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The effect of grafting on the antioxidant properties of tomato (*Solanum lycopersicum* L.)

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Abstract

The use of grafted plants in vegetable crop production is now being expanded greatly. However, few data are available on the nutritional composition of grafted vegetables with emphasis on antioxidant properties. Therefore, the major objective of this study was to evaluate antioxidant components of tomatoes influenced by grafting technique. The tomato plants were grown in a greenhouse located at Križevci, Croatia. The cultivars 'Efialto', 'Heman', and 'Maxifort' were used as rootstocks, while 'Tamaris' was used as scion. Grafting resulted in increase of number of marketable fruits per plant by 30%. Content of total vitamin C and total phenolics significantly decreased after grafting. The concentration of total extractable phenolics in tomatoes ranged from 287.1 to 977.4 mg gallic acid equivalents (GAE) kg^{-1} fresh weight, whereas lycopene content ranged from 11.44 to 60.99 mg kg^{-1} fresh weight. Antioxidant activities determined by 1,1-diphenyl-2 picrylhydrazyl (DPPH) method of grafts were significantly different compared to their respective rootstocks. The overall results showed that tomato grafting on suitable rootstocks has positive effects on the cultivation performance, but decreases nutritional quality of tomatoes.

Additional key words: antioxidant activity; greenhouse; lycopene; plant production; total phenolics; vitamin C.

Resumen

Efecto del injerto en las propiedades antioxidantes del tomate (*Solanum lycopersicum* L.)

En la producción de cultivos hortícolas se está expandiendo actualmente de forma considerable el uso de plantas injertadas. Sin embargo, hay pocos datos disponibles sobre la composición nutricional de las hortícolas injertadas, especialmente sobre sus propiedades antioxidantes. El principal objetivo de este estudio fue evaluar los componentes antioxidantes de tomates influenciados por la técnica de injerto. Se cultivaron plantas de tomate en un invernadero de Križevcii, Croacia. Se utilizaron como portainjertos los cultivares 'Efialto', 'Heman', y 'Maxifort', mientras que 'Tamaris' fue utilizado como injerto. El resultado del injerto fue un aumento del 30% en el número de frutos comerciales por planta, mientras que el contenido de vitamina C y de fenoles totales disminuyó significativamente. La concentración del total de fenoles extraíbles en los tomates varió entre 287,1 y 977,4 mg de equivalentes de ácido gálico (GAE) por kilo sobre la base de peso fresco, mientras que el contenido de licopeno varió desde 11,44 hasta 60,99 mg kg^{-1} de peso fresco. Las actividades antioxidantes determinadas por el método DPPH (2,2-difenil-1-picrilhidrazilo) de los injertos fueron significativamente diferentes respecto de sus respectivos patrones. Los resultados globales muestran que el injerto de tomate sobre patrones adecuados tiene efectos positivos sobre el rendimiento de cultivo, pero la calidad nutricional de los frutos disminuye.

Palabras clave adicionales: actividad antioxidante; fenoles totales; injerto; licopeno; tomate; vitamina C.

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Abbreviations used: AA (antioxidant activity); DPPH (1,1-diphenyl–2 picrylhydrazyl); fwt (fresh weight); GAE (gallic acid equivalents); TEAC (Trolox equivalent antioxidant activity).

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular and widely used vegetable crops in the world. Many epidemiological studies have suggested that the regular consumption of tomatoes may lead to a decreased incidence of various forms of cancer and heart disease (Giovannucci, 1999). The fruits of *S. lycopersicum* have valuable nutritional components with antioxidant activity like vitamin C, carotenoid pigments and phenolic compounds. A large variation in these nutrients is mainly attributed to factors such as plant nutrition, environment and genotype (Abushita *et al.*, 2000; George *et al.*, 2004; Dorais *et al.*, 2008). Temperature and light intensity exert a direct influence on the quality attributes of tomato fruit (Dorais *et al.*, 2008). On the other hand, the effects of various environmental stresses are known to affect the antioxidant content of tomatoes (Dumas *et al.*, 2003). Further insight into the factors likely to affect their composition should help to define the quality of tomatoes more clearly (Dumas *et al.*, 2003). Since consumers demand more varieties of higher quality, strategies focused on increasing fruit quality continue to be of great interest (Flores *et al.*, 2010 and references therein). In the Mediterranean area, where land use is very intensive and continuous cropping is in common practice, vegetable grafting is considered an innovative technique with an increasing demand by farmers (Pogony *et al.*, 2005; Khah *et al.*, 2006; Martínez-Rodríguez *et al.*, 2008; Flores *et al.*, 2010; King *et al.*, 2010). Grafting is widely used in horticulture for a variety of reasons. With field grown vegetables, this technique is now expanded to reduce infections caused by pathogens (Rivard and Louws, 2008; Lee *et al.*, 2010). Greenhouse tomato growers are using grafting to decrease susceptibility to root disease and to increase fruit production through increased plant vigour. However, only very few information is available on the influence of grafting methods on nutritional composition of tomato fruits with emphasis on their antioxidant properties. Also there are some contradictory results concerning the fruit quality traits and how grafting affects those (Khah *et al.*, 2006; Dorais *et al.*, 2008; Martínez-Rodríguez *et al.*, 2008; Flores *et al.*, 2010; Rouphael *et al.*, 2010). Therefore, the major objective of this study was to evaluate the effect of grafting technique on the antioxidant activity and antioxidant components (vitamin C, lycopene, total phenolics) of the greenhouse-grown tomatoes.

Material and methods

Plant material

The tomato plants (*Solanum lycopersicum* L.) were grown in a greenhouse located at Križevci, Croatia. The indetermined commercial cultivar 'Tamaris' (Clause Semences, France) was used as scion and grafted on the commercial rootstocks 'Efialto' (Enza Zaden, Netherland), 'Heman' (Syngenta, Switzerland), and 'Maxifort' (De Ruiter Seeds, Netherland). The experiments comprised then four non-grafted controls (a scion and three rootstocks) and three grafted combinations. The seeds of the scion cultivar were sown 3 days earlier than the seed of the 3 rootstocks to ensure similar stem diameters at the grafting time because of the differences in growth vigour. Grafting was done 20 days after planting of the scion using tube grafting technique. Plants were pruned to one stem and transplanted to the soil in a greenhouse on 15/04/2009. A randomized block design was adopted with 4 replications, each consisting of 6 plants. Common cultural practices were followed for irrigation and application of fertilizers and pesticides. Tomato fruits were picked and weighed at intervals throughout the growing season and total yield was determined at the end of experiment. A freshly harvested, uniformly ripened healthy fruit at the full ripe stage as usually occurs for marketing were taken for nutritional analysis. For each plant, one sampling made up of at least four ripe fruits from the third-fifth trusses was taken at the same time for analysis of the fruit quality characteristics. Before performing analysis, the samples were washed, first with running water and then with distilled water. All analysis was performed immediately after harvesting.

Soil analysis

Before transplanting of seedlings, three soil samples from the upper 30 cm were taken in three repetitions for every treatment. These soil samples were mixed and analyzed for every repetition separately according to standard procedures for nutrients and pH-value. The soil exhibited slightly acidic reaction ($pH_{KCl} = 6.22 \pm 0.04$), low humus content ($1.84 \pm 0.20\%$) and averagely suppliend with available P_2O_5 (133.1 ± 13.4 g kg^{-1}), $0.15 \pm 0.01\%$ total N and low K_2O level (78.0 ± 5.2 g kg^{-1}).

Determination of vitamin C content

Disintegration of the samples with quartz sand in a crucible mortar with pestle before extraction was necessary. The aqueous extract of the fresh tomato was prepared by homogenizing 5 g of the tomato in 100 mL of distilled water for 10 min. The homogenates were filtered and centrifuged at 4,000 rpm for 20 min. The supernatant was used for the determination of vitamin C content.

Total vitamin C content of the aqueous extracts was determined using the method of Benderitter *et al*. (1998). Briefly, dinitrophenyl hydrazine (DNPH) solution was prepared by mixing 2 g DNPH, 230 mg thiourea and 270 mg $CuSO_4 \times 5H_2O$ in 100 mL of 5 mol L^{-1} H_2SO_4. A volume of 120 µL DNPH solution were added to 800 µL reaction mixture [240 µL of the aqueous extract with 160 µL 13.3% trichloroacetic acid (TCA) and 400 µL water]. The reaction mixtures were subsequently incubated for 3 h at 37°C, then 800 µL of 65% (v/v) H_2SO_4 was added to the medium; their absorbance was measured at 520 nm and the vitamin C content of the samples was subsequently calculated, using a vitamin C standard.

Determination of total phenolics content and antioxidant activity

The weighed samples (25 g) were added to a glass beaker and homogenized with 25 mL of 80% (v/v) methanol (with addition of 1% (v/v) HCl) at 24°C for 2 h. Extracts were then filtered and centrifuged at 2,500 rpm for 20 min. The supernatant was sealed and stored at 4°C until use.

Total phenolics content of the tomato extract was determined using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965). Each methanol extract solution (0.5 mL) was mixed with 6 mL of distilled water and 0.5 mL of Folin-Ciocalteu's phenol reagent. After 5 min, 2 mL of a 2% (w/v) sodium carbonate solution was added and vortexed vigorously. The reaction mixture was diluted to a final volume of 10 mL. The same procedure was also applied to the standard solutions of gallic acid. The absorbance at 750 nm of each mixture was measured after incubation for 2 h at 20°C. Results were expressed as milligrams of gallic acid equivalents (GAE) per kg of fresh weight (fwt).

Relative antioxidant activity was measured in methanol tomato extracts using the DPPH (1,1-diphenyl-2 picrylhydrazyl) assay. The free radical scavenging ability of the extracts against DPPH free radical was evaluated as described by Gyamfi *et al*. (1999). Briefly, the methanol tomato extract (70 µL) was mixed with 2 mL of 0.1 mmol L^{-1} methanol solution containing DPPH radicals. The mixture was left in the dark for 25 min and the absorbance was measured at 518 nm using the UV-Visible spectrophotometer. The DPPH free radical scavenging ability was subsequently calculated with respect to the Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), which was used as a standard reference to convert the inhibition capability of each extract solution to the µmol L^{-1} Trolox equivalent antioxidant activity (TEAC). The radical was freshly prepared and protected from the light. A blank control of methanol/water mixture was run in each assay. All determinations were carried out in triplicate. Absorbance measurements were recorded on VARIAN Cary 50 UV-Visible Spectrophotometer. The ratio between the slopes of the regression lines of tomato extraction solution and the Trolox solution was defined as the TEAC, which was used to indicate the scavenging free radical capability of kg fresh tomato. Results are expressed in mmol TEAC per kg of fwt.

Determination of lycopene

Lycopene was extracted from fresh tomato samples after homogenisation of whole fruits. The homogenised sample (5 g) was weighed into a 125 mL flask wrapped with aluminium foil to exclude light, and 50 mL of a mixture of hexane/acetone/ethanol (2:1:1, v/v/v) was added to solubilise the lycopene (Sadler *et al.,* 1990). Samples were shaken for 30 min, and then 10 mL of distilled water was added. The solution was left to separate into a distinct polar (35 mL) layer and a non-polar (25 mL) layer containing lycopene. The content of total lycopene was obtained by measuring the absorbance of the lycopene hexane solution against a hexane blank at 472 nm. The conversion of absorbance into lycopene concentration was based on lycopene specific extinction coefficient ($E_{1cm}^{1\%}$) of 3.450 in hexane (Sharma and Le Maguer, 1996).

Statistical analysis

Analysis of variance (ANOVA) was used; the least significant difference (LSD) at $p \leq 0.05$ was calculated

using the STATISTICA, Version 9 (StatSoft, Inc., 2009) to determine significant differences between the grafted and non-grafted tomatoes in their antioxidant components and antioxidant activities.

Results and discussion

To develop information on the effect of grafting on tomato quality, we compared the antioxidant profile (vitamin C, lycopene, total phenolics and antioxidant activity) of greenhouse tomatoes, grown in Croatia for one year of production. 'Tamaris' was selected as early cultivar suitable for greenhouse production, producing good yields and characterized with firm quality fruits. Numerous rootstocks were developed, which have quite different characteristics and resistance (Pogonyi *et al.,* 2005; King *et al.*, 2010; Lee *et al.*, 2010). All selected rootstocks 'Efialto', 'Heman' and 'Maxifort' for this study are characterised by a strong generative tendency and high disease resistance. Rootstock efficacies are influenced by compatibility to the selected scion, existing disease pressure, and climate conditions. Our grafting technique and subsequent plant handling procedure was successful in all three variants, 'Tamaris' × 'Efialto', 'Tamaris' × 'Heman', and 'Tamaris' × 'Maxifort'. The productivity of the grafted tomatoes was increased, which agreed with previously published results (Pogonyi *al.*, 2005; Khah *et al*., 2006; Rouphael *et al.*, 2010), but is contrary to the results of Martínez-Rodríguez *et al*. (2008) and Flores *et al.* (2010). Grafting resulted in an increase of a number of marketable fruits per plant by *ca.* 30% (Table 1). Averaged grafted fruits weight was decreased by 10%, whereas averaged marketable yield per grafted plant was increased by 18% (Table 1).

The composition of the tomato varieties analyzed here is similar, in qualitative terms, to that observed in other *S. lycopersicum* varieties examined in the literature (Giovannucci, 1999; Dumas *et al*., 2003; Brandt *et al.*, 2006; Raffo *et al*., 2006; Dorais *et al.*, 2008). Total phenolics showed the greatest variations between grafted and non-grafted plants (Table 2). In plants, phenolic biosynthesis is particularly sensitive to induction by various biotic and abiotic stresses (Dixon and Paiva, 1995; Dorais *et al.*, 2008). For instance, flavanols can be synthesized in response to a pathogen attack or to wounding, whereas wound-induced chlorogenic acid and phenolic esters may act directly as defence compounds or may serve as a precursor for the synthesis of polyphenolic barriers (Dixon and Paiva, 1995). Noticeable variations in phenolics pattern between different sampling times were also observed (Raffo *et al*., 2006). The concentration of total extractable phenolics in samples examined ranged from 287.1 (in 'Efialto' × 'Tamaris') to 977.4 mg GAE per kg fwt (in 'Maxifort'). Under standard growth conditions applied in these experiments, grafting significantly reduced the total phenolics content of fruits. It is interesting to note that no significant differences were found among the different graft combinations. However, total phenolics content changes significantly among the rootstocks used in our experiment as well as between different rootstocks and the scion. These values are only indicative of the concentration of polyphenols in tomatoes, since there is no single analytical method that, collectively and accurately, is able to measure the total polyphenol content of a food. The Folin-Ciocalteau reagent used here usually overestimates the content of phenolic compounds, since other reducing agents present in food, such as ascorbic acid, can interfere (Martínez-Valverde *et al*., 2002). The lycopene content of analyzed tomatoes is presented in Table 2. When expressed on fresh weight basis, highest lycopene content was found in 'Heman' (61 mg kg^{-1}) and lowest in 'Maxifort' (14 mg kg^{-1}). These values are well agreed with those reported by Martínez-Valverde *et al.* (2002) and Clinton (1998). Total phenolics did not correlate with lycopene content. As previously observed, toma-

Table 1. Effect of tomato grafting on plant productivity[1]

Trial variants	Fruits per plant	Average fruit weight (g)	Yield per plant (kg)
Scion (Tamaris)	40.2[a]	132[a]	4.4[a]
Graft (Efialto × Tamaris)	53.1[b]	119[b]	5.2[b]
Graft (Heman × Tamaris)	54.1[b]	121[b]	5.4[b]
Graft (Maxifort × Tamaris)	54.1[b]	118[b]	5.0[b]

[1] Values with different letters in each column differ statistically ($p \leq 0.05$).

Table 2. Effect of grafting on lycopene concentrations, total phenolics, total vitamin C content and antioxidant activity (*AA*) in tomatoes[1]

Treatments	Total phenolics, mg GAE kg^{-1} of fwt	Lycopene mg kg^{-1} of fwt	Total vitamin C, mg kg^{-1} of fwt	*AA*, mmol TEAC kg^{-1} of fwt
Rootstock (Heman)	821.07^{a} (21.50)	60.99^{a} (1.45)	1,277.11^{a} (10.94)	793.15^{a} (0.75)
Rootstock (Efialto)	508.29^{b} (30.86)	54.04^{b} (1.02)	1,106.20^{b} (4.60)	804.90^{b} (5.79)
Rootstock (Maxifort)	977.36^{c} (15.93)	14.11^{c} (0.14)	1,316.93^{a} (7.00)	811.04^{b} (9.61)
Scion (Tamaris)	428.86^{d} (10.43)	49.56^{d} (0.60)	772.92^{c} (29.32)	823.51^{c} (5.41)
Graft (Heman × Tamaris)	365.79^{e} (6.64)	48.84^{d} (0.89)	739.22^{d} (26.70)	813.06^{b} (0.75)
Graft (Efialto × Tamaris)	287.14^{f} (2.57)	49.38^{d} (0.78)	733.09^{d} (9.19)	724.70^{d} (6.43)
Graft (Maxifort × Tamaris)	325.14^{e,f} (5.14)	49.20^{d} (0.75)	683.41^{d} (39.17)	769.45^{e} (1.21)

[1] Data show the mean of 4 plants, while standard deviations are given in parenthesis. Values with different letters in each column differ statistically ($p \leq 0.05$). GAE: gallic acid equivalents. TEAC: Trolox equivalent antioxidant activity. fwt: fresh weight.

toes showing similar fruit colour do not necessarily contain similar amounts of total phenolics or lycopene (Clinton, 1998). An ANOVA test indicated that there were no significant difference in lycopene content between the scion and the grafted plants (see Table 2). On the other hand, lycopen concentrations in all rootstocks were significantly different from grafted plants as well as scions. Rootstock 'Maxifort' showed significantly decreased lycopene concentration, whereas lycopene contents of 'Heman' and 'Efialto' were significantly higher compared to all other samples analyzed. George *et al*. (2004) and Helyes *et al*. (2009) have concluded that the variety of tomato is one of the most important determinants of lycopene content.

All rootstocks showed significantly increased vitamin C content compared to grafts (Table 2). However, it is important to emphasise that all three rootstocks were characterised with small, cherry-like tomato fruits. Smaller fruits have generally higher vitamin C content while the greater skin/volume ratio of smaller tomatoes may enhance their flavonol content, which is mainly found in the skin (Dorais *et al.*, 2008). It is very interesting that all the values for vitamin C amounts (presented in Table 2) were higher than those habitually mentioned in the literature. Vitamin C content ranged from 683 to 1,317 mg kg^{-1} fwt. This fact could be considered as a good nutrient characteristic for each tomato variety analyzed here. For example, Halevy *et al*. (1957) reported 210 mg kg^{-1} for generic tomatoes. However, total vitamin C content (*i.e.* ascorbic plus dehydroascorbic acid) was extracted and measured in this study. Raffo *et al*. (2006) have found a relatively high amount of dehydroascorbic acid (40-60% of total ascorbic acid), whereas lower proportions have been previously reported for ripe tomatoes: 20-30% according to Davies and Hobson (1981) and Vanderslice *et al*. (1990). Even lower proportions have been observed by Jimenez *et al*. (2002) and Cano *et al*. (2003). On the contrary, over 90% of the vitamin C was found as dehydroascorbic acid in fresh tomatoes grown in warm climates (De Serrano *et al*., 1993). In any case, the simple quantification of only ascorbic acid would lead to a substantial underestimation of total vitamin C content.

Vitamin C acts as an antioxidant through a catalytic reaction with ascorbic acid peroxidase (Shigeoka *et al*., 2002). Despite its advantages, grafting cannot avoid injury stress. Plants are injured by the cut in the grafting process, and the active oxygen concentration increases as a result of this injury stress (García *et al*., 2004). This early negative effect of grafting has also been reported by other authors (Khah *et al*., 2003). In tomato plants, the concentration of active oxygen increases after grafting, and activity of antioxidant enzymes such as ascorbic acid peroxidase (APX) and catalase increase to eliminate the active oxygen (García *et al*., 2004). Hyper-production of vitamin C is thought to counteract injury stress, because this vitamin has been shown to protect plants from oxidation (Tabata *et al*., 2001). However, our results showed that vitamin C content in fruits was significantly lower in grafts compared with their respective rootstocks and scion (Table 2). This could be due to the fact that grafted plants were initially subjected to stress following the grafting operation. Ascorbic acid is known to control cell differentiation (Arrigoni, 1994) and to promote callus division

and growth (Tabata *et al*., 2001). Vitamin C treatment may result in callus formation at the cut surface of the scion *via* ascorbic acid's promotion of cell division (Joy *et al*., 1988). Observed significant decrease in content of total vitamin C after grafting could also be a result of redistribution or accumulation of vitamin C in other parts of grafted plants. Thus, Wadano *et al*. (1999) have found that ascorbic acid concentration is increased after grafting in leaves of tomato plants. Another casual factor of decreased vitamin C in grafted plants is probably higher plant shoot biomass in grafts compared to non-grafts. Indeed, it can be seen from the results presented in Table 1 that total yield and number of fruits per plant were significantly higher after grafting although the average fruit weights were decreased.

Rather than determining the concentration of each antioxidant molecule individually, evaluation of total antioxidant activity (*AA*), using different model assay systems has become increasingly important. Total *AA* is a measure of the capacity of substances extracted from the food matrix to delay the oxidation process in a controlled system (George *et al*., 2004). The data presented in Table 2 show that the antioxidant activities of two grafts ('Efialto' and 'Maxifort' grafted on 'Tamaris') are significantly lower compared to their respective rootstocks and scion. The *AA*, measured by DPPH method, of the scion (cv. 'Tamaris') was significantly higher than *AA*s of rootstocks and grafted plants. It has been noted before that antioxidant properties of tomatoes depend largely on lycopene content (Martínez-Valverde *et al.*, 2002). Raffo *et al*. (2002) indicate that together with phenolic compounds, ascorbic acid represents the main water-soluble antioxidant in tomatoes and contributes to the antioxidant activity of the water-soluble fraction. However, no correlation was found between total lycopene content and *AA* or between total phenolics and *AA*. This might have resulted from the production of some other undetermined antioxidants as well as from production of phenolic substances with higher antioxidant activities; further investigation is required.

As a rapid alternative to the relatively-slow breeding methodology, commercial cultivars onto selected rootstocks could be a promising tool (Flores *et al.*, 2010; Rouphael *et al.*, 2010). With regard to the possibility of using grafting in order to increase fruit quality, previous studies suggest that the traits associated with fruit quality are translocated to the scion through the xylem (Lee, 1994). However, depending on the scion-rootstock combination, either a decrease or an increase in fruit quality seemed to occur (Flores *et al.*, 2010; King *et al.*, 2010). The simultaneous increase of both fruit yield and nutritional quality in commercial tomato cultivars has already proved difficult (Flores *et al.*, 2010). Under standard growth conditions, grafting increased fruit yield of studied tomatoes, but higher fruit quality of rootstocks compared to the scion did not affect nutritional value of grafted plants. Actually, total phenolic, lycopene and vitamin C contents of grafted peppers are more similar to the scion than to the selected rootstocks. This could be due to the fact that control of fruit quality resides mainly in the shoot, not in the root, as was published previously when Martínez-Rodríguez *et al*. (2008) observed also that the rootstock effect on fruit quality depends on the genotype used as scion.

Conclusion

Understanding the relationship between the content of compounds with antioxidant potential and varietal, agronomic, geographical and seasonal factors is necessary if the potential benefits to human health of the consumption of tomatoes and tomato products are to be exploited. In the Mediterranean area, where the vegetable cultivation is still carried out mostly by traditional methods and modern cultivated techniques are adopted slowly, the grafting technique could help in the solution of many problems. The use of grafting is a simple step for more developed cultivation forms, like hydroponics. Therefore, we consider the advantages of grafted plants to be of value for farmers. The results showed that tomato grafting on suitable rootstocks has positive effects on the cultivation performance, especially in the greenhouse conditions. Grafting offered higher yield and consequently higher profit. Nutritional properties of grafted tomatoes indicated satisfactory quality as well. However, since the preliminary results obtained showed that grafting was ineffective agricultural approach to improve fruit nutritional value, future work will be aimed at the selection of suitable rootstock-scion combination able to reach this goal.

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