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THE EFFECT OF FERTILIZATION AND FRUIT ORDER ON STRAWBERRY FRUIT QUALITY

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Abstract

Fertilization is an important cultural practice in modern strawberry production, including the use of different types of fertilizer. The present research is aimed to evaluate the effect of fertilizer type and order in the truss on the fruit quality of strawberry cultivar 'Clery'. Fruit quality (mass, dimensions, shape index, firmness, soluble solids and total phenols) was monitored in three fertilizer treatments (biofertilizer, the combination of bio- and mineral fertilizer and mineral fertilizer) and four fruit positions in the truss (primary, secondary, tertiary and quaternary). Application of mineral fertilizer resulted in a significant increase in fruit firmness (10.4 N) while the use of biofertilizers containing bacteria of the genus *Bacillus* enabled fruits with significantly higher total phenolic content (276.6 mg GA/100 g fw) with the positive effect also exerted in secondary positioned fruits (486.7mg GA/100 g fw). Differences in quality traits were greater among different fruit positions, compared to fertilizer treatments although primary fruits were characterized by the highest values of mass and dimensions. Tertiary positioned fruits had the highest soluble solids content (11.7%) that did not significantly differ from the fourth batch of strawberry fruits (11.1%). Overall, the result of this study suggests that fruits at a lower position in the truss have better organoleptic composition while fruits on higher positions have better physical properties. Biofertilizers used in combination with mineral fertilizers have the potential to increase physical strawberry properties and the use of biofertilizers as supplements to mineral fertilizers can be considered an appropriate practice to ensure safe strawberry fruit production and help overcome environmental problems caused by the overuse of mineral fertilizers.

Keywords: *Strawberry, Biofertilizer, Mineral Fertilizers, Fruit order, Fruit Quality.*

Introduction

Strawberries are widely and highly consumed fresh and in processed forms, and their increasing production under glass makes them available all year round (Anttonen et al., 2006). Micronutrients and phenolic compounds concentration in berries, as well as commercial and organoleptic attributes, have been reported to change according to many pre-harvest conditions, such as genotype, environment, and cultivation techniques (Cordenunsi et al., 2002; Kafkas et al., 2007).

In modern strawberry production, fertilization is one of the most important cultural techniques because balanced fertilization has an important effect on both the fruit yield and fruit quality. An extensive body of research shows that the continuous use of mineral fertilizers leads to environmental contamination, with more than 50% of applied mineral fertilizers remaining unabsorbed, resulting in mineral loss, and thus posing a serious threat to the environment. Therefore, proper use and partial or complete substitution of mineral fertilizers with microbial inoculants i.e. biofertilizers can help overcome environmental problems caused by the overuse of mineral fertilizers. Also, food scientists study production and processing to develop new technologies that improve the quality and quantity of healthy food products, with the main

objective of increasing food production without affecting food quality and environment, while fulfilling consumer expectations (González-Aguilar et al., 2010).

The flowers of the strawberry are collected in cymose inflorescence called diciasia. Diciasia consists of one primary, two secondary, four tertiary, and eight quaternary flowers that open according to the time of origin. Despite changes in strawberry fruit phytochemical content during ripening (Kosar et al., 2004; Ferreyra et al., 2007), many published studies employ a commercial type of picking (by color) to determine harvest date and ignore developmental time, truss position and the effect these parameters have on fruit chemical composition (Tsormpatsidis et al., 2011). Thus, this study aimed to evaluate the potential of fertilizers as well as fruit order on the quality of the strawberry fruit.

Materials and methods

Experimental layout and orchard management

The field experiment was carried out in the strawberry plantation at the Fruit Research Institute, Čačak, located in central Čačak (43° 54' N latitude, 20° 21' E longitude, 242 m altitude), in the Western Morava valley (Western Serbia). Strawberries were planted in August 2015 in double rows on beds covered with black polyethylene foil. Plant spacing was 30×30cm, and spacing between beds was 40 cm. 'Clery', one of the predominant strawberry cultivars in the Republic of Serbia, was selected for this study in the first year after planting (2016). The layout of the experiment was a completely randomized design, with the effect of two factors, viz. fertilizer and fruit order analyzed. In addition to standard cultivation practices, the plants were regularly irrigated according to soil humidity. The fertilizers were applied through fertigation.

The experimental procedure included three fertilization treatments: B, the bacterial inoculum of *Bacillus* sp. ('Bacilomix'); B+M, half of the standard recommendations mineral fertilizers and bacterial inoculum of *Bacillus* sp.; M, mineral fertilizers with different formulation ratios according to standard recommendations for strawberry production. Microbiological fertilization with a bacterial inoculum of *Bacillus* sp. involved fertigation with 10–12 L/ha of the inoculum three times per month during the growing season. The bacterial titer in the inoculum was 20–40×10⁶ cm³. Mineral fertilizers were applied according to the phenological stage of the plant, as follows: at the beginning of the growing season, starter fertilizer NPK Poly-Feed Drip 11-44-11 with micronutrients; during intensive plant growth and flower bud emergence, two applications of NPK Poly-Feed Drip 20-20-20 with micronutrients at a seven-day interval; during flowering, fruit set, growth, and ripening, five applications of the complex mineral fertilizer NPK Poly-Feed Drip 16-8-32+2MgO at ten-day intervals; during intensive fruit growth and ripening, in addition to the former formulation, two applications of Multi-Cal (15.5% N and 26.5% CaO) and Multi-KMg (12% N; 43% K and 2% MgO) at a 10-day interval. Combined application of microbiological and mineral fertilizers consisted of a half amount of aforementioned microbiological and mineral fertilizers each. The treatments were represented by 60 plants each (3 replicates of 20 plants).

The strawberries were harvested at commercial maturity stage. Samples for analysis were taken during the whole harvest and consisted of primary (I), secondary (II), tertiary (III) and quaternary fruits (IV) (sixty strawberries from each order of fruit, 20 fruits per 3 replications). Fresh fruits were used for initial physicochemical measurements and then the samples were stored at -20°C till analysis of total phenolic content (TPC).

Determination of fruit quality

Twenty fruits in each replication were randomly selected to determine average fruit weight using the 'Metler' balance (±0.01 g accuracy) and the data were expressed in g per fruit. The fruit dimensions (length and width) were measured by a digital moving scale (Carl Roth,

Germany) with an accuracy of ± 0.05 mm. The index of the fruit shape was obtained by calculation, determining the ratio of the length and width of the fruit. The firmness of the fruit was determined by a penetrometer, and the values were expressed in N. Soluble solids (SS) was determined using a hand refractometer (Pocket PAL-1, Atago, Japan), and values were expressed in %.

Total phenolic content was measured using a modified Folin-Ciocalteu assay (Liu et al., 2002). Samples weighing 4 g of fresh strawberries were blended in a food processor for 1 minute with 40 ml of 80% of aqueous methanol. The well-blended solution was centrifuged at 10 000 g for 15 minutes at 20°C, and the supernatant was separated. Shortly, 40 μ L of fruit extracts or gallic acid standard solution were mixed with 3.16 mL of distilled water. In the next phase, 200 μ L of Folin-Ciocalteu reagent was added and allowed to stand for 8 minutes before adding 600 μ L of 20% sodium carbonate solution. The solution was well mixed and absorbance of the samples and standards were measured spectrophotometrically (Jenway 6300, UK) at 765 nm. TPC was calculated as mg of gallic acid equivalents per 100 g fresh weight of the sample (mg GAE/ 100 g FW). The total phenolic concentration was derived as a function of the equivalent absorbance of gallic acid in the range 50 to 500 mg/L ($R^2=0.99$).

Results and discussion

Fruit size is one of the most important traits in the selection of new genotypes (Di Vittori et al., 2018). Describing the characteristics of strawberry cultivars grown in northern Italy, Lucchi et al. (2015) point out that the regular conical shape of cultivar 'Clery' is its good characteristic, while the tendency to reduce the dimensions of the fruit during the second part of the harvest is a bad characteristic of this cultivar. Similar results were obtained in our study where the dimensions of the fruit in strawberry cultivar 'Clery' on third and tertiary positioned were lower. In the research of Milivojević et al. (2015), the fruit weight of the cultivar 'Clery' (22.8 g) grown in the open field was close to the fruit weight recorded in a previous study by the same authors (Milivojević et al., 2009) in the cultivar 'Clery' cultivated in greenhouse conditions (22.9 g). However, the fruit weight of the cultivar 'Clery' in our studies was lower (from 20.9 in mineral to 21.4 g in microbiological fertilizer treatment) compared to the above values.

A fertilization regime was found to influence the fruit nutritional quality of cultivated strawberry (Di Vittori et al., 2018). The data presented in our study show that fertilizers did not affect most of the fruit quality parameters, except in firmness and total phenols (Table 1). The high fruit firmness obtained in mineral fertilizer treatment may be related to the application of fertilizers with high calcium content. The obtained results are in agreement with those of Treder (2004) and Wójcik and Lewandowski (2003), who point out that the basic element responsible for fruit firmness is calcium.

According to Jouquand (2008), the sensory quality of strawberry fruit is the result of complex balance among sweetness, aroma, texture and fruit appearance. The same authors point out that genotypes assessed as "not sweet" also had low soluble solids content and this parameter is generally a good indicator of consumer acceptability./for the acceptability of consumers. The average values of soluble solids content as a function of the fertilizer types and fruit order ranged from 9.7 to 11.7%. Soluble solids content did not vary significantly among the tested fertilizers while the position of the fruit significantly influenced these properties. Tertiary fruits had the highest soluble solids content (11,7%) that did not differ significantly from the soluble solids content of the fourth-order strawberry fruits (11,1%).

Table 1. Fruit quality of strawberry cultivar 'Clery' depending on fertilizer and fruit order.

		Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Shape index	Firmness (N)	Soluble solids (%)	Total phenols (mg GAE/100 g FW)
Fertilizer (A)	B	21.4±4.5 a	39.6±3.0 a	34.3±2.2 a	1.1±0.0 a	7.3±0.9 b	10.3±0.4 a	276.6±38.4 a
	B + M	21.3±3.9 a	39.9±2.7 a	33.4±2.0 a	1.2±0.0 a	8.5±1.0 ab	10.5±0.3 a	234.3±15.7 b
	M	20.9±3.9 a	39.6±2.9 a	33.3±2.2 a	1.2±0.0 a	10.4±1.1 a	11.1±0.5 a	204.6±11.5 c
Fruit order (B)	I	41.6±2.6 a	52.3±1.1 a	43.8±1.3 a	1.2±0.0 a	7.3±0.5 a	10.0±0.4 bc	227.1±11.3 b
	II	22.3±0.8 b	42.4±0.4 b	35.0±0.5 b	1.2±0.0 a	9.0±1.0 a	9.7±0.2 c	283.7±51.0 a
	III	13.2±0.5 c	37.3±0.9 c	30.5±0.6 c	1.2±0.1 a	8.4±1.8 a	11.7±0.6 a	206.6±25.4 c
	IV	7.8±0.4 d	26.8±0.5 d	25.4±0.6 d	1.1±0.0 b	10.2±1.3 a	11.1±0.4 ab	236.7±7.5 b
A	ns	ns	ns	ns	*	ns	*	
B	*	*	*	*	ns	*	*	
A×B	ns	ns	ns	ns	ns	ns	*	

Data represent the means of three replicates±standard error. The different lower-case letters in the columns indicate statistically significant differences among the mean values relative to fertilizer and fruit position at P≤ 0.05 level (Duncan's test).

Recently, also the nutritional value of berries, as the content of bioactive compounds with healthy effects on the final consumer, is being considered to better characterize the fruit quality (Di Vittori et al., 2018). Strawberry fruits, compared to other fruit species, contain high amounts of phenolic compounds, which have shown a positive effect against free radicals in *in vitro* tests (Aaby et al., 2007; Seeram et al., 2009). The phenolic components in berries have many different biological functions, including a role in plant growth, development, and protection (Nile and Park, 2014). They play a role in pigmentation, have antimicrobial and antifungal functions, provide protection against insects and ultraviolet radiation, chelate toxic heavy metals and defend against free radicals formed in the process of photosynthesis (Beer et al., 2004; Parry et al., 2005). In regard to the content of total phenols in our study, the influence of the fertilizer treatment was significant. Co-inoculation of strawberry plants showed better results in concentration of the total phenols compared to those recorded in plants cultivated following the standard fertilization protocol. Microbial biostimulants can improve root activity, increase water and nutrient uptake, and enhance crop tolerance to environmental stresses, yield and product quality, which may explain the high phenolic content in microbial inoculation treatment in our study. It is also known that beneficial soil microorganisms, like bacteria and arbuscular mycorrhizal (AM) fungi can influence the plant secondary metabolic pathways (Zeng et al., 2013).

Anttonen et al. (2006) found that fruit order significantly affected the phenolic content in fruits. The same authors stated that the levels of the total phenolics, ellagic acid, and antioxidant activity were found to increase from primary to tertiary fruits. Fruit order had a significant effect on the content of total phenols in our study. Second-order fruits had a significantly higher content of total phenols compared to other position of fruits. However, it is important to point out that the highest content of the aforementioned parameter was recorded in interaction between secondary fruit and microbiological fertilizer (Figure 1).

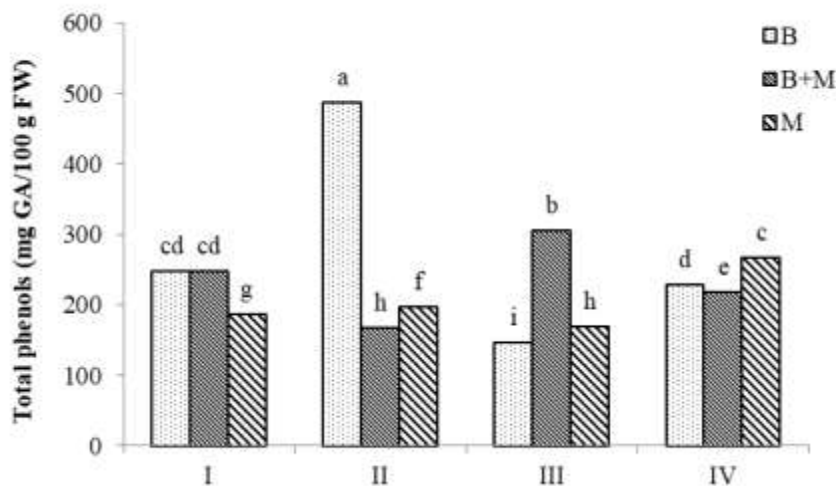


Figure 1. Total phenols in the fruit of strawberry cultivar 'Clery'. The different small letters at the top of columns indicate significant differences in total phenols at $P \leq 0.05$ by Duncan's test.

Principal components analysis (PCA) is a way of identifying the patterns in the data and expressing the data in such a way as to highlight their similarities and differences. In this study, the PCA analysis was applied to the complete data set (mass, dimensions, firmness, SS, TPC) to explain the correlations between the combination fertilizer/fruit order and to identify group patterns (Figure 2). The first group was composed of fruits with a high fruit mass and dimensions. This group includes primary and secondary fruits in mineral and a combination of mineral and microbiological fertilizer. Tertiary and quaternary fruits in combination with mineral and microbiological fertilizer belong to a group of fruits with good firmness and soluble solids content. The PCA analysis confirmed that the differences, which occurred in the physical and chemical composition of fruit, depended on the fruit position more than fertilizer type. Based on the presented data, it can be concluded that specific fruit properties depend on developmental time which is important for their potential commercial (fresh) or industrial use.

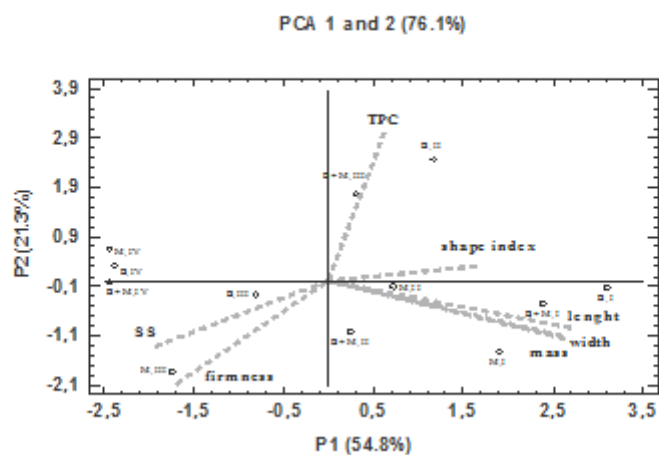


Figure 2. Biplot based on principal components analysis (PCA) for fruit quality properties in strawberry cultivar 'Clery'.

Conclusion

Bearing in mind that there was no difference in the fruit quality in combined use of mineral and bio fertilizers compared to the treatment with mineral fertilizers, fertilization modifications may provide a potential means to increase the fruit quality (weight, dimensions and firmness) as well as health-related value of strawberry by altering the phenolic contents of the fruit. Choosing cultivar as well as fruit order can affect the quality of fresh fruits and strawberry end products.

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