaCO_3$ may have created a suitable environment for various nematode communities, as the organic matter supports microbial activity and enhances nutrient availability. Additionally, moderate $CaCO_3$ helps stabilize the soil structure even more.

On the other hand, Briqa presented the lowest Shannon-Wiener index (H′ = 0.39), corresponding to its high salinity (EC = 25 dS m^{-1}) and low organic matter content (5.7%). High salinity is known to suppress nematode diversity, as many species are sensitive to saline conditions. Similarly, Al-Jubail and Al-Shiraa, with lower H′ values (0.64), also had lower OM content (6.5% and 5.5%, respectively) and higher salinity (EC = 5.0 dS m^{-1} in Al-Jubail and 5.0 dS m^{-1} in Al-Shiraa), further supporting the negative impact of salinity on nematode diversity.

Evenness and nematode distribution. The Evenness index (E), which measures the relative abundance of nematode species, was highest in Al-Omran (E = 0.96) and Al-Hofuf (E = 0.83), indicating a more balanced distribution of nematode species in these regions. It aligns with their higher OM and moderate $CaCO_3$ levels, which likely support a more stable and diverse nematode community. In contrast, Briqa had the lowest Evenness (E = 0.28), reflecting a less balanced nematode community, likely due to its high salinity and lower OM content.

Average number of PPNs. The average number of PPNs differs significantly across the regions, with the highest average (171) documented from Al-Jubail, followed by Al-Bataliyah (165) and Al-Bustan (162). In addition, moderate OM content (6.6% in Al-Bataliyah, 5.2% in Al-Bustan, and 6.5% in Al-Jubail) and lowered salinity (EC = 5.0 dS m^{-1} in Al-Jubail, 10.0 dS m^{-1} in Al-Bataliyah, and 9.5 dS m^{-1} in Al--Bustan) were reported, which designates moderate OM and lower salinity as attractive for higher nematode populations. Conversely, Al-Hofuf and Al-Tarabil had the lowest average number of PPNs (24 and 21, respectively), perhaps due to their lower OM content (5.0% and 6.4%) and higher salinity (EC = 4.5 dS m^{-1} in Al-Hofuf and 6.0 dS m^{-1} in Al-Tarabil).

A clear association has been demonstrated between soil physicochemical characteristics, nematode diversity, and abundance in Hasawi Lumi cultivation regions. Diverse and balanced nematode communities are associated with higher organic matter and moderate calcium carbonate levels in Al-Omran, Al-Hofuf, and Al-Tarabil. Conversely, high salinity and low organic matter, as seen in Briqa, induce a decrease in nematode diversity and abundance.

DISCUSSION

Extensive studies were conducted in the principal growth regions of Hasawi Lumi trees to determine the distribution of PPNs in Al-Ahsa Oasis. Based on the morphological and morphometric features, four genera of PPNs were identified, with *Tylenchulus semipenetrans* being the predominant PPN in Hasawi Lumi orchards. Comparable observations were reported in other regions, such as Iran, Egypt, and Spain [Maafi and Damadzadeh 2008, Abd-Elgawad et al. 2016, Sorribas et al. 2008]. This species stands out due to its strong connection with citrus and the Hasawi Lumi trees' ability to sustain high nematode populations until they reach senescence. The spread of the citrus nematode may have been facilitated by contaminated seedlings, crop debris, organic manure, irrigation, and machinery [Abd-Elgawad et al. 2016].

The spiral nematode, *Helicotylenchus* spp., accounted for the second most prevalent PPN, ranging from 76% in Al-Hofuf to 72% in Al-Tarabil, and 4% in Al-Halila. The Hasawi Lumi-growing zones of Al-Ahsa Oasis have more of this genus than Spain [Sorribas et al. 2008] and Egypt [Abu Habib 2020]. According to Kumar and Das [2019], 80% of *H. dihystera* were found in 80% of samples from Tinsukia. Population densities of lesion nematode (*Pratylenchus* spp.) spanned from 125 to 15 per 250 g of soil in Al-Omran and Briqa, respectively, and from 21 to 3 per 25 g of roots in Al-Jubail and Al-Tarabil, respectively. Numerous investigations have found these nematodes in citrus crops worldwide [Badii et al. 2015]. The coffee species infests 1% of Brazilian citrus nurseries and plantations, and is prevalent in Florida [Freitas et al. 2008]. These organisms can substantially de-

crease root mass, thereby reducing yield [Abd-Elgawad 2020].

The top 10 economically relevant PPNs include the dagger nematode *Xiphinema* spp. [Jones et al. 2013]. As migrating ectoparasites, these nematodes can transmit plant viruses, posing a significant threat to crops. Even in low populations, they can cause substantial harm to plants [MacFarlane and Robinson 2004]. This study found significant variances in *Xiphinema* spp. distribution across Hasawi Lumi cultivation areas. This nematode spreads the Arabis mosaic virus, which causes grapevine fan leaf degeneration [Mokrini et al. 2014]. *Xiphinema pachtaicum* was initially found with Hasawi Lumi in Al-Ahsa Oasis. This finding is particularly significant due to the nematode's economic impact, quarantine implications, and potential to devastate Hasawi Lumi orchards.

Al-Omran, Al-Hofuf, and Al-Tarabil, regions with long dry seasons, have the highest Shannon (H′) and Evenness (E) indexes. Freitas et al. [2008] found that the dry season and 0–10 cm soil depth increased Brazil's citrus plant PPN population.

The soil physicochemical properties in the Hasawi Lumi cultivation regions of Al-Ahsa Oasis exhibit significant variability, particularly in texture, salinity, organic matter, and calcium carbonate content. These factors likely influence the distribution and abundance of PPNs. For example, sandy soils with high porosity, such as those in Al-Halila, may facilitate nematode movement and proliferation. High salinity in Al-Omran and Briqa could limit nematode diversity, as many species are sensitive to saline conditions. Regions with higher organic matter, such as Al-Bataliyah, may support more diverse nematode communities due to improved soil fertility and microbial activity. Alkaline soils with moderate $CaCO_3$ content, as seen in Al-Bustan, may favor nematodes like *Xiphinema*. The higher salinity and carbonate content often favor the prevalence of *Tylenchulus* nematodes. The preference of Helicotylenchus nematodes for specific soil pH conditions suggests that higher salinity might suppress Helicotylenchus abundance, or that this nematode prefers less saline conditions. Both nematodes, *Pratylenchus* and *Xiphinema*, can coexist in soil environments with moderate salinity and sufficient carbonate levels. Grasping the relationship between soil physicochemical attributes and PPNs is crucial for efficient and sustainable management. The citrus nematode *T. semipenetrans* demonstrated a favorable association with soil mineral elements (Na, Ca, K, and C) and the concentration of organic matter. A significant association was observed between these traits and the occurrence of *T. semipenetrans* in citrus farming regions, including Spain [Sorribas et al. 2008]. Conversely, Benjlil et al. [2020] discovered an inverse relationship between the occurrence of PPNs affecting saffron and OM in the soil. Increasing soil organic matter may considerably decrease PPN amounts in wheat by compromising their fundamental characteristics [Hu and Qi 2013]. The presence of *Pratylenchus* spp., *Helicotylenchus* spp., and *Xiphinema* spp. was strongly associated with the mineral composition, particularly Ca, Fe, and Na [Yavuzaslanoglu et al. 2012, Karuri et al. 2017].

Francl [1993] also identified a direct link between the abundance of *Heterodera glycines* and magnesium (Mg) levels. In the current research, most detected PPNs exhibited an inverse association with nitrogen (N). The accumulation of nitrate during nitrification is regarded as harmful to PPNs [Rodríguez-Kábana 1986]. In the current study, the phosphorus concentration positively correlated with the *Xiphinema* spp. and *Pratylenchus* spp. populations. Similarly, Nisa et al. [2021] observed the same positive trend between the soil phosphorus content and nematode populations. The pH of the soil markedly influenced the occurrence of citrus nematodes – decreased pH enhanced nematode population and diversity [Al-Sayed et al. 1993]. However, according to Salahi Ardakani et al. [2014], the citrus nematode was most abundant in soils with a pH of 7. Additionally, Van Gundy and Martin [1962] observed that the population of *T. semipenetrans* in citrus was fourfold greater in neutral soils than in acidic conditions. Soil structure and texture have significant effects on the mobility of soil nematodes. Based on our findings, fine sandy soil texture enhances the movement and dispersion of nematodes. Clay soils exhibited the highest prevalence of citrus nematodes [Salahi Ardakani et al. 2014]. Recently, Laasli et al. [2022] demonstrated that sandy and silt types of soils found in wheat fields contained *Aphelenchoides* spp., *Merlinius* spp., and *Pratylenchus* spp. However, *Longidorus* spp. and *Xiphinema* spp. appeared to have a greater prevalence in soil samples with increased clay content. Mokrini et al. [2019] concluded that several PPNs im-

pacting raspberries significantly correlated with soil granulometry. According to Kim et al. [2017], sandy soils facilitate the proliferation of nematodes like *M. incognita* by enabling their locomotion and feeding behaviors.

CONCLUSIONS

This study sheds light on the PPNs' diversity. Citrus nematodes were found to predominate in soil and root matrices, likely due to favorable local soil and environment in Hasawi Lumi farming areas. Other commercially essential nematodes, including *Pratylenchus*, *Helicotylenchus*, and *Xiphinema*, were initially described in *C. aurantiifolia* orchards within Al-Ahsa Oasis. Farmers may use the relationship between these nematodes and soil qualities to manage PPNs effectively. The findings of this study will help researchers and pest management authorities control and mitigate PPNs to increase Hasawi Lumi production in Al-Ahsa Oasis.

The relationship between soil properties such as OM, $CaCO_3$, and EC and nematode communities in Hasawi Lumi growing areas reveals that higher organic matter and moderate calcium carbonate levels increase nematode diversity, while high salinity suppresses it. Effective soil management strategies, such as increasing organic matter and regulating salinity, are crucial for mitigating nematode infestations and improving productivity. Additional research is needed to investigate the direct relationships between soil properties and nematode species, and to develop strategies for balancing soil health with sustainable agricultural practices.

AUTHORS' CONTRIBUTIONS

HHK was responsible for the conception and design, data acquisition, data analysis, data interpretation, and article drafting; MAA-S handled sample collection, nematode extraction, and enumeration. Molecular analysis and data interpretation and article drafting: SME-G; Soil analysis and data interpretation: LMH; Statistical analysis and data interpretation: BVC. All authors have reviewed and consented to the final version of the manuscript.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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REFERENCES

Abd-Elgawad, M.M.M. (2020). Managing nematodes in Egyptian citrus orchards. Bull. Nation. Res. Centre, 44(1), 135–136. https://doi.org/10.1186/s42269-020-00298-9

Abd-Elgawad, M.M.M., Koura, F.F.H., Montasser, S.A., Hammam, M.M.A. (2016). Distribution and losses of *Tylenchulus semipenetrans* in citrus orchards on reclaimed land in Egypt. Nematology, 18(10), 1141–1150. https://doi.org/10.1163/15685411-00003020

Abu Habib, A., Younes, H., Ibrahim, I., Khalil, A. (2020). Plant parasitic nematodes associated with citrus trees and reaction of two citrus cultivars to *Tylenchulus semipenetrans* in northern Egypt. J. Adv. Agric. Res., 25(1), 166–175. https://doi.org/10.21608/jalexu.2020.161764

Al Sayed, A.A., Abdel-Hameed, S.H., El-Nagar, H.I. (1993). Population dynamics of *Tylenchulus semipenetrans* in relation to citrus species and soil temperature. Bull. Fac. Agric. Cairo Univ., 44, 183–190.

Al-Hazmi, A.S. (1997). Status of plant nematology in Saudi Arabia. In: M.A. Maqbool, B.R. Kerry (eds.). Plant nematode problems and their control in the Near East Region: Proceedings of the Expert Consultation on Plant Nematode Problems and Their Control in the Near East Region, Karachi, Pakistan, 22–26 November 1992. Food and Agriculture Organization (FAO), pp. 315.

Al-Yahya, F.A., Al-Hazmi, A.S., El-Saedy, M.A. (1988). Effect of soil texture on reproduction of *Tylenchulus semi-*

penetrans on lime seedlings irrigated with treated sewage water. Alexandria J. Agric. Res., 33(1), 183–192.

Badii, K.B., Billah, M.K., Afreh Nuamah, K., Obeng Ofori, D., Nyarko, G. (2015). Review of the pest status, economic impact, and management of fruit-infesting flies (Diptera: Tephritidae) in Africa. Afr. J. Agric. Res., 10(12), 1488–1498. https://doi.org/10.5897/ajar2014.9278

Bello, T.T., Coyne, L.D., Rashidifard, M., Fourie, H. (2020). Abundance and diversity of plant-parasitic nematodes associated with watermelon in Nigeria, with focus on *Meloidogyne* spp. J. Nematol., 22, 781–797. https://doi.org/10.1163/15685411-00003340

Benjlil, H., Elkassemi, K., Aït Hamza, M., Mateille, T., Furze, J.N., Cherifi, K., Mayad, E.H., Ferji, Z. (2020). Plant-parasitic nematodes parasitizing saffron in Morocco: Structuring drivers and biological risk identification. Appl. Soil Ecol., 147, 103362. https://doi.org/10.1016/j.apsoil.2019.103362

De Ley, P., Félix, M.A., Frisse, L.M., Nadler, S.A., Sternberg, P.W., Thomas, W.K. (1999). Molecular and morphological characterisation of two reproductively isolated species with mirror-image anatomy (Nematoda: Cephalobidae). Nematology, 1(6), 591–612. https://doi.org/10.1163/156854199508559

Duncan, L.W. (2005). Nematode parasites of citrus. In: R.A. Sikora, M. Luc, J. Bridge (eds.), Plant parasitic nematodes in subtropical and tropical agriculture. 2nd ed. CAB International, pp. 437–466. https://doi.org/10.1079/9780851997278.0437

Eissa, M.F.M., Mustafa, M., Al-Kahtani, M.S. (1979). Susceptibility of some citrus rootstocks to the citrus nematode, *Tylenchulus semipenetrans* Cobb, 1913 and spiral nematode, *Helicotylenchus dihystera* (Cobb, 1893) Sher, 1961 in Saudi Arabia. Bull. Zool. Soc. Egypt, 28, 62–65.

Francl, L.J. (1993). Multivariate analysis of selected edaphic factors and their relationship to *Heterodera glycines* population density. J. Nematol., 25(2), 270–276.

Freitas, V., Cares, J., Huang, S.P. (2008). The influence of *Citrus* spp. on the community of soil nematodes in the dry and rainy seasons in Distrito Federal of Brazil. Nematol. Brasil., 32(1), 20–32.

Gee, G.W., Bauder, J.W. (2018). Particle-size analysis. In: A. Klute (ed.), Methods of soil analysis, part 1: Physical and mineralogical methods. 2nd ed., Am. Soc. Agron./Soil Sci. Soc. Am., 383–411. https://doi.org/10.2136/sssabookser5.1.2ed.c15

Hammam, M., Abdel Gawad, M., Ruan, W., El-bahrawy, A. (2021). Management of pests and pathogens affecting citrus yield in Egypt with special emphasis on nematodes. Egypt. J. Agronematol., 20(1), 64–84. https://doi.org/10.21608/ejaj.2021.183231

Holterman, M., van der Wurff, A., van den Elsen, S., van Megen, H., Bongers, T., Holovachov, O., Bakker, J., Helder, J. (2006). Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Mol. Biol. Evol., 23(9), 1792–1800. https://doi.org/10.1093/molbev/msl044

Hooper, D.J., Hallmann, J., Subbotin, S.A. (2005). Methods for extraction, processing, and detection of plant and soil nematodes. In: M. Luc, R.A. Sikora, J. Bridge (eds.), Plant parasitic nematodes in subtropical and tropical agriculture. CAB International, 53–86. https://doi.org/10.1079/9780851997278.0053

Hu, C., Qi, Y. (2013). Effective microorganisms and compost favor nematodes in wheat crops. Agron. Sustain. Dev., 33(3), 573–579. https://doi.org/10.1007/s13593-012-0130-9

Jones, J.T., Haegeman, A., Danchin, E.G.J., Gaur, H.S., Helder, J., Jones, M.G.K., Kikuchi, T., Manzanilla-López, R., Palomares-Rius, J.E., Wesemael, W.M.L. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. Mol. Plant Pathol., 14(9), 946–961. https://doi.org/10.1111/mpp.12057

Karuri, H.W., Olago, D., Neilson, R., Njeri, E., Opere, A., Ndegwa, P. (2017). Plant parasitic nematode assemblages associated with sweet potato in Kenya and their relationship with environmental variables. Tropic. Plant Pathol., 42(1), 1–12. https://doi.org/10.1007/s40858-016-0114-4

Kassambara, A., Mundt, F. (2020). *Factoextra*: Extract and visualize the results of multivariate data analyses [R package factoextra version 1.0.7]. https://doi.org/10.32614/cran.package.factoextra

Khan, U.M., Sameen, A., Aadil, R.M., Shahid, M., Sezen, S., Zarrabi, A., Ozdemir, B., Sevindik, M., Kaplan, D.N., Selamoglu, Z., Ydyrys, A., Anitha, T., Kumar, M., Sharifi-Rad, J., & Butnariu, M. (2021). *Citrus* genus and its waste utilization: A review on health-promoting activities and industrial application. Evid.-Based Compl. Altern. Med., 2021, 2488804. https://doi.org/10.1155/2021/2488804

Kim, E., Seo, Y., Kim, Y. S., Park, Y., Kim, Y.H. (2017). Effects of soil textures on infectivity of root-knot nematodes on carrot. Plant Pathol. J., 33(1), 66–74. https://doi.org/10.5423/ppj.oa.07.2016.0155

Kumar, K.K., Das, A.K. (2019). Diversity and community analysis of plant parasitic nematodes associated with

citrus at citrus research station, Tinsukia, Assam. J. Entomol. Zool. Stud., 7(1), 187–189.

Laasli, S.-E., Mokrini, F., Lahlali, R., Wuletaw, T., Paulitz, T., Dababat, A.A. (2022). Biodiversity of nematode communities associated with wheat (*Triticum aestivum* L.) in Southern Morocco and their contribution as soil health bioindicators. Diversity, 14(3), 194. https://doi.org/10.3390/d14030194

Lê, S., Josse, J., Husson, F. (2008). FactoMineR: An R package for multivariate analysis. J. Stat. Soft., 25(1), 1–18. https://doi.org/10.18637/jss.v025.i01

Maafi, Z.T., Damadzadeh, M. (2008). Incidence and control of the citrus nematode, *Tylenchulus semipenetrans* Cobb, in the north of Iran. Nematology, 10(1), 113–122. https://doi.org/10.1163/156854108783360096

MacFarlane, S.A., Robinson, D.J. (2004). Transmission of plant viruses by nematodes. In: S.H. Gillespie, G.L. Smith, A. Osbourn (eds.), Microbe-vector interactions in vector-borne diseases. Cambridge Univ. Press, 263–286. https://doi.org/10.1017/cbo9780511754845.012

Mai, W.F., Mullin, P.G., Lyon, H.H., Loeffler, K. (1996). Plant-parasitic nematodes: A pictorial key to genera. 5th ed. Cornell Univ. Press. http://www.jstor.org/stable/10.7591/j.ctv5rdz0t

Maqbool, Z., Khalid, W., Atiq, H.T., Koraqi, H., Javaid, Z., Alhag, S.K., Al-Shuraym, L.A., Bader, D.M.D., Almarzuq, M., Afifi, M., Al-Farga, A. (2023). Citrus waste as source of bioactive compounds: Extraction and utilization in health and food industry. Molecules, 28(4), 1636. https://doi.org/10.3390/molecules28041636

Mokrini, F., Abbad Andaloussi, F., Waeyenberge, L., Viaene, N., Moens, M. (2014). First report of the dagger nematode *Xiphinema diversicaudatum* in citrus orchards in Morocco. Plant Dis., 98(4), 575. https://doi.org/10.1094/pdis-07-13-0764-pdn

Mokrini, F., Janati, S., Andaloussi, F.A., Essarioui, A., Houari, A., Sbaghi, M. (2018). Importance and distribution of the main citrus parasitic nematodes in Morocco. Rev. Maroc. Sci. Agron. Vét., 6(4), 558–564.

Mokrini, F., Laasli, S.E., Iraqui, D., Wifaya, A., Mimouni, A., Erginbas-Orakci, G., Imren, M., Dababat, A.A. (2019). Distribution and occurrence of plant-parasitic nematodes associated with raspberry (*Rubus idaeus*) in Souss-Massa region of Morocco. Relationship with soil physico-chemical factors. Russ. J. Nematol., 27(2), 107–121. https://doi.org/10.1163/15685411-00003286

Nisa, R.U., Tantray, A.Y., Kouser, N., Allie, K.A., Wani, S.M., Alamri, S.A., Alyemeni, M.N., Wijaya, L., Shah, A.A. (2021). Influence of ecological and edaphic factors on biodiversity of soil nematodes. Saudi J. Biol. Sci., 28(5), 3049–3059. https://doi.org/10.1016/j.sjbs.2021.02.046

Page, A.L., Miller, R.H., Keeney, D.R. (1982). Methods of soil analysis, part 2: Chemical and microbiological properties. 2nd ed. Am. Soc. Agron. https://doi.org/10.1002/jpln.19851480319

Richard, L.A. (1954). Diagnosis and improvement of saline and alkali soils. Agricultural Handbook No. 60. U.S. Department of Agriculture. https://doi.org/10.1097/00010694-195408000-00012

Rodríguez-Kábana, R. (1986). Organic and inorganic nitrogen amendments to soil as nematode suppressants. J. Nematol., 18(2), 129–135.

Ryss, A.Y. (2017). A simple express technique to process nematodes for collection slide mounts. J. Nematol., 49(1), 27–32. https://doi.org/10.21307/jofnem-2017-043

Salahi Ardakani, A., Tanha Mafi, Z., Mokaram Hesar, A., Mohammadi Goltappeh, E. (2014). Relationship between soil properties and abundance of *Tylenchulus semipenetrans* in citrus orchards, Kohgilouyeh va Boyerahmad Province. J. Agric. Sci. Technol., 16(6), 1699–1710.

Sorribas, F.J., Verdejo-Lucas, S., Pastor, J. (2008). Population densities of Tylenchulus semipenetrans related to physicochemical properties of soil and yield of clementine mandarin in Spain. Plant Dis., 92(3), 445–450. https://doi.org/10.1094/pdis-92-3-0445

Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E. (eds.). (2020). Methods of soil analysis, Part 3: Chemical methods. Vol. 3. John Wiley & Sons. https://doi.org/10.2136/sssabookser5.3

SPSS (2016). 2a IBM SPSS Statistics Processes for PC. Version 23.0. IBM SPSS Statistics 23 Step by Step, 22–39. https://doi.org/10.4324/9781315545899-7

Van Gundy, S.D., Martin, G.P. (1962). Soil texture, pH, and moisture effect on the development of citrus nematode (*Tylenchulus semipenetrans*). Phytopathology, 52(1), 31.

Yavuzaslanoglu, E., Elekcioglu, H.I., Nicol, J.M., Yorgancilar, O., Hodson, D., Yildirim, A.F., Yorgancilar, A., Bolat, N. (2012). Distribution, frequency, and occurrence of cereal nematodes on the central Anatolian plateau in Turkey and their relationship with soil physicochemical properties. Nematology, 14(7), 839–854. https://doi.org/10.1163/156854112x631926

Zou, Z., Xi, W., Hu, Y., Nie, C., Zhou, Z. (2016). Antioxidant activity of citrus fruits. Food Chem., 196, 885–896. https://doi.org/10.1016/j.foodchem. 2015.09.072

Zoubi, B., Mokrini, F., Dababat, A.A., Amer, M., Ghoulam, C., Lahlali, R., Laasli, S.-E., Khfif, K., Imren, M., Akachoud, O., Benkebboura, A., Housseini, A.I., Qaddoury, A. (2022). Occurrence and geographic distribution of plant-parasitic nematodes associated with citrus in Morocco and their interaction with soil patterns. Life, 12(5), 637. https://doi.org/10.3390/life12050637

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