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The response to bacterial inoculation is cultivar-related in strawberries

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Abstract: This study was carried out to evaluate the impact of biofertilizer and cultivar on vegetative potential (height of the plant, number of crowns and leaves per plant, and the area of a single leaf), leaf mineral composition (micro- and macroelements), yield potential (number of inflorescences and fruit set per plant, yield per plant, and yield per square meter), fruit characteristics (mass, length, width, and fruit shape index), and chemical traits (soluble solids content, titratable acidity (TA), vitamin C, total anthocyanins (TACY), total phenolic content (TPC), and total antioxidant capacity (TAC)) in the fruits of Clery, Joly and Dely strawberry plants. Two types of biofertilizer were applied: Biofertilizer 1 (inoculums of the mixture of liquid bacteria cultures of the genera *Azotobacter*, *Derxia*, and *Bacillus*) and Biofertilizer 2 (inoculums of liquid culture of diazotrophic bacteria belonging to the genus *Klebsiella*). The applied biofertilizers made a significant impact on the parameters of vegetative potential and contents of some macroelements and microelements in the leaf, as well as the values of TA, TACY, TPC, and TAC. Cultivar demonstrated a significant impact on plant height and the number of crowns in it, leaf macroelement and microelement content, generative potential parameters, and morphometric and chemical fruit parameters.

Key words: Biofertilization, *Fragaria* × *ananassa* Duch., fruit quality, productivity, vegetative development

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) production is in constant increase, primarily due to increasing consumption of the fruit and its high profitability. Intensive farming practices that result in high yield and quality also require extensive use of chemical fertilizers, which are costly and create environmental problems. Therefore, there has been a recent, growing interest in various biofertilizers (microbe inoculants). Use of biofertilizers containing various genera of bacteria, like *Pseudomonas*, *Azotobacter*, *Bacillus*, *Derxia*, and *Klebsiella*, has been found to be beneficial for plant growth, yield, fruit quality of strawberries, and leaf P and Zn content (Esitken et al., 2010; Pešaković et al., 2013).

The other equally important aspect of intensifying strawberry production is the selection of high-yielding cultivars with flavorful fruits that are more desirable to consumers. In this regard, breeding programs are based on improving yield and fruit quality characteristics (sensorial and nutritional), adapting to different growing systems, and ecological production (Capocasa et al., 2008; Magnani et al., 2009; Luković et al., 2012). Furthermore, technologies should be adjusted to a single cultivar or a group of cultivars with similar requirements.

Considerations of biofertilizer impacts on chemical fruit characteristics and health benefits of strawberry cultivars have also received attention and are important directions for future research (Anttonen et al., 2006; Agulheiro-Santos, 2009).

The objective of this study was to highlight how various bacteria can be used in biofertilization aimed at increasing strawberry production through regulation of vegetative development and how these bacteria can improve the nutritional fruit quality of three strawberry cultivars. In addition to this, we wanted to investigate the combined effect of biofertilizer and cultivar on certain vegetative, generative, and fruit quality traits in strawberries.

2. Materials and methods

2.1. Plant material

An open field trial was conducted at the experimental plantation of the Fruit Research Institute, Čačak, Serbia (43°53′N, 20°20′E, 225 m a.s.l.). Soil physicochemical analysis was performed prior to trial establishment. The soil's macronutrient content was determined according to standard laboratory protocols and methods. The trial was conducted on alluvial soil with a sandy-loam texture

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(51.9% sand and 48.1% loam), pH_{KCl} 5.48, humus 3.95%, N_{TOT} 0.20%, easily accessible potassium 27.00 mg g^{-1}, easily accessible phosphorus 22.95 mg g^{-1}. The field was planted on 18 July 2011 in double rows on raised beds covered with black polyethylene mulch. Certified frigo plants (A+ class) of the three newly introduced short-day strawberry cultivars Clery, Joly, and Dely (Consorzio Italiano Vivaisti, Italy) were planted. Planting distance was 30 × 30 cm.

2.2. Experimental design

The experiment was conducted in 3 treatments (Biofertilizer 1, Biofertilizer 2, and nonfertilized control) on 3 varieties (Clery, Joly, and Dely) with 20 plants in each treatment in 3 replications. The research was carried out in 2011 (autumn yield) and 2012 (spring yield). Since the examined period was characterized by low yield in 2011 and higher yield in the spring of 2012, the results are shown as 2-year average values.

Biofertilizer 1 was a commercial microbiological fertilizer composed of nitrogen-fixing and phosphorus-mineralizing bacteria (*Azotobacter chroococcum*, *A. vinelandii*, *Derxia* sp., *Bacillus megaterium*, *B. licheniformis*, and *B. subtilis*). Biofertilizer 2 was a pure culture of gram-negative diazotrophic nitrogen-fixing bacterium *Klebsiella planticola*, obtained from the Microorganism Collection of the Laboratory of Microbiology, Faculty of Agronomy, Čačak, Serbia. The bacteria titer in the inoculum ranged from 20 to 40 × 10^6 mL^{-1}. The biofertilizer was first applied during plant establishment by dipping the roots in a liquid inoculum of bacteria for 30 min. The plants were fertigated through the irrigation system three times per month during the vegetation period (9 times in total) in accordance with the corresponding phenophase of the plant development, using 10–12 L ha^{-1} of bacterial inoculum (20 to 40 × 10^6 CFU mL^{-1}). The control plants were treated with the same amount of pure water. Additionally, the plants were regularly irrigated according to soil humidity. A tensiometer was set up in the root zone at a depth of 30 cm.

2.3. Plant growth

Strawberry vegetative potential was calculated by measuring the plant height (cm), number of crowns per plant, number of leaves per rosette, and size of the single leaf area (cm^2). Measurements were taken after harvesting in both years of investigation using counting and standard morphometric methods. The one exception was leaf area, which was determined by scanning the leaves and measuring their area in the AutoCAD program (Rico-Garcia et al., 2009). For average leaf area, we estimated 180 leaves in three replications (60 leaves of each cultivar). Leaves were randomly selected. The results were presented as 2-year average values of three replications.

Samples for determining the mineral composition of the leaf were taken in both years of investigation during the full maturity of the fruits. The leaves were dried at 50 °C for 24 h until obtaining constant mass before they were ground. The ground particles were then subjected to digestion in a microwave digester, using an HNO_3-H_2O_2 acid mixture (2:3 v/v). The content of the macroelements (N, P, K, Ca, Mg) and microelements (Fe, Cu, Mn, Zn, B, and Mo) in the leaves was determined using inductively coupled plasma optical emission spectroscopy according to the method of Nikolic et al. (2011). The concentration of mineral elements in the solution is expressed in μg mL^{-1}. Method AOAC 972.43:2000 and an elementary analysis ELEMENTAR VARIO III were used to determine nitrogen content in the leaves. The corresponding values are expressed in percentages.

2.4. The yield and characteristics of fruits

The generative potential of the strawberry plants was determined by establishing the number of the inflorescences and fruits per plant, yield per plant, and yield per square meter in both years of investigation. Inflorescences and fruits were counted for each plant. Yield per plant was obtained by collecting and weighing fruit (g/plant; kg/m^2) during the first and second harvesting seasons. Means of 2-year values obtained for each generative parameter are presented.

To assess the morphometric fruit properties, a sample of 25 fruits per replication was randomly selected in both harvesting seasons. For average fruit weight, a METTLER balance (±0.01 g accuracy) was used. Data are expressed in g/fruit as 2-year mean values. Fruit dimensions (length and width) were also determined in the same samples by an INOX Vernier scale (±0.05 mm accuracy) and the corresponding data are expressed in millimeters. The fruit shape index was determined using a computer program and expressed as the ratio between the length and width of the fruit.

Ripe fruits of selected strawberry cultivars were sampled per each treatment separately in the first and second picking season. Samples were immediately frozen in liquid nitrogen and stored for up to 1 month at –20 °C until chemical analyses. Results are expressed as the mean of 2-year values with three replications for each year.

2.5. Fruit chemical analyses

Soluble solids content (SSC) was determined by hand refractometer (ATC, Belgium). A drop of homogenized and filtrated sample was placed on the lens and the reading was expressed as % of SSC in the fruit.

Titratable acidity (TA) was measured using a burette containing 0.1 N NaOH. Homogenized and filtered samples (25 mL) were titrated using phenolphthalein as an acid-base sensitive color indicator to achieve the pink endpoint. The sodium hydroxide solution was added drop by drop to the flask and mixed until the color turned a persistent pink for at least 30 s (approximately 8.1 pH). The

total TA of the samples was calculated using the following equation:

$$\frac{V\,(mL) \times N \times 0.268}{25\ mL\ of\ sample} \times 100 = \%\ malic\ acid\ equivalent$$

where V (mL) is the amount of sodium hydroxide solution used, N is the normality of sodium hydroxide solution, and 0.268 is the milliequivalent factor for malic acid, the predominant acid found in strawberries. TA was expressed as percentage of malic acid equivalent.

Vitamin C was quantified with the refractometer set of Merck Co. (Merck Rqflex) as described by Pantelidis et al. (2007). Results are expressed as milligram of ascorbic acid per 100 grams of fresh weight (mg 100 g^{-1} FW).

The monomeric anthocyanin pigment content of the aqueous extracts was determined using the pH-differential method described previously (Liu et al., 2002). Pigment content was calculated as milligrams of cyanidin-3-glucoside equivalents per 100 grams of fresh weight (mg C3G eq 100 g^{-1} FW), using an extinction coefficient of 26.900 L cm^{-1} mol^{-1} and a molecular weight of 449.2 g mol^{-1}.

The total phenolic content was determined using a modified Folin–Ciocalteu colorimetric method (Liu et al., 2002), with results expressed as milligrams of gallic acid equivalent per 100 grams of fresh weight (mg GAE 100 g^{-1} FW). First, 40 µL of fruit extract or gallic acid standard solution was mixed with 3.16 mL of distilled water. In the next phase, 200 µL of Folin–Ciocalteu reagent was added and allowed to stand for 8 min before adding 600 µL of 20% Na_2CO_3 solution. The solution was mixed well and absorbance at 765 nm against an appropriate blank was determined after 2 h.

Antioxidant capacity was determined using the DPPH method reported by Brand-Williams et al. (1995) with modifications (Sanchez-Moreno et al., 1998). An aliquot of 0.1 mL of the fruit phenol extraction was added to 3.9 mL of DPPH solution in methanol (0.060 mM) and vortexed. A control sample, containing the same volume of solvent in the place of the extraction, was used to measure the maximum DPPH absorbance. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 515 nm was recorded to determine the concentration of the remaining DPPH. The results were expressed as the Trolox equivalent antioxidant capacity per 100 grams of fresh weight (µmol TE 100 g^{-1} FW). Trolox standard solutions were prepared at a concentration ranging from 50 to 300 µM.

Data were subjected to analysis of variance using the MSTAT-C statistical computer package (Michigan State University, East Lansing, MI, USA), and the treatment means were compared by least significance difference (LSD) test at P = 0.05.

3. Results

3.1. Vegetative potential

Fertilizers, cultivars, and fertilizer × cultivar interactions had an effect on the majority of investigated parameters of strawberry vegetative growth in this study (Table 1). No

Table 1. The influence of fertilizer type and cultivar on the vegetative potential of strawberries.

Factor		Plant height (cm)	Number of crowns per plant	Number of leaves per rosette	Single leaf area (cm^2)
Fertilizer (A)	Biofertilizer 1	33.9 ± 1.2 a	5.4 ± 0.5 a	43.5 ± 2.4 a	311.7 ± 28.8 a
	Biofertilizer 2	33.3 ± 0.5 a	5.4 ± 0.7 a	39.8 ± 2.6 a	243.2 ± 10.2 a
	Control	31.4 ± 0.6 b	4.2 ± 0.4 b	28.9 ± 1.2 b	233.7 ± 21.0 a
Cultivar (B)	Clery	35.0 ± 1.0 a	3.2 ± 0.2 b	35.3 ± 2.7 a	225.1 ± 31.6 a
	Joly	33.0 ± 0.3 b	6.0 ± 0.4 a	39.3 ± 3.9 a	243.1 ± 12.7 a
	Dely	30.6 ± 0.5 c	5.8 ± 0.2 a	37.7 ± 2.3 a	297.5 ± 21.8 a
ANOVA					
A		*	*	*	ns
B		*	*	ns	ns
A × B		*	*	*	ns

Mean of 2-year values with three replications in each year ± standard error are presented.
Values within each column followed by the same letter are not significantly different at P ≤ 0.05 by LSD test. *: Statistically significant differences at P ≤ 0.05; ns: nonsignificant differences.

significant differences were observed only in the size of a single leaf area as affected by both tested factors, whereas the number of leaves per rosette did not differ significantly by cultivar alone.

Plant height increased significantly with bacterial treatments compared with the control. Clery had significantly higher plant heights than Joly and Dely, whereas the number of crowns per plant in Clery was significantly lower. The numbers of crowns and leaves per plant were significantly higher in Biofertilizer 1 and 2 treatments compared to the control treatment.

Fertilizer × cultivar interaction had a significant impact on plant height, number of crowns per plant, and number of leaves per rosette (Figures 1–3). The highest plant vegetative potential was recorded in the application of Biofertilizer 2 to the Joly cultivar.

3.2. Leaf mineral composition

Macroelement content in strawberry leaves in relation to the fertilizer, cultivar, and their interactive effect is shown in Table 2. The cultivar, as well as the fertilizer × cultivar effect, demonstrated a significant impact on the content of all of the examined macroelements in the strawberry leaves, while the fertilizer exhibited a significant impact only on N and K content. Moreover, the application of Biofertilizer 1 led to significantly higher concentrations of N in the leaves compared to the treatment without fertilization. Among the tested cultivars, Joly had a significantly higher concentration of P in the leaf, whereas a significantly lower concentration of N, Ca, and Mg was observed in Dely.

The content of microelements in strawberry leaves was significantly affected by fertilizer, cultivar, and their interaction (Table 3). Some exceptions were found, such as the impact of fertilizer on Zn and B content, the impact of cultivar on Mn content, and the impact of the interaction of these two factors on the Zn content in the leaves. The highest concentration of Fe was recorded in Biofertilizer 1 and the control treatment, while concentrations of Cu, Mn, and Mo were found to be the highest in the leaves from the control treatment. Significant differences among the cultivars were registered in relation to the content of microelements, with the exception of Mn. Cultivar Joly had considerably higher Fe and Cu contents than the other two examined cultivars, whereas significantly higher Mo content was detected in Dely.

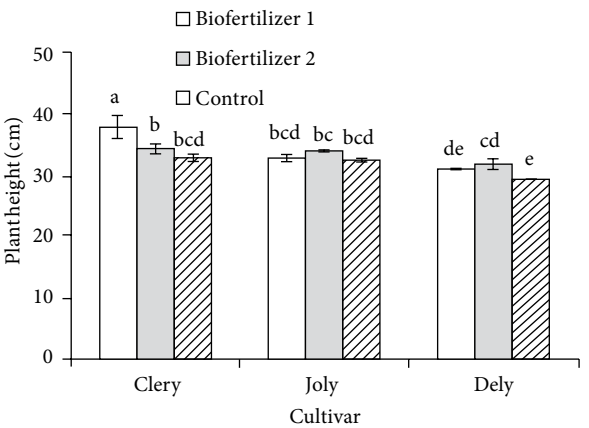

Figure 1. Interaction effect of fertilizer type and cultivar on plant height.

Data are means of 2-year values with three replications in every year ± standard error. The same letters represent nonsignificant differences at P ≤ 0.05 by LSD test.

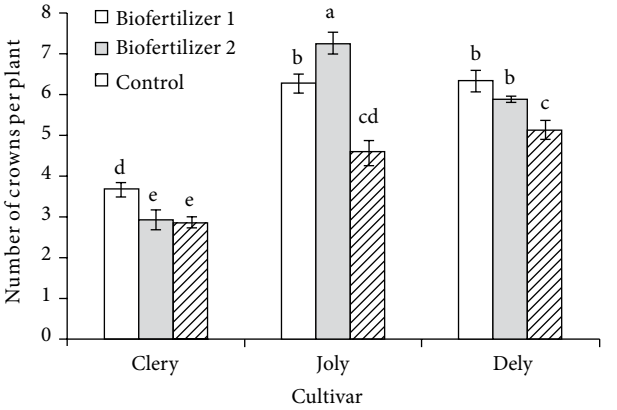

Figure 2. Interactive effect of fertilizer type and cultivar on the number of crowns per plant.

Data are means of 2-year values with three replications in each year ± standard error. The same letters represent nonsignificant differences at P ≤ 0.05 by LSD test.

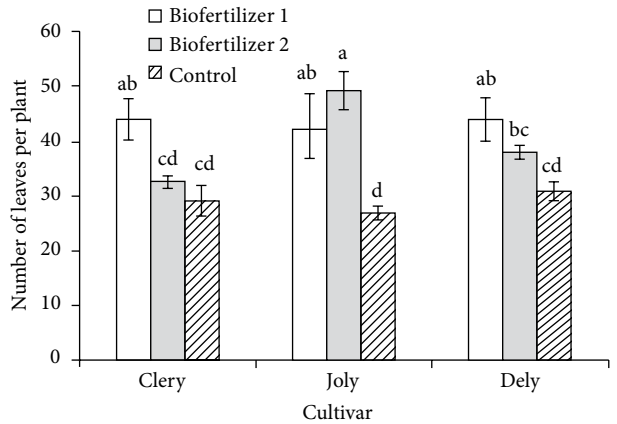

Figure 3. Interactive effect of fertilizer type and cultivar on the number of leaves per rosette.

Data are means of 2-year values with three replications in each year ± standard error. The same letters represent nonsignificant differences at P ≤ 0.05 by LSD test.

Table 2. The influence of fertilizer type and cultivar on macroelement content in strawberry leaves.

Factor		N (%)	P (μg mL^{-1})	K (μg mL^{-1})	Ca (μg mL^{-1})	Mg (μg mL^{-1})
Fertilizer (A)	Biofertilizer 1	2.03 ± 0.03 a	22.81 ± 0.90 a	63.20 ± 2.99 b	127.16 ± 14.83 a	32.79 ± 3.46 a
	Biofertilizer 2	1.98 ± 0.03 ab	21.04 ± 1.64 a	62.24 ± 3.74 b	113.98 ± 7.01 a	28.69 ± 2.59 a
	Control	1.94 ± 0.01 b	22.07 ± 1.32 a	70.94 ± 5.35 a	113.36 ± 3.24 a	29.01 ± 0.66 a
Cultivar (B)	Clery	2.00 ± 0.03 a	20.17 ± 1.03 b	52.58 ± 1.27 b	125.84 ± 7.15 a	32.68 ± 1.98 a
	Joly	2.03 ± 0.03 a	25.10 ± 1.10 a	72.06 ± 2.67 a	133.54 ± 11.07 a	33.71 ± 2.68 a
	Dely	1.93 ± 0.02 b	20.65 ± 1.16 b	71.74 ± 4.17 a	95.12 ± 4.20 b	24.10 ± 1.17 b
ANOVA						
A		*	ns	*	ns	ns
B		*	*	*	*	*
A × B		*	*	*	*	*

Means of 2-year values with three replications in each year ± standard error are presented.
Values within each column followed by the same letter are not significantly different at P ≤ 0.05 by LSD test. *: Statistically significant differences at P ≤ 0.05; ns: nonsignificant differences.

Table 3. The influence of fertilizer type and cultivar on microelement content in strawberry leaves.

Factor		Fe (μg mL^{-1})	Cu (μg mL^{-1})	Mn (μg mL^{-1})	Zn (μg mL^{-1})	B (μg mL^{-1})	Mo (μg mL^{-1})
Fertilizer (A)	Biofertilizer 1	0.72 ± 0.03 a	0.04 ± 0.00 b	0.24 ± 0.01 b	0.08 ± 0.01 a	0.28 ± 0.03 a	0.004 ± 0.000 b
	Biofertilizer 2	0.58 ± 0.04 b	0.04 ± 0.00 b	0.21 ± 0.01 b	0.07 ± 0.00 a	0.25 ± 0.02 a	0.004 ± 0.000 b
	Control	0.68 ± 0.03 a	0.04 ± 0.00 a	0.27 ± 0.01 a	0.08 ± 0.00 a	0.28 ± 0.00 a	0.007 ± 0.000 a
Cultivar (B)	Clery	0.61 ± 0.02 b	0.04 ± 0.00 b	0.23 ± 0.01 a	0.08 ± 0.00 a	0.24 ± 0.02 a	0.004 ± 0.000 b
	Joly	0.74 ± 0.04 a	0.05 ± 0.00 a	0.25 ± 0.01 a	0.09 ± 0.00 a	0.31 ± 0.02 a	0.004 ± 0.000 b
	Dely	0.62 ± 0.04 b	0.04 ± 0.00 b	0.24 ± 0.02 a	0.07 ± 0.00 b	0.22 ± 0.02 b	0.005 ± 0.000 a
ANOVA							
A		*	*	*	ns	ns	*
B		*	*	ns	*	*	*
A × B		*	*	*	ns	*	*

Means of 2-year values with three replications in each year ± standard error are presented.
Values within each column followed by the same letter are not significantly different at P ≤ 0.05 by LSD test. *: Statistically significant differences at P ≤ 0.05; ns: nonsignificant differences.

3.3. Generative potential

The applied biofertilizers, as well as the fertilizer × cultivar interactions, did not have a significant impact on the parameters of the generative potential (Table 4). On the other hand, cultivar had a significant impact on the number of inflorescences and fruits per plant, yield per plant, and yield per square meter. Among the cultivars, Joly had a significantly higher number of inflorescences, yield per plant, and yield per square meter. No significant difference was found between Clery and Dely in the number of inflorescences per plant, yield per plant, and yield per square meter.

Table 4. The influence of fertilizer type and cultivar on the generative potential of strawberry.

Factor		Number of inflorescences per plant	Number of fruits per plant	Yield per plant (g)	Yield per m^2 (kg)
Fertilizer (A)	Biofertilizer 1	4.1 ± 0.5 a	22.3 ± 1.3 a	565 ± 63.5 a	4.5 ± 0.5 a
	Biofertilizer 2	4.3 ± 0.4 a	24.1 ± 1.5 a	581 ± 80.1 a	4.6 ± 0.6 a
	Control	4.0 ± 0.5 a	19.5 ± 1.6 a	503 ± 88.5 a	4.0 ± 0.7 a
Cultivar (B)	Clery	3.1 ± 0.1 b	23.8 ± 1.51 a	451 ± 28.9 b	3.6 ± 0.2 b
	Joly	5.8 ± 0.2 a	23.1 ± 1.52 ab	840 ± 67.3 a	6.4 ± 0.5 a
	Dely	3.5 ± 0.1 b	19.0 ± 1.32 b	393 ± 31.1 b	3.2 ± 0.3 b
ANOVA					
A		ns	ns	ns	ns
B		*	*	*	*
A × B		ns	ns	ns	ns

Means of 2-year values with three replications in each year ± standard error are presented.
Values within each column followed by the same letter are not significantly different at $P \leq 0.05$ by LSD test. *: Statistically significant differences at $P \leq 0.05$; ns: nonsignificant differences.

3.4. Morphometric fruit traits

The fertilizers did not demonstrate a significant impact on the morphometric characteristics of the fruits, nor were these traits significantly affected by the fertilizer × cultivar interaction (Table 5). Significant differences established in all of the examined morphometric parameters were only affected by the cultivar. A significantly larger fruit weight, length, and width were observed in Joly, whereas lower values of the fruit weight and width were found in Clery. No significant differences were found in the fruit length between Clery and Dely.

3.5. Chemical fruit traits

Fertilizer, cultivar, and fertilizer × cultivar interaction had a significant influence on TA, TPC, TACY, and TAC (Table 6). Vitamin C content was also affected by the cultivar. Biofertilizer 2 demonstrated a positive impact on TA, TPC, and TACY, while a significantly higher TAC value was recorded in the Biofertilizer 1 treatment. Clery had a considerably higher TA level, while Joly ranked the highest concerning vitamin C content, TPC, TACY, and TAC.

The fertilizer × cultivar interaction significantly influenced TA, TPC, TACY, and TAC of the fruits (Figures 4–7). The strongest impact on TA was due to the Biofertilizer 2 × Clery interaction, while the Biofertilizer 1 × Joly interaction significantly influenced TPC and TAC. TACY was found to be considerably higher in the fruits of cultivar Joly considering the interactive effect of both biofertilizers.

4. Discussion

Our results indicated that fertilizer had a significant influence on plant height, number of crowns, and number of leaves per plant. Seo et al. (2009) stated that the use of three different commercial microbiological fertilizers (Ofarmguard, O-sis, and EXTN), implemented in seven applications at 15-day intervals resulted in an increased number of leaves per plant as well as in larger leaf area compared to the control treatment. Results presented by Umar et al. (2009) also indicated that the largest leaf area in strawberry plant was recorded after the gradual addition of mineral nitrogen together with *Azotobacter*. Moreover, the use of *Azotobacter* contributes to a more efficient absorption of nitrogen by plants, which was previously reported by Bambal et al. (1998).

According to our research, cultivar had a stimulating effect on plant height and the number of crowns per plant, while the cultivar × fertilizer interaction influenced the number of leaves per plant. However, Shaw (1993) reported that an overly vigorous plant can actually lead to a reduced yield, due to the shift of the assimilatory function from the generative to the vegetative potential. Enhancing plant growth is probably not the only factor that influences yield components, as can be seen from our results: the cultivar (Joly) with a lower plant height exhibited the highest number of inflorescences per plant, yield per plant, and yield per square meter.

In this study, both of the tested biofertilizers had a significant impact on the mineral composition of

Table 5. The influence of fertilizer type and cultivar on morphometric fruit traits.

Factor		Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Index of fruit shape
Fertilizer (A)	Biofertilizer 1	25.6 ± 2.8 a	42.4 ± 2.4 a	37.8 ± 1.6 a	1.12 ± 0.03 a
	Biofertilizer 2	23.8 ± 2.6 a	41.9 ± 1.9 a	36.6 ± 1.4 a	1.14 ± 0.02 a
	Control	25.0 ± 2.7 a	43.0 ± 2.7 a	37.3 ± 1.4 a	1.14 ± 0.04 a
Cultivar (B)	Clery	19.0 ± 0.7 c	38.7 ± 0.7 b	32.9 ± 0.5 c	1.17 ± 0.02 a
	Joly	34.8 ± 1.6 a	51.3 ± 1.1 a	42.3 ± 0.9 a	1.21 ± 0.02 a
	Dely	20.6 ± 0.5 b	34.7 ± 0.6 b	36.5 ± 0.3 b	0.95 ± 0.01 b
ANOVA					
A		ns	ns	ns	ns
B		*	*	*	*
A × B		ns	ns	ns	ns

Mean of 2-year values with three replications in every year ± standard error are presented.
Values within each column followed by the same letter are not significantly different at P ≤ 0.05 by LSD test. *: Statistically significant differences at P ≤ 0.05; ns: nonsignificant differences.

Table 6. The influence of fertilizer type and cultivar on chemical fruit properties.

Factor		Soluble solids content	Titratable acidity	Vitamin C	Total anthocyanins	Total phenolics	Total antioxidant capacity
		(%)	(% malic acid)	(mg 100 g^{-1} FW)	(mg C3G eq 100 g^{-1})	(mg GAE 100 g^{-1} FW)	(mmol TE 100 g^{-1} FW)
Fertilizer (A)	Biofertilizer 1	10.56 ± 0.36 a	0.70 ± 0.02 b	17.46 ± 0.79 a	26.65 ± 2.54 b	199.60 ± 16.73 b	1.85 ± 0.19 a
	Biofertilizer 2	10.68 ± 0.14 a	0.81 ± 0.05 a	16.40 ± 0.88 a	33.30 ± 1.36 a	231.12 ± 5.45 a	1.61 ± 0.10 b
	Control	10.38 ± 0.29 a	0.73 ± 0.02 b	17.16 ± 0.49 a	24.76 ± 2.45 c	197.08 ± 8.55 b	1.45 ± 0.10 c
Cultivar (B)	Clery	10.75 ± 0.37 a	0.86 ± 0.04 a	16.05 ± 0.46 b	29.22 ± 1.30 b	182.92 ± 12.06 c	1.31 ± 0.06 c
	Joly	10.34 ± 0.25 a	0.71 ± 0.02 b	19.46 ± 0.49 a	34.37 ± 1.42 a	229.93 ± 12.16 a	2.08 ± 0.11 a
	Dely	10.51 ± 0.17 a	0.67 ± 0.01 c	15.51 ± 0.32 b	21.13 ± 2.12 c	214.94 ± 5.59 b	1.52 ± 0.10 b
ANOVA							
A		ns	*	ns	*	*	*
B		ns	*	*	*	*	*
A × B		ns	*	ns	*	*	*

Means of 2-year values with three replications in each year ± standard error are presented.
Values within each column followed by the same letter are insignificantly different at P ≤ 0.05 by LSD test. *: Statistically significant differences at P ≤ 0.05; ns: nonsignificant differences.

strawberry leaves. A significantly higher content of N was recorded in the treatment with Biofertilizer 1, which may be associated with N_2-fixing abilities of bacteria contained in this fertilizer type. Furthermore, Biofertilizer 1 insignificantly promoted P, Ca, and Mg uptake in strawberry by slightly increasing their contents in the leaves. This effect may be explained by the phosphate-solubilizing capacity of the bacteria that decreases the soil pH and stimulates the availability of P by producing organic acids. Esitken et al. (2010) found significantly increased leaf P content in plant growth-promoting bacteria (*Pseudomonas* BA-8, *Bacillus* OSU-142, and *Bacillus* M-3) treatments. In the present study, only the K content was significantly lower compared to the control, although the value was much higher (63.20 μg mL^{-1}) than normal (15–25 μg ml^{-1}) according to the adequate range of mineral nutrient content in strawberry proposed by Bergmann (1992). The deficiency of leaf K content might be attributed to excessive N levels, which may interfere with the uptake of this element. In microelements, the highest Fe content was recorded in the treatment with Biofertilizer 1, while the contents of Cu, Mn, and Mo were

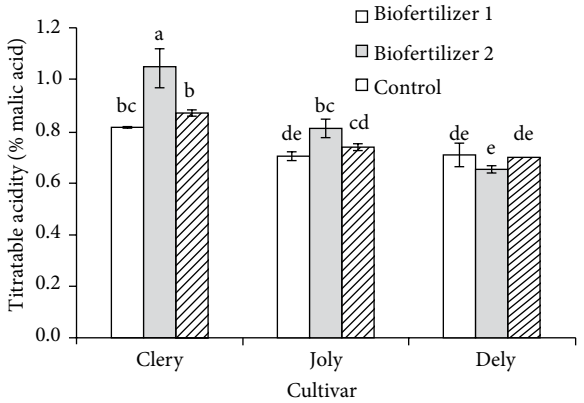

Figure 4. Interactive effect of fertilizer type and cultivar on titratable acidity in strawberry fruits.
Data are means of 2-year values with three replications in each year ± standard error; FW is fresh weight. The same letters represent nonsignificant differences at P ≤ 0.05 by LSD test.

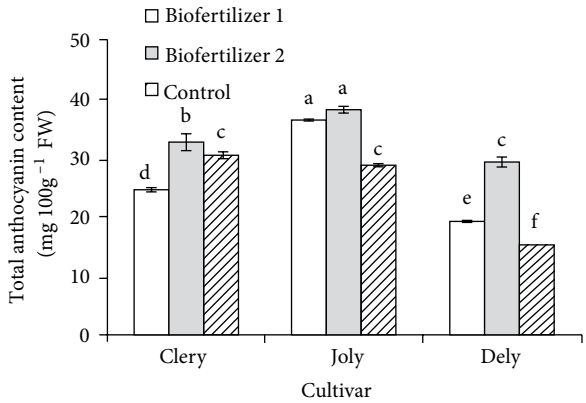

Figure 5. Interactive effect of fertilizer type and cultivar on total anthocyanin content in strawberry fruits.
Data are mean of 2-year values with three replications in each year ± standard error; FW is fresh weight. The same letters represent nonsignificant differences at P ≤ 0.05 by LSD test.

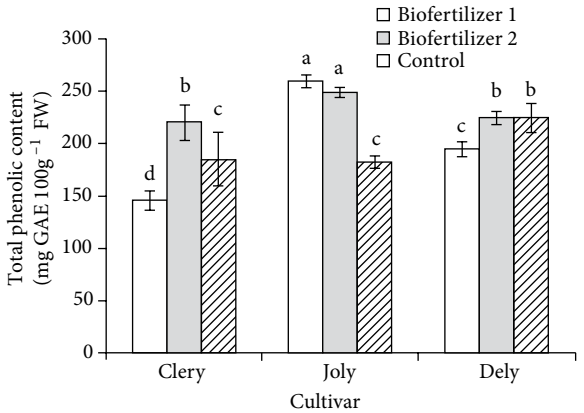

Figure 6. Interactive effect of fertilizer type and cultivar on total phenolic content in strawberry fruits.
Data are means of 2-year values with three replications in each year ± standard error; FW is fresh weight. The same letters represent nonsignificant differences at P ≤ 0.05 by LSD test.

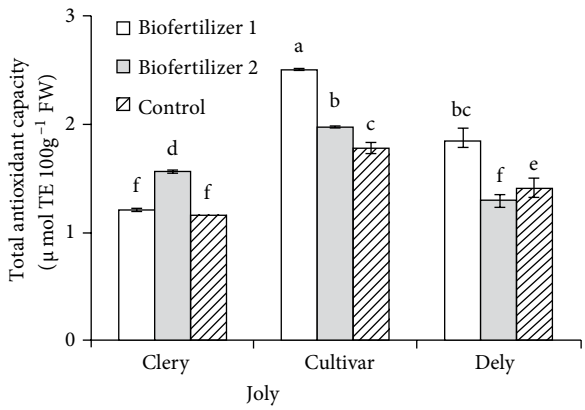

Figure 7. The interactive effect of fertilizer type and cultivar on total antioxidant capacity of strawberry fruits.
Data are means of 2-year values with three replications in every year ± standard error. The same letters represent nonsignificant differences at P ≤ 0.05 by LSD test.

considerably higher in the treatment without fertilization. Leaf Cu and Mn deficiency can be associated with the immobility of these elements in the plant (Papadakis et al., 2007), i.e. the uptake of Cu may be blocked by excessive P levels (Leece, 1975). Perkins-Veazie (2004) also revealed that excessive P content can lead to deficiency in some microelements, and Zn in particular. The availability of Mo to plants is highly dependent on soil pH, making its uptake markedly enhanced under alkaline conditions (Reddy et al., 1997). This may be one of the reasons why we observed lower leaf Mo content. The observed Mn and Mo deficiencies in the biofertilizer treatments may also be explained by the fact that the desired effects of applying cultured beneficial microorganisms appear only after they are established and become dominant in the soil. Antagonistic microorganisms already present in the soil compete with microbial inoculants and sometimes do not allow their effective establishment by outcompeting the inoculated populations (Mahdi et al., 2010). Therefore, the positive effects of biofertilizers can be expected only after a certain time.

The analysis of strawberry leaves also revealed that amounts of all the macro- and microelements, with the exception of Mn, are cultivar-related. Hakala et al. (2003) also found that cultivar and origin are the main factors affecting macro- and microelement leaf content in several cultivars.

Our results have confirmed the significant impact of cultivar on the generative potential of strawberry plants. The highest average number of inflorescences per plant

and the largest yields per plant and per square meter were recorded in cultivar Joly. Joly and Clery also had a significantly higher fruit set per plant as compared to Dely. Handley and Dill (2003) stated that the fruit set of a cultivar may have a stronger impact on the yield than the fruit weight. Despite the fact that Clery and Joly demonstrated a slight difference in the average number of fruit set, Joly had a significantly higher fruit weight and, consequently, the highest yield per plant.

Fruit weight is an important trait in highly productive strawberry cultivars. In our study, cultivar expressed a significant influence not only on fruit weight but also on the other examined morphometric fruit traits. Among the cultivars, Joly had the largest fruit weight and dimensions, which is in accordance with the description reported by Martinelli and Leis (2012). Milivojević et al. (2009) observed a significantly higher fruit weight in Clery planted in a cultivation system on raised beds compared to those obtained in two soilless cultivation systems (substrate-filled bags with a different volumes and number of plants per square meter). Slightly lower results were observed in our study, which can be attributed to the differences of the agroecological conditions in the examined cultivation regions. Pešaković and Milivojević (2014) also stated that cultivar affected the fruit shape index, indicating that the lowest value recorded in Dely corresponded to the rounded conical form, while Joly and Clery tended to have a long conical form. Similar results were obtained in our research.

Our research established that TA is determined by the cultivar as well as by the type of the applied biofertilizer. The impact of the applied biofertilizer on TA was also confirmed by Pešaković et al. (2013), who observed a positive effect of biofertilizer on SSC, TA, and total and reducing sugars in Senga Sengana strawberry fruits. As opposed to the expressed variation in TA among the examined cultivars, as well as in the function of the biofertilizer application, there were no major differences among obtained SSC values with respect to either the different cultivars or the various fertilization treatments. Phenolic content (TACY, TPC) and TAC levels were found to be greatly influenced by both of the tested factors and their interaction. Pešaković and Milivojević (2014) observed greater values of TPC and TAC in the biofertilizer treatment, which might be due to highly intensive mineralizing processes in the substrate and the increased physiological functions and activity of the plant root. Likewise, our results confirmed the positive impact of Biofertilizer 2 on TA, TACY, and TPC and the positive impact of Biofertilizer 1 on TAC. Among the natural antioxidant substances contained in strawberry fruit, vitamin C has also been shown to play an important role in controlling oxidative reactions in the human body and thereby exhibits anticarcinogenic activities (Sun et al., 2002). In our study, the only variable that determined the quantity of vitamin C in the fruits was the cultivar.

Concerning the effect of the cultivar, significantly higher values of vitamin C, TACY, TPC, and TAC were registered in Joly compared to the other two examined cultivars. Cordenunsi et al. (2002) examined six strawberry cultivars at a commercial plantation in Brazil and demonstrated that significant changes in SSC, vitamin C, TPC, and TACY in the fruits during the ripening phase were also cultivar-dependent. The high TPC of the Joly fruit obtained in our study could be additionally explained as the response to the nutrient application as well as being due to the genetically controlled accumulation of individual phenolics.

Our comparative study of three cultivars under two different biofertilizer treatments indicated that the response to bacterial inoculation is cultivar-related in strawberries. The investigation into vegetative potential showed that the Biofertilizer 1 application provided not only the most favorable conditions for plant growth but also exhibited a pronounced effect on the content of macroelements in leaves. On the other hand, Biofertilizer 2 gave the best results in terms of the chemical properties of strawberry fruits. Although the Clery, Joly, and Dely cultivars are characterized by outstanding physical and chemical fruit characteristics, which are reflected primarily in their high antioxidant activity, Joly was ranked the highest according to most of the examined parameters. Finally, the significant impact of the fertilizer × cultivar interaction on plant growth and nutritional fruit quality may promote bacterial inoculation as an appropriate technique of field application in commercial strawberry production.

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