

The effect of using grafted seedlings on the yield and quality of tomatoes grown in greenhouses

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Abstract

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This study was aimed at observing the effect of the grafting of tomato plants on morphological (vegetative growth), production and nutritive characteristics (quantity and quality of production). For this purpose, the 'Lorely F1' cultivar was used as a scion grafted onto the 'Beaufort' rootstock. Plants were cultivated with a stem and two stems. The observations collected in this study were concerned with the characteristics of plant growth. The studied morphological characteristics were plant height, stem diameter and number of leaves, and the studied production characteristics were the characteristics of fructification and productivity (the average number of fruit per plant, the average weight of the fruit, production per plant). Particular attention was paid to the nutritional characteristics of the fruit, to the fruit quality (total soluble solids, total sugar, acidity, vitamin C, antioxidant activity (by the Trolox method) and the contents of lycopene and beta-carotene). The results showed that grafting positively influenced the growth and production characteristics. Grafting of tomato plants had an appreciable effect on the vegetative growth of the variant 2-grafted tomatoes with a stem. The best option in terms of productivity and production was the variant 3-grafted tomatoes with two stems, which yielded 9.2 kg per plant. Fruit quality was not improved in any of the grafted variants.

Keywords: hybrids; rootstock; two stems; lycopene; antioxidants

Tomatoes (*Solanum lycopersicum* L.) are one of the most consumed species of fruit worldwide. Fruits are rich in vitamins and minerals and make an important contribution to human health (DINU et al. 2015). The pleasant taste associated with the content of vitamins, sugars and amino acids in tomatoes, has resulted in a rapid expansion of tomato cultivation, and the fruit is now considered one of the main cultivated species (BEI 2015).

In Romania, tomatoes are grown in greenhouses under conditions of continuous monoculture, which reduces yield efficiency, both quantitatively and qualitatively. Studies have shown that after four years of continuous culture, production efficiency dropped to 48%, making it necessary to adopt soil disinfection

practices or other technical methods (BOGOESCU et al. 2011). The use of grafted plants is recommended as a measure to avoid these negative phenomena, and this innovative technique is increasingly in demand with farmers (KHAH et al. 2006).

Modern methods have been introduced into tomato culture with the aim of increasing tomato production and quality. The use of high-quality hybrids, grafting methods which reduce the number of chemical treatments and other technological methods represent viable alternatives for sustainable agriculture. Tomato production is influenced by many factors, including temperature, humidity, fertilisation, pests and diseases, etc. The most serious problems in greenhouse tomato crops are nematodes and soil pathogens, in

particular *Fusa-rium* and *Verticillium*. The grafting of tomatoes with hybrids of high quality and productivity onto rootstocks that are susceptible to attack by soil pests and diseases has been known for some years ago, but the technique has been improved and has spread rapidly in recent times. The main result of the process of grafting is the increased resistance against diseases and pests in the soil (*Melodogyne* spp.) (BOGOESCU et al. 2011). KHAH et al. (2006) showed that tomato grafting onto compatible rootstocks has positive effects on culture and on production efficiency, determining leading to higher profits for farmers. According to these authors, the increased efficiency is the result of fruit size in grafted plants (TURHAN et al. 2011). Grafting is a viable alternative strategy for improving tolerance to saline and water stress in tomato plants (AL-HARBI et al. 2016), and, according to other authors, grafting is considered an important technique for sustainable production in greenhouses (KACJAN MARŠIĆ, OSVALD 2004).

The grafted plants used in the production of tomatoes increase the productive efficiency and quality of the fruit depending on the rootstocks. For example, higher values of micronutrients such as potassium, phosphorus, calcium and magnesium were found in self-grafted plants compared to non-grafted ones. However, these differences were not found to be significant (BOGOESCU et al. 2011; AL-HARBI et al. 2016). Increased total soluble solids concentration and titratable acidity are important characteristics both for varieties used for processing and for fresh consumption and increase the nutritional value of the fruit.

Grafting the vegetables can affect various aspects of the fruit quality. Combinations of rootstock/graft (scion) should be carefully selected according to the climate and geographical conditions of the region. The proper selection can help control soil-borne diseases (soil) and can also increase the efficiency and the quality of fruit (DAVIS et al. 2008). The possibility of applying grafting to improve fruit quality and quantity has been poorly investigated. In this regard, this study was aimed at highlighting the positive effects of grafting on plant growth and on production quantity and quality.

MATERIALS AND METHODS

The experiments were carried out at the teaching facilities of the University of Craiova, Romania

(44°19'N and 23°48'E) in 2013–2014, in an unheated greenhouse. 'Lorely F1' was chosen as the biological material, and 'Beaufort' was used as rootstock. This rootstock was chosen because it is widely used in Romania for grafted tomato plants grown in greenhouses. Plants were grafted according to the slant-cut grafting technique. The experimental model included the following variants: V₁ non-grafted 'Lorely F1' (control), V₂ 'Beaufort' × 'Lorely F1' with a stem and V₃ 'Beaufort' × 'Lorely F1' with two stems. Planting was carried out in equidistant rows spaced at 100 cm and with distances between plants of 30 cm for variants 1 and 2 and 60 cm between plants for variant 3. The experiment was performed in three repetitions in randomised blocks. Practices specific to the culture of tomato crops in greenhouses, such as drip irrigation, application of pesticides and fertilisers, were followed. The culture ended after six stages of fructification.

Biometric measurements were made of plant height, stem diameter and number of leaves per plant, as well as to determine quantitative and qualitative production. Weighing was performed at each harvest to determine the average fruit weight and production per plant. In order to assess the nutritional quality, 10 fruits of each repetition were harvested and formed average samples. The samples of fruit were harvested at the mature stage when ready for consumption (red stage). The fruits were brought to the Laboratory of Biochemistry, washed, wiped with paper towels, cut to remove the seeds and introduced into a blender for 1 min, resulting in a smooth puree. The analyses were carried out in three repetitions, and the results were expressed as the mean of the repetitions. From that mixture, the following parameters were determined: total soluble solids (TSS), titratable acidity, reducing sugars, vitamin C, β-carotene, lycopene, total polyphenols and antioxidant activity.

Chemical analysis. The total soluble solid content (TSS) was determined using a digital refractometer and expressed as °Brix at 20°C.

The titratable acid content (acidity) was determined by titration with 0.1 N sodium hydroxide (NaOH), using phenolphthalein as an indicator and expressed as % citric acid.

Reducing sugars (%) were extracted in distilled water (1:50 w/v) and assayed colorimetrically with 3,5 dinitrosalicylic acid.

Ascorbic acid was extracted in 2% hydrochloric acid (HCl) (1:50 w/v) and measured by iodomet-

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ric redox titration. Ascorbic acid content was expressed as mg /100 g (f.w.).

Lycopene and β -carotene were extracted in 2:1:1 hexane:methanol:acetone. The non-polar layer was collected and spectrophotometrically analysed (Evolution 600 UV-Vis; Thermo Scientific, UK). The contents of lycopene and β -carotene were calculated according to the following equations: lycopene mg/100 ml = $-0.0458 \times A_{663} + 0.204 \times A_{645} + 0.372 \times A_{505} - 0.0806 \times A_{453}$; β -carotene mg /100 ml = $0.216 \times A_{663} - 1.220 \times A_{645} - 0.304 \times A_{505} + 0.452 \times A_{453}$. The results were expressed in mg/kg (f.w.).

Extracts for the determination of phenols and antioxidant activity were prepared in 80% aqueous methanol (1:10 w/v) at 24°C for 16 hours. The resulting slurries were centrifuged at 4,000 g for 5 min and the supernatants were analysed. Total phenolic content (TPC) was determined colorimetrically using the Folin-Ciocalteu method (as described by DANNEHL et al. 2011). The absorbance was recorded at 765 nm using a Thermo Scientific Evolution 600 UV-Vis spectrophotometer. The total phenolic content (TPC) was calculated using a standard curve prepared using gallic acid and expressed as mg of gallic acid equivalents (GAE/kg).

Antioxidant activity. DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The capacity of tomato extracts to reduce the radical 2,2-diphenyl-1-picrylhydrazyl was assessed using the method of BABBAR et al. (2014) with some modifications. A 0.075-mM (final concentration) DPPH solution in ethanol was mixed with sample extracts and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 20 min at 2 min intervals. The absorbance of the remaining DPPH radicals was measured at 519 nm. A blank reagent was used to track the stability of DPPH over the timeframe of the experiment. The scavenging activity of extracts was evaluated as a percentage of DPPH discoloration using the following formula: % scavenging = $[A_0 - (A_1 - A_S)] / A_0 \times 100$, where A_0 is the absorbance

of DPPH alone, A_1 is the absorbance of DPPH + extract and A_S is the absorbance of the extract alone. A Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as μ mol Trolox (TE/kg).

Data analysis: Statistical analysis was performed using ANOVA measures and the differences between the means were compared using the criterion of Duncan's multiple range test and LSD ($P = 0.05$) to assess productivity and quality elements. For morphological characteristics, mean and standard deviation were calculated using MS Excel software.

RESULTS AND DISCUSSION

The results of the effect of grafting on the vegetative growth of plants show differences based on the studied variants (Table 1). Plant size ranged between 142.8 cm in variant 3 to 211.5 cm in variant 2 for the grafted plants, and it was 175.7 cm in the non-grafted plants. The plant size variability found in the present study is consistent with the results obtained by LEE (1994) and KHAH et al. (2006).

Stem diameters were larger in the grafted variants than in the control variant and non-grafted variant. Values were 11.62 in V3 and 12.40 mm in V2, respectively, while diameter was 10.39 mm in the non-grafted variant. The average number of leaves per plant was between 21.3 and 30.1 in the grafted variants and 17.9 in the control variant. The marked increase in variant 3 is positively correlated with plant height, stem diameter and the number of stems. PULGAR et al. (1998) reported an increase in the number of leaves, which was probably due to the better absorption of water and minerals.

Our results are supported by the findings of KHAH et al. (2006) and KARACA et al. (2012) who showed that grafted tomato plants were more vigorous than the non-grafted plants. In a study where they used grafted and non-grafted plants to determine the most efficient use of water, AL-HARBI et al. (2016)

Table 1. Effect of tomato plant grafting on morphological characteristics (averages)

Variant	Plant height (cm)	Stem diameter (mm)	No. of leaves/plant
V1 – non-grafted Lorely F1 (control)	175.7 \pm 7.74	10.39 \pm 0.41	17.9 \pm 0.76
V2 – Beaufort \times Lorely F1 with a stem	211.50 \pm 4.83	12.40 \pm 0.54	21.3 \pm 0.63
V3 – Beaufort \times Lorely F1 with two stems	142.8 \pm 5.67	11.62 \pm 0.48	30.1 \pm 0.43

values are means \pm standard deviation

Table 2. Effect of grafting on tomato plant yields

Variants	Average No. of fruits/plant	Fruit average weight (g)	Production (kg/plant)
V1 – non-grafted Lorely F1 (control)	23.5 ^b	140.3 ^b	3.3 ^b
V2 – Beaufort × Lorely F1 with a stem	33.2 ^{ab}	204.7 ^a	6.8 ^{ab}
V3 – Beaufort × Lorely F1 with two stems	43.5 ^a	210.5 ^a	9.2 ^a
LSD (5%)*	13.7	23.2	4.5

*means followed by the same letter are not significantly different from each other (Duncan's multiple range test, $P = 0.05$)

observed that the grafted plants had a higher growth rate than the non-grafted ones as determined by plant height (195.7 cm), stem diameter (13.08 mm) and root weight (971.06 g).

The production of tomato fruits can be influenced by the cultivar, the method of grafting, the rootstocks and the number of stems per plant (one or two) (PULGAR et al. 1998; KACJAN MARSIC, OSVALD 2004; HOZA 2013). Many authors have shown that grafted tomatoes showed higher productivity than non-grafted plants (KHAH et al. 2006; KARACA et al. 2012).

KACJAN MARSIC and OSVALD (2004), in a study on the effects of grafting on production in two tomato cultivars, showed that the total number of fruits per plant and production was significantly increased in the grafted plants in comparison to the non-grafted plants, and for the grafted plants the production was influenced by the rootstock. Also, TURHAN et al. (2011) found that the grafting of tomato plants increased the productive efficiency of the species, and AL-HARBI et al. (2016) showed that the grafting of tomato plants resulted in an increased production which was primarily associated with the average weight of fruit and fruit number per plant, both of which were higher.

In our study, productivity and the production of tomatoes per plant was significantly increased in the grafted plants with two stems in comparison to the grafted ones with one stem and to the non-grafted ones (Table 2). The average number of fruits per plant ranged between 23.5 in V1 (V1 – non-grafted Lorely) and 43.5 in V3 (V3 – Beaufort × Lorely F1 two stems). This difference is explained by the fact that the plants had two stems in V3 compared with one in V1 and V2 (V2 – Beaufort × Lorely F1 with a stem), which positively influenced production, and the 'Beaufort' rootstock which allowed a better development of the grafted plants. The influence of the rootstock was observed also

in V2 where the average number of fruits per plant was 33.2, an increase of nearly 10% over the control non-grafted variant. Significant differences were found for the average weight of grafted plant fruits compared to the non-grafted ones. In the grafted plants with two stems, average fruit weight was 210.5 g, while in non-grafted plants, average fruit weight was 140.3 g. The grafted plants with two stems in this study formed larger fruits in V3, when plants are exposed to light of greater intensity that enhances photosynthesis.

RAHMATIAN (2014), in a study on the effect of tomato grafting, reported a significant 11% increase in average fruit weight and a 17.8% increase in the number of fruits, which together led to a 27% spike in production efficiency in grafted plants with one stem compared to non-grafted plants. IBRAHIM et al. (2001) found that the total number of fruit on non-grafted plants was lower than the total number on the grafted plants. In this study, plants with two stems exhibited a higher average number of fruits per plant and weight compared to the control variant and the non-grafted variant. The average yield per plant (kg/plant) ranged from 3.3 kg/plant in V1 up to 9.2 kg/plant in V3, reflecting the effect of the grafting (Table 2).

The production recorded in V3 was higher than that of V2 because the plants had two stems. We explain the difference between the production of the grafted and non-grafted control variant as being due to the root system of the rootstock that used a larger volume of soil compared to that of 'Lorely F1'. A higher degree of root branching and enhanced depth of growth led to a better supply of the plant with water and mineral salts and not surprisingly to a higher production per plant. Similar results were observed by IBRAHIM et al. (2001) and KACJAN-MARSIC and OSVALD (2004), who showed that the highest yield of fruits per grafted tomato plant was most likely due to the vigorous root system of the rootstock.

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Table 3. Effect of grafting on tomato plant on the chemical composition

Parameters	Cultivation method (variants)			LSD (5%)*
	V1 – non-grafted Lorely F1 (control)	V2 – Beaufortx Lorely F1 with a stem	V3 – Beaufortx Lorely F1 two stem	
TSS (°Brix)	4.6 ^{ns}	4.1 ^{ns}	4.3 ^{ns}	0.54
Titratable acidity (% citric acid/l)	0.260 ^{ns}	0.200 ^{ns}	0.240 ^{ns}	0.099
Reducing sugars (%)	3.37 ^{ns}	2.94 ^{ns}	2.89 ^{ns}	0.512
Vitamin C (mg/100 g)	17.6 ^{ns}	14.0 ^{ns}	17.5 ^{ns}	8.38
β-carotene(mg/kg)	19.84 ^a	11.26 ^b	10.46 ^b	4.64
Lycopene(mg/kg)	112 ^{ns}	110.3 ^{ns}	112.4 ^{ns}	20.35
Total phenolic content (mg GAE/kg)	358 ^a	306.7 ^b	294 ^b	31.67
AO (μmol Trolox/kg)	1432.5 ^{ns}	1045.8 ^{ns}	941.9 ^{ns}	368.3

*means followed by the same letter are statistically not significant (Duncan's multiple range test, $P = 0.05$); ns – not significant

Fruit quality can be influenced by many factors such as cultivar or the use of grafted or non-grafted plants, but also by the rootstock used for grafting (TURHAN et al. 2011).

The soluble dry matter content is one of the most important parameters of tomato quality. The majority (50–65%) of soluble solids in tomatoes are constituted by the sugars glucose and fructose, and their quantity and ratio influence the organoleptic properties of tomatoes (MANASHI 2011). Taking the °Brix as a measure of soluble solids in this study, values were lower in the fruit from the grafted plants. The TSS values were 4.60°Brix in V1 and 4.1–4.3°Brix in V2 and V3 and therefore were lowered by grafting. Similar results were reported by TURHAN et al. (2011) and KARACA et al. (2012).

According to CAMPOS et al. (2006), SSC in fresh tomato fruit is about 4.5. In protected cultures, TSS values differ between the grafted and non-grafted plants, being higher in the non-grafted ones than the grafted ones. Lee (1994) found that the TSS of fruit was influenced by the rootstock. In agreement with the results of this study, several authors have reported that the use of rootstocks led to a decrease in TSS in fruit (LOPEZ-GALARZA et al. 2004).

The titratable acidity influences the organoleptic properties, the aroma (taste), colour, as well as how well fruits hold their quality over time (MANASHI 2011). In this study, the acidity ranged from 0.20 to 0.24% in the grafted variants, and it was 0.26% in the non-grafted variant. Regarding the average content of reducing sugars in tomato fruits, the value was 3.37% in the non-grafted variants and ranged from 2.89 to 2.94% in the grafted variants. Similar results were observed by TURHAN et al. (2011) who

described a decreased sugar content of between 2.03% and 4.34% in grafted variants compared to non-grafted ones.

The vitamin C content was 17.6 mg/100 g in the variant 1 non-grafted tomato; content of this vitamin was non-significantly lower in variant 2, grafted tomato plants with one stem and in variant 3, grafted tomatoes with two stems, with values of 14 mg/100 g (f.m.) and 17.5 mg/100g (f.m.), respectively (Table 3). These observations are also supported by BOGOESCU et al. (2011) and according to AL-HARBI et al. (2016), the nutritional value of tomatoes, as determined by the vitamin C content, shows a slight but non-significant decrease in grafted tomatoes compared with non-grafted one.

Another important factor in the analysis of tomato fruits is the colour, which is determined by the ratio of two pigments, lycopene and β-carotene (TURHAN et al. 2011). β-carotene levels were decreased in the fruits of grafted plants in comparison to those from non-grafted plants. Content of beta-carotene was 19.84 mg/kg (f.m.) in V1 non-grafted tomatoes, 10.47 mg/kg (f.m.) in V3 grafted tomato plants with two stems and 11.26 mg/kg (f.m.) in V2 grafted tomato plants.

The nutritional value as expressed by the content of lycopene, an antioxidant that is formed during fruit ripening, determining their red colour, was 112 mg/100 g in V1 non-grafted tomato, and in grafted variants ranged from 110.3 mg/100 g in V2 grafted tomato plants with one stem to 112.4 mg/100 g in V3 grafted tomatoes with two stems. The values obtained in this study are consistent with those of MOHAMMED et al. (2009) demonstrating a significant decrease in the content of lycopene and β-carotene

in fruits obtained from grafted plants. The decrease in lycopene content was reported also by HELYES et al. (2009), who found a significant decrease in lycopene levels in tomato and concluded that it may be due to the used cultivar. The low β -carotene levels in this study are also supported by the results of KHAH et al. (2006) who carried out tests on tomato plants grafted onto 'Beaufort' rootstock. The high content of β -carotene in fruits from plants grafted onto 'Beaufort' in the study of MOHAMMED et al. (2009) is not in agreement with the results reported by KHAH et al. (2006) and the results of this study. This leads us to assert that the differences in the results between these two studies was probably due to the different tomato hybrids or the different grafting methods.

The content of antioxidants in tomato depends on a number of factors, in particular on the cultivar, the stage of maturity and on the culture conditions (DINU et al. 2015). The total content of phenolic compounds in this study ranged from 294 mg GAE/kg in V3 grafted tomatoes with two stems to 358 mg/kg in V1 non-grafted tomatoes. The differences between the average values of the total phenolic compounds found in tomatoes were not significant. The antioxidant activity in tomato fruits from non-grafted plants was higher than in fruits from grafted plants, but the difference was not statistically significant.

Grafting the of tomatoes not different from each other AL-HARBI et al. (2016) showed that the grafting of tomatoes lowers the content of vitamin C, titratable acidity (TA) and total soluble solids (TSS), but can mitigate the impact of unwanted salinity stress on growth and fruit quality. ROUPHAEL et al. (2010) and TURHAN et al. (2011) also found a reduction in tomato fruit quality in grafted plants compared to non-grafted plants, in terms of total soluble solid content and acidity of the fruit, while the firmness of the fruit was enhanced. KHAH et al. (2006) reported that tomato grafting onto compatible rootstock has a positive effect on cultivation performance, especially for greenhouse culture, and improves the production efficiency in different soil and climatic conditions. However, other reports have described a slight decrease in the nutritional quality of tomato fruits in response to grafting. BALLIU et al. (2008) and FLORES et al. (2010) concluded that, overall, quality parameters are higher in the fruits of the grafted plants. In other studies, the nutritional quality of fruits was described to be variable and depended on the stud-

ied variants. The varied results reported by different authors can be attributed mostly to the type of combination rootstock/scion, to the differences in production and culture systems and to the harvest date (DAVIS et al. 2008).

CONCLUSIONS

Grafting tomato plants had significant effects on the morphological characteristics and on production. The yield per plant increased most in variants 2 and 3 compared to the control plant. We explain the difference in production between the grafted variants and the non-grafted control plant as being due to the root system of the 'Beaufort' rootstock which explored a larger volume of soil compared to the 'Lorely F1'. The higher degree of root branching and the greater depth at which it develops, lead to a better supply of plants with water and mineral salts and naturally to higher production per area unit. Therefore, grafting with two stems is a powerful method for increasing the production of tomatoes, and it decreases the costs per area unit by reducing by half the number of plants and by solving the problems regarding soil disinfection.

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