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The Effect of Scion/Rootstock Combination and Ripening Stage on the Composition of Carotenoids and some Carpometric Characteristics of Tomato Fruit

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Summary

In this study, the impact of scion/rootstock combinations on the composition of carotenoids and fruit firmness during three ripening stages was investigated. Two tomato cultivars were assessed as scions: 'Amati F1', and 'Gardel F1', grafted on two rootstocks: 'Body F1' and 'Robusta F1'. To evaluate the effect of grafting and ripening stages on firmness and colour evolution of tomato fruits, the lightness coefficient L* and a*/b* index were monitored in tomatoes, harvested at technological ripening stages (orange to light red and red). The effect of scion/rootstock combinations on the coloration of fruits was more pronounced than on the firmness of fruits. Significantly lower a*/b* ratio and higher L* values were measured on fruits from 'Body F1' rootstock, which could indicate slower ripening compared to 'Robusta F1' rootstock and non-grafted plants. Fruit firmness was only significantly affected by the scion cultivars and ripening stages. In tomato fruit, three carotenoids were detected; lycopene represented the major, β-carotene the second prevailing and lutein the minor carotenoid. Grafting significantly affected the composition of carotenoids in fruits of 'Amati F1' scion, where a significant increase of lycopene and a decrease of β-carotene were measured in fruits correlated with a change from orange to red colour. The composition of carotenoids in tomato fruit is usually under genetic control, which was confirmed also in our study. However, our results also indicate that the levels of carotenoids and their composition can additionally be affected by scion/rootstock regulation and ripening stages.

Key words. Carotenoids – compositional data analysis – grafting – *Lycopersicon lycopersicum* – scion/rootstock combination – tomato

Introduction

The global vegetable market has shown a considerable increase in demand for crops with high antioxidant characteristics, since antioxidants in vegetables and fruits can greatly reduce the risk of many types of chronic diseases (Levy and Sharoni 2004). Among plants constituents, carotenoids have been identified as antioxidants that are beneficial to human health (Shi and Le Maguer 2000). They are compounds comprised of eight isoprenoid units, and are classified as hydrocarbon carotenes, such as lycopene and β-carotene; and oxycarotenoids – xanthophylls, that have carbon, hydrogen and additionally oxygen, typified by lutein (Delgado-Vargas et al. 2000). Under appropriate conditions, lycopene can function as the most efficient singlet oxygen quencher in the human body and thus plays an important role in reducing cardiovascular diseases, digestive tract tumours and incidence of prostate cancer (Giovannucci 1999; Delgado-Vargas et al. 2000).

Tomato is known as an important source of carotenoids and as the second most important vegetable in the world after potato, with an annual production around 145,6 million tonnes fresh weight (Fao 2010 (http://faostat.fao.org)). Therefore, tomato fruit represents the predominant source of carotenoid antioxidants in the human diet. The content and composition of primary and secondary metabolites in tomatoes could be improved through the manipulation of genetic factors (Fanasca et al. 2006),

modification of nutrients and salinity in the root zone (Bénard et al. 2009; Borghesi et al. 2011) and by grafting. The latter is frequently used as a tool to induce plant resistances to different biotic and abiotic stresses (Fernández-Garcia et al. 2004; Rouphael et al. 2010) to improve nutrients uptake. Grafting is also used to improve water-use efficiency, and thereby to increase crop yield under normal and stressed conditions (Leonardi and Giuffrida 2006). The possibility of carotenoid content regulation in tomato fruit induced by grafting has scarcely been investigated until now and moreover, the few results obtained have largely been inconsistent (Davis et al. 2008). For example, an increase in carotenoid content levels has been reported in fruits harvested from grafted plants, compared to non-grafted plants (Fernández-Garcia et al. 2004), and in other studies no significant differences in lycopene content has been confirmed in fruits of grafted and non-grafted plants (Khah et al. 2006; Marković et al. 2010, Turhan et al. 2011). The content of antioxidants in fruits and vegetables may differ according to cultivar, environmental conditions and agronomical practices (Veberic et al. 2005; Usenik et al. 2008).

In order to obtain tomato cultivars with improved nutritional characteristics, germplasm from wild-type relatives is currently being used by molecular marker-assisted technique and genetic transformation strategies (Ronen et al. 1999; Rosati et al. 2000). However, these studies and manipulations are somewhat limited, due to the negative attitude of many consumers, especially Europeans, towards the acceptability of the use of genetically-modified organisms in food production. For that reason, grafting of commercial tomato cultivars on selected rootstocks seems to be a useful technological measure striving to improve the levels of health promoting compounds in tomato fruit (Flores et al. 2010). Recently, consumers are increasingly concerned about tomato fruit quality, since the current practice in the horticultural chain promotes tomato fruit harvest at breaker stage (pink to orange) and a global long-distance transport (Schouten et al. 2007).

In the present study, the commercial tomato hybrid cultivars ('Amati' and 'Gardel') grafted on two rootstocks ('Body' and 'Robusta') which differed in compatibilities with the scions (Kacjan Maršić and Osvald 2004), were selected as a model. Scion/rootstock combinations were used to investigate the potential of grafting to alter the levels of fruit coloration, firmness and carotenoids content and composition in fruits of grafted plants compared to the non-grafted. The objective was firstly, to evaluate the composition and content levels of the main carotenoids in tomatoes harvested at three commercial ripening stages (orange, light red and red). Secondly, the influence of scion/rootstock combinations on the composition of carotenoids, fruit firmness and colouration at these stages was observed, since these traits are reportedly time dependant and correlated during the ripening process (Fraser et al. 1994). Carotenoid composition of tomato fruit for fresh consumption has not been analysed from that point of view until now. The results will be interesting for a further detailed research on to improve of carotenoids content and composition with one of the technological tool as well as for vegetable growers, who require additional information on the usefulness of the scion/rootstock combinations.

Materials and Methods

Plant culture

The experiment was conducted in an unheated, three-span greenhouse (each span was 8 m wide and 25 m long) on the experimental field (298 m above sea level) of Biotechnical Faculty in Ljubljana, Slovenia (latitude 46°2' N, longitude 14°28' E) from March till October 2006. Two hybrid tomato (*Lycopersicon esculentum* Mill.) cultivars 'Amati F1' and 'Gardel F1' were used as scions and as non-grafted control and two hybrid tomatoes 'Body F1' and 'Robusta F1' were used for rootstocks. Descriptions of cultivars used for scions and rootstocks with a list of practical work and characteristics of the experiment are presented in Table 1 and 2. Grafted and non-grafted plants were hand planted into two rows of 1.5 m long plots, on polyethylene mulch raised beds, with- and between-row spacing of 0.5 m and 0.5 m, respectively (to a plant density of 30.000 plants ha^{-1}). Each plot contained six plants and each treatment was replicated four times, which means 24 plants per treatment, so 144 plants were included in the experiment. Normal cultural practices were followed for irrigation, fertilization and pesticide application (Table 2).

At harvest in September, 18 fruits were randomly selected (6 less ripe – orange stage, 6 medium ripe – light red stage and 6 fully ripe fruits – red stage), among marketable and undamaged fruits, which have been taken from the third up to the fifth inflorescences, for each graft combination and on non-grafted plants. The average carpometric characteristics (colour, firmness) and the content and composition of carotenoids were monitored on these samples.

Weather conditions

In the greenhouse, air temperatures were measured during the vegetation period and samplings using a temperature data logger (Voltcraft DL-120TH) located one meter above plants and at the center of the experiment flats. Average daily temperature, maximum and minimum temperature for the description of weather conditions during the experiment, from May to September 2006 are presented in Fig. 1. It can be assumed that during the growing period the environmental conditions in the greenhouses were very similar to those in the open-field, especially during the hot summer period, when all windows and doors were opened in order to make the environmental conditions suitable for normal growth and development of tomato plants. The weather in the year 2006 was comparable to a

Table 1. Cultivars of tomato scions and rootstocks included in the experiment.

Cultivar Scion	Type/Shape of fruit	Resistances	Seed company	Country of origin
'Amati F1'	For fresh consumption/ Round	Fusarium wilt Verticillium dahliae Tobacco Mosaic Virus Nematodes Leaf mould	Royal Sluis	The Netherlands
'Gardel F1'	Beef tomato/flattened globe-shaped	Fusarium wilt Verticillium dahliae Tobacco Mosaic Virus Tomato Yellow Leaf Curl Virus Nematodes Leaf mould	Royal Sluis	
Rootstock	Characteristics	Resistances	Seed company	Country of origin
'Body F1'	Enhances vigour and endurances of the scions, especially on long crop	Fusarium wilt, races 1–2 Fusarium crown and root rot Tomato mosaic virus Verticillium wilt Southern root-knot Nematodes Corky root Leaf mold	Royal Sluis	The Netherlands
'Robusta F1'	Strong and cold tolerance strong-branching root system resulting in a very strong plant	Fusarium wilt, races 1–2 Fusarium crown and root rot Tomato mosaic virus Verticillium wilt Nematodes Corky root	Bruinsma seeds	The Netherlands

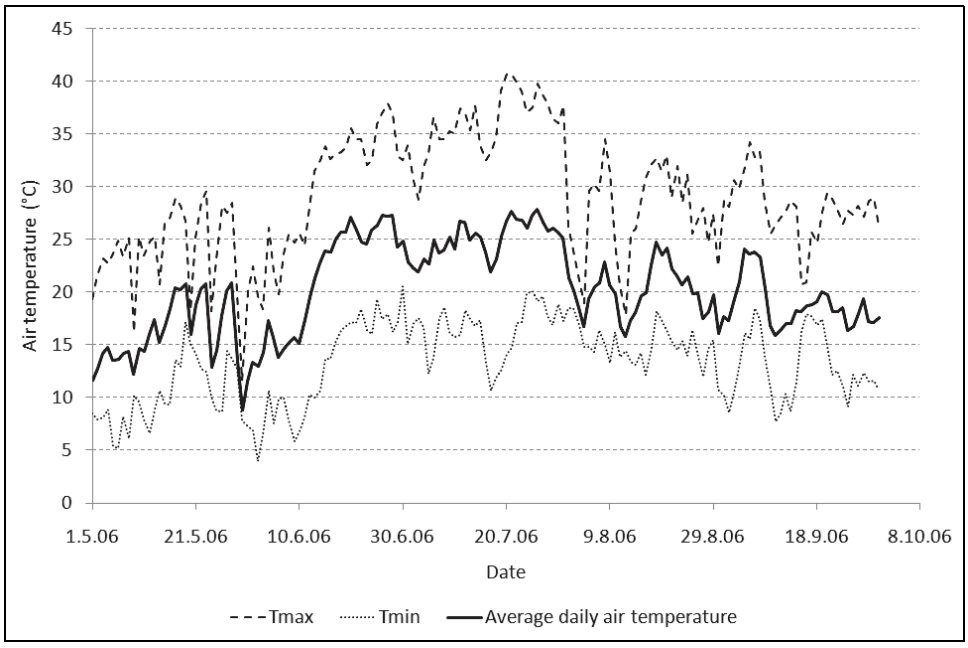

Fig. 1. Average daily air temperature, mean daily temperature minimum (T_{min}) and mean daily temperature maximum (T_{max}), measured using a temperature data logger (Voltcraft DL-120TH) during the experimental period from May to October 2006 in Ljubljana, Slovenia.

Table 2. List and description of the experimental work.

Experiment	Description
Experimental design	Complete randomized design 6 replicates 18 treatments (2 scion, 2 rootstocks + non-grafted plants, 3 ripening stages, 2*3*3)
Location of the experiment	Multi-span greenhouse with polycarbonate covering
Soil texture	Heavy clay loam
Date of rootstock and scion sowing	March 3, 2006 (rootstocks) March 10, 2006 (scions)
Date of grafting	April 25, 2006/(cleft grafting approach was applied)
Date of transplanting	May 27, 2006
Fertilization	
Basic application	May 22, 2006/500 kg ha^{-1}/NPK 7-20-30 (granulated)
Fertigation	Every 7 days with water soluble fertilizer $Ca(NO_3)_2$ (Yara, Norway) and 'Ferticare 10-5-26' (Yara, Norway) alternatively
Plant protection	August 3, 2006/Bulldock (0.05 %)
Harvest date	September 11, 2006 (103 DAT) (DAT: days after transplanting)

long-term average regarding the temperatures. The mean daily solar radiation ranged from 17.94 MJ m^{-2} in May to 22.74 MJ m^{-2} in June, and was the lowest in September (14.30 MJ m^{-2}). In August slightly cooler temperatures and less sunshine (18.40 MJ m^{-2}) compared to the long-term average were measured.

Measurement of CIELAB colour values

The skin colour variables were measured using a Minolta CR 300 Chroma portable colorimeter (Minolta Co., Osaka, Japan) with C illuminant. Fruit chromaticity was expressed in L*, a*, b* colour space coordinates (CIELAB). The colorimeter was calibrated with a white standard calibration plate (Y = 93.9, x = 0.3134, y = 0.3208) before use. L* corresponds to a dark/light scale (0 = black, 100 = white) and represents the relative lightness of colours, being low for dark colours and high for light colours (Lancaster et al. 1997). The parameters a* and b* extend from −60 to +60 where a* is negative for green and positive for red and b* is negative for blue and positive for yellow. Colour was described by lightness (L*) and a*/b* index, the latter being frequently used as a reference for maturity stages evaluation of vine ripened fruits (Raffo et al. 2002; Batu 2004). Four measurements on the equatorial region of each fruit were performed and an average was calculated.

Fruit firmness measurements

Flesh firmness was measured immediately after harvest on orange, light red and red fruits. One cm^2 of fruit skin on the opposite sides of the fruit were removed and a Chatillion penetrometer (model DFG 50), equipped with an 11-mm diameter round stainless steel probe with flat end (John Chatillion & Sons, U.S.A.) was utilized. The force needed to penetrate the mesocarp tissue was measured twice for each fruit and an average was calculated and expressed in Newtons/mm (N mm^{-1}).

Sample preparation

After colour and firmness measurements had been performed, individual tomato fruits were chopped, frozen in liquid nitrogen and stored at −20 °C. For lipophilic antioxidant analysis, frozen samples were grounded to a fine powder using a planetary micro mill (Fritsch, Pulversette 7) and stored at −20 °C in humidity proof plastic containers until further analysis.

Extraction and HPLC analysis of carotenoids

Carotenoids were determined using the method described by Šircelj and Batič (2007). Pigments were extracted from the dry fruit powder with ice-cold acetone. All extraction procedures were performed in dim light. Acetone extracts were filtered through 0.2 μm Minisart SRP 15 filter (Sartorius Stedim Biotech GmbH, Goettingen, Germany) and then subjected to HPLC gradient analysis (column Spherisorb S5 ODS-2 250 × 4.6 mm with pre-column S5 ODS-2 50 × 4.6 mm) (Alltech Associates, Inc., Deerfield, USA), using the following solvents: solvent A: acetonitrile/methanol/water (100/10/5, v/v/v); solvent

B: acetone/ethylacetate (2/1, v/v), at a flow rate of 1 ml min^{-1}, employing linear gradient from 10 % solvent B to 70 % solvent B in 18 min, with a run time of 30 min, and photometric detection at 440 nm. The HPLC analysis was performed on a Spectra-Physics HPLC system with Spectra Focus UV-VIS detector (Fremont, USA). Identification of compounds was achieved by comparing retention times and the spectra as well as by addition of external standards. The concentrations of pigments were calculated with the help of corresponding external standards. The following standards were used for the determination of pigments: β-carotene, lutein and lycopene, all from DHI LAB products (Hoerholm, Denmark). The solvent acetone, ethylacetate, methanol and acetonitril were purchased from Merck (all HPLC grade).

Statistical analysis

Statistical analysis was done with R program (http://www.R-project.org). The experiment was treated as a three factorial experiment in a complete randomized design with six replications. The first factor (scion) had two levels, (two tomato cultivars 'Amati F1' and 'Gardel F1'); the second factor (rootstock) had three levels, (two cultivars 'Body F1', 'Robusta F1') and non-grafted plants (control) and the third factor (ripening stage) had three levels, (three categories of fruit maturity orange, light red, red). The data of colour parameter L* and a*/b* index, firmness and carotenoid contents were analysed by discriminant analysis to find out which variable separate the treatments the most. In the next step, ANOVA was performed for each variable separately. The Duncan's multiple comparisons test at a significance level of $P < 0.05$ was performed where needed. The average structure of carotenoids was analyzed using compositional data analysis (Pawlowsky-Glahn and Egozcue 2001; Aitchinson 2003). The geometric mean was used as the measure of the central tendency for three carotenoid components. The data were transformed with isometric log ratio transformation (ilr) before discriminant analysis was performed.

Results and Discussion

Fruit colour

Fruit colour measurements (parameter L* and a*/b* index) were performed to uniform the fruits collected at three subjectively determined ripening stages: orange, light red and red. Discriminant analysis showed that ripening stages differed more by the colour parameter L* than by a*/b* index. Further ANOVA for each colour variables separately showed no statistically significant differences for two and three factor interactions, so further analysis was done for the main effects of three factors. The average a*/b* index, which expresses the intensity of red colour of the fruit skin, showed a significant increase during the ripening process, for both scions and all three rootstocks (Table 3, Fig. 2, left). It ranged from 0.96 ± 0.03 in orange fruits, to 1.18 ± 0.03 in light red fruits and 1.38 ± 0.03 in red fruits, according to the colour scale reported by Raffo et al. (2002) or to colour stages of pink to red, according to the USDA colour scale used by Grierson and Kader (1986). Significantly higher average a*/b* index values were measured on fruits of 'Amati F1' cultivar compared to the fruits of 'Gardel F1' cultivar, for all three levels of ripening stages and all three rootstocks. Moreover, statistically significant influences of rootstocks on the average a*/b* index were determined for both scions and in all three ripening stages: lower a*/b* index was measured on tomatoes harvested from plants grafted on 'Body F1' rootstock than on tomatoes from non-grafted plants or tomatoes from plants grafted on 'Robusta F1' rootstock, respectively. No significant differences in a*/b* index were detected between the tomatoes from non-grafted plants and plants grafted on 'Robusta F1' rootstock (Fig. 2, left).

Table 3. Average value ($\bar{x}$) and standard error (SE) of colour parameter L* and a*/b* index for tomato fruits for all scion/rootstock combinations and for non-grafted plants.

Ripening stage		L*						a*/b*					
Scion	Rootstock	Orange		Light red		Red		Orange		Light red		Red	
		$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
'Amati F1'	Non-grafted	41.9	0.9	36.5	0.6	34.8	0.2	1.05	0.06	1.34	0.04	1.52	0.01
	'Body F1'	40.7	0.8	38.9	0.7	35.1	0.6	1.07	0.06	1.19	0.04	1.40	0.02
	'Robusta F1'	39.8	0.7	36.1	0.4	35.2	0.2	1.04	0.06	1.36	0.02	1.47	0.02
'Gardel F1'	Non-grafted	41.8	0.9	38.5	0.8	35.6	0.4	0.89	0.06	1.14	0.04	1.33	0.03
	'Body F1'	42.4	0.7	39.9	0.6	34.9	0.3	0.85	0.06	1.04	0.03	1.26	0.02
	'Robusta F1'	42.5	1.1	38.5	0.5	35.6	0.4	0.87	0.05	1.03	0.02	1.34	0.01

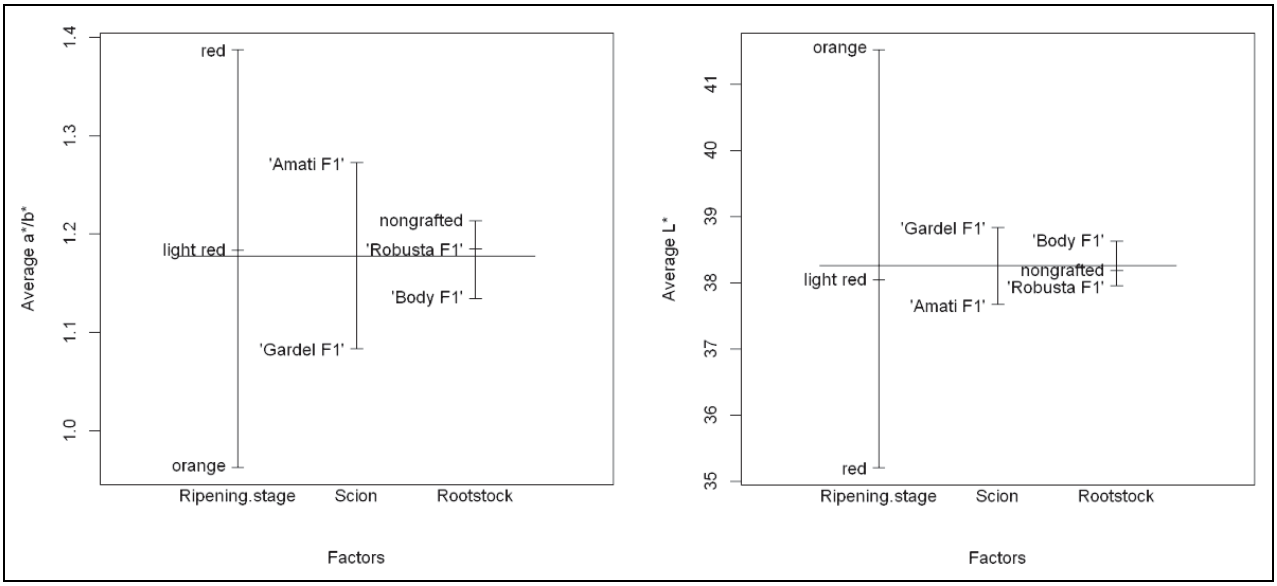

Fig. 2. Main effect averages of colour index a*/b* (left) and L* (right) for three factors were presented. Because no interactions between factors were found, the averages of ripening stages were calculated across both scions and across all three rootstocks, and similar were calculated the averages of scions and rootstocks.

Average L* values significantly decreased during tomato ripening: L* = 41.5 ± 0.3 for orange fruits, 38.1 ± 0.3 for light red fruits and 35.2 ± 0.3 for red fruits (Fig. 2, right). This is in accordance with previously published data on lightness decline during tomato ripening, where average L* values for cherry tomato were 44.8 for fruits in orange to light red ripening stages, 43.2 for fruits in light red to red stages and 41.6 for fruits in the red stage (Raffo et al. 2002). The average L* was also significantly higher on fruits of 'Gardel F1' cultivar than 'Amati F1' cultivar. Similar to a*/b* index, statistically significant influences of grafting on average L* values were observed. Tomatoes from plants grafted on 'Body F1' rootstock had higher average L* values than those from non-grafted plants or plants grafted on 'Robusta F1' rootstock (Fig. 2, right). This suggests a slower fruit ripening on 'Body F1' rootstock, since it has been reported that higher L* values indicate lighter fruit skin and lightness decreases with fruit ripening (Lancaster et al. 1997).

Fruit firmness

In addition to fruit colour, fruit firmness is an important quality attribute of fresh tomatoes. The texture of tomato fruit is namely directly influenced by flesh firmness and the relation of pericarp and locular tissue. These parameters are subjected to declination of consistency and serum viscosity during the ripening process, which consequently leads to fruit softening (Barrett et al. 1998). The average firmness measured on fruits from grafted and non-grafted plants are presented in Fig. 3.

The ANOVA for three factorial experiment showed that average firmness of tomato fruits was significantly affected by the cultivar ($P < 0.0001$). Firmer fruit was harvested from the 'Gardel F1' cultivar than from 'Amati F1' cultivar, respectively (Fig. 3). The interaction between the ripening stage and rootstock was the only statistically significant interaction ($P < 0.0001$), and the average fruit firmness measurements for the treatments determined with ripening stage/rootstock combinations are presented in Fig. 3.

Statistically significant differences in average fruit firmness from grafted plants among three ripening stages (orange, light red and red) were established. Average firmness decreased with the subsequent ripening stage, which is in accordance with the results of Batu (2004), who reported, that during the ripening process, the firmness of tomato fruits decreases.

When the fruit firmness of grafted and non-grafted plants was compared during ripening, statistically significant differences were confirmed only for fruits in the red (fully ripe) stage. At this stage were fruits from non-grafted plants significantly firmer than fruits from grafted plants. A lower fruit firmness of grafted plants indicated higher softening of tomatoes (Barrett et al. 1998). This could be a consequence of faster development and ripening process, which are characterized for grafted plants, compared to the non-grafted, where the growth and development are more successive (Leonardi and Giuffrida 2006).

Content and composition of carotenoids

Three carotenoids were identified in tomato fruit samples: lycopene, β-carotene and lutein. Their average contents in fruits from grafted and non-grafted plants are presented

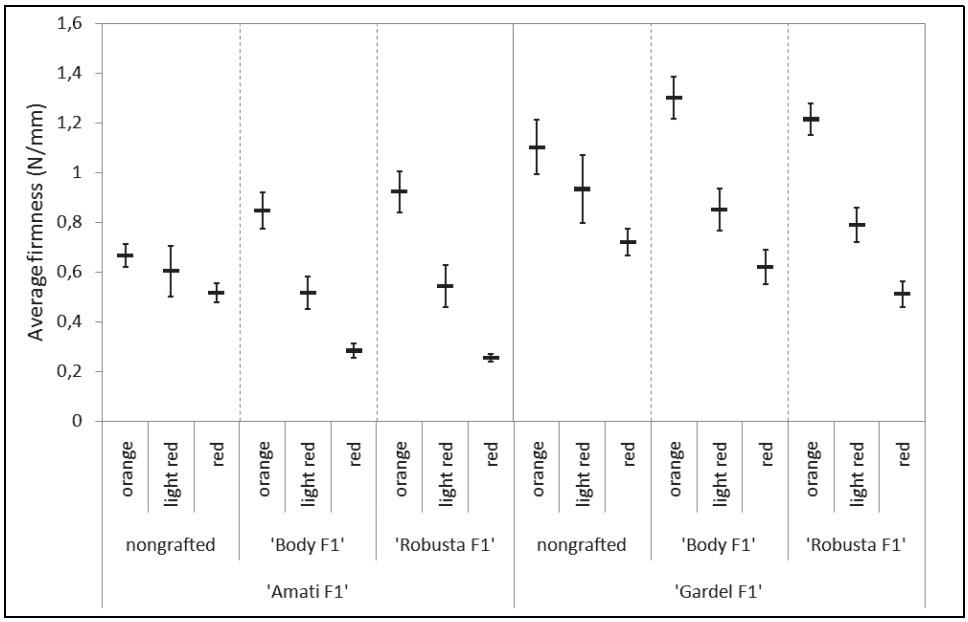

Fig. 3. The average firmness with the intervals of ± SE of tomato fruits at different ripening stages picked from grafted and non-grafted plants.

in Table 4 and Fig. 4. In both cultivars, lycopene was the major carotenoid component, representing between 40 and 70 % total carotenoids in the tomato fruits. The relative amount of β-carotene ranged from 20 to 40 % and lutein represented the smaller part, between 7 and 20 % of total carotenoids in tomato fruit (Table 4). In general, an increase in relative amount of lycopene during tomato fruit ripening and a decrease of relative amounts of β-carotene and lutein was observed.

Statistical analysis of the influence of ripening stage and scion/rootstock combination on carotenoid content and their composition was done separately for each cultivar. Namely the fruits of 'Amati F1' and 'Gardel F1' cultivars differed significantly in colour parameters and in fruit firmness (Table 3 and Fig. 3), and according to their original description (Table 1), a distinct fruit size, shape, colour and time of maturation has been reported for these two cultivars (Syngenta seeds 2011). The average content levels of total carotenoids in fruits of 'Amati F1' cultivar ranged between 30 and 60 μg g^{-1} dw, and in fruits of 'Gardel F1' cultivar between 30 and 46 μg g^{-1} dw. This is in accordance with the results of Kuti and Konuru (2005), who reported that the differences in total carotenoid content in fruits of round type tomatoes are genetically regulated and range from 70 to as much as 700 μg/g dw in fully ripe fruits, depending on the cultivar. In fruits of 'Amati F1' cultivar, ANOVA for all three carotenoids showed that the impact of grafting on the average contents of lycopene, β-carotene and lutein was not the same at all three ripening stages (Table 4). In fruits of 'Gardel F1' cultivar, a similar impact was confirmed only for lycopene, while the average amount of β-carotene was significantly affected only by the ripening stage, and the average amount of lutein was affected by both factors: grafting combination and ripening stage (Table 4).

Relationship between the carotenoids

As carotenoid composition in tomatoes is rapidly modified during fruit ripening (Bramley 2002) and tomatoes are usually harvested and offered to the consumers at the technological maturity (orange to light-red and red stage) (Schouten et al. 2007) a thorough stage-dependant analysis of carotenoids has been conducted in our study. In order to better understand the effect of grafting combinations on the relationship between the main carotenoids in tomatoes during the ripening period, the compositional data statistical analysis was done for determination of lycopene and β-carotene log-ratio and for the log-ratio between hydrocarbon carotenes, lycopene and β-carotene and lutein. The results indicate a statistically significant impact of ripening stage ($P < 0.000$) on log-ratio between the amount of lycopene and β-carotene (ilr_1 coordinate) for both cultivars (Table 4). Grafting only had a significant influence on this ratio for 'Amati F1' scion ($P = 0.0001$). The average relative amount of lycopene in grafted 'Amati F1' fruit increased compared to non-grafted plants and plants grafted on 'Body F1' rootstock, from 54 % in orange fruits to 70 % in red fruits. Simultaneously, the average relative amount of β-carotene decreased during tomato fruit ripening from 33 to 22 %. The relative amount of lycopene in orange fruits from plants grafted on 'Robusta F1' rootstock was significantly lower (48 %) and it increased to 67 % in red fruits, which was associated with the decrease of β-carotene from 36 to 24 % at the same ripening stage. For 'Gardel F1' scion, a more prominent increase in the average relative amount of lycopene during the ripening process was recorded: from 44 % in orange fruits to 70 % in red fruits compared to 'Amati F1' scion. Also, a decrease in the average relative amount of β-carotene was more pronounced in this fruit, and amounted from 40 % to 23 %

Table 4. The average composition of carotenoids in tomato fruits and the average content ($\bar{x}$) and its standard error (SE) of each component of carotenoids for all treatments.

Scion	Root-stock	RS	Average composition					Lycopene			β-carotene			Lutein		
			Lyco-pene	β-caro-tene	Lutein	ilr_1	ilr_2	(μg/g dw)								
								$\bar{x}$	SE		$\bar{x}$	SE		$\bar{x}$	SE	
'Amati F1'	non-grafted	orange	0.51	0.34	0.15	Aa	a	28.2	2.3	abc	18.7	1.6	ab	8.5	0.6	a
		light red	0.62	0.28	0.10	Ba	bc	30.8	3.5	ab	14.3	1.9	bcd	5.2	0.5	b
		red	0.69	0.23	0.08	Ca	cde	20.0	2.3	c	6.8	1.0	c	2.2	0.3	c
	'Body F1'	orange	0.56	0.31	0.13	Aa	ab	30.7	2.1	ab	17.1	1.2	bc	7.4	0.8	a
		light red	0.66	0.27	0.07	Ba	de	36.7	1.9	a	14.8	1.0	bcd	4.0	0.4	bc
		red	0.71	0.21	0.08	Ca	cde	37.9	6.0	a	11.9	2.6	d	4.2	0.9	b
	'Robusta F1'	orange	0.48	0.36	0.17	Ab	a	21.9	0.9	bc	16.4	0.9	bcd	7.8	0.8	a
		light red	0.54	0.39	0.07	Bb	e	31.4	1.2	ab	23.1	0.9	ab	4.5	0.7	b
		red	0.67	0.24	0.09	Cb	cd	34.7	4.9	a	12.8	2.0	cd	4.7	0.6	b
'Gardel F1'	non-grafted	orange	0.39	0.40	0.21	A	a	12.1	1.7	d	12.0	0.6	A	6.3	0.3	Aa
		light red	0.57	0.32	0.11	B	b	22.2	2.3	bc	13.0	2.1	A	4.7	0.8	Aa
		red	0.67	0.25	0.08	C	bc	26.6	2.5	ab	10.5	1.9	B	3.3	0.5	Ba
	'Body F1'	orange	0.52	0.38	0.10	A	b	20.6	1.7	c	15.6	1.9	A	4.4	0.5	Ab
		light red	0.54	0.35	0.11	B	b	20.3	0.9	c	13.9	2.0	A	4.3	0.5	Ab
		red	0.72	0.21	0.07	C	c	29.6	1.7	a	8.7	0.7	B	2.8	0.4	Bb
	'Robusta F1'	orange	0.41	0.41	0.18	A	a	14.3	2.0	d	13.8	0.8	A	6.2	0.3	Aa
		light red	0.54	0.34	0.12	B	b	24.6	1.5	abc	15.7	1.4	A	5.6	0.5	Aa
		red	0.71	0.22	0.07	C	c	29.7	1.7	a	9.6	0.8	B	3.0	0.3	Ba

ilr_1 = ln(lycopene/β-carotene), ilr_2 = ln(lycopene*β-carotene/lutein). There is no statistical significant difference according to Duncan's multiple range test (α = 0.05) between the averages denoted with the same capital letters (A, B, C) for ripening stage and small letters (a, b, c, d, e) for different grafting levels or in the case when interaction between grafting and ripening stage is presented.

of total carotenoids (Table 4). Our results are in accordance with previously published data on the ripening process and carotenoid synthesis of tomato fruits (Bramley 2002; Raffo et al. 2002). In some earlier studies, lycopene content in fully ripe tomatoes was compared among cultivars but not during the ripening process (Fernández-Garcia et al. 2004; Kuti and Konuru 2005). Tomato carotenoid composition during fruit ripening is subjected to intensive synthesis of enzymes controlling the synthesis of carotenoids, such as phytoene synthase and phytoene desaturase (Bramley 2002). These ultimately enable the production of lycopene and reportedly increase content 10 to 20 fold at breaker stage of ripening (green to yellow). At the same time lycopene cyclases (lycopene β-cyclase and lycopene ε-cyclase), which promote the production of β-carotene, are deactivated (Hirschberg 2001). We should pointed out that changes in the content of both major carotenoids could be a consequence not only of the ripening stages, but also of the effect of different 'history' of the fruits, since the fruits have been taken from different inflorescences and thus they were grown and developed in different climate conditions, which reportedly influence carotenoid synthesis in tomatoes (Brandt et al. 2006). Our results are also in accordance with those reported by Raffo et al. (2002) who detected a similar decrease in the relative amount of β-carotene during the ripening period, from 31 to 14 % of total carotenoids in fruits of cherry tomatoes. In this phase a*/b* colour index increased from 0.6 to 1.4.

Although lycopene and β-carotene represent the most abundant carotenoids in tomato, the amount of lutein, which has been found to offer effective protection against the age-related macular degeneration (Seddon et al. 1994), has also been detected in fruit samples. For a more detailed

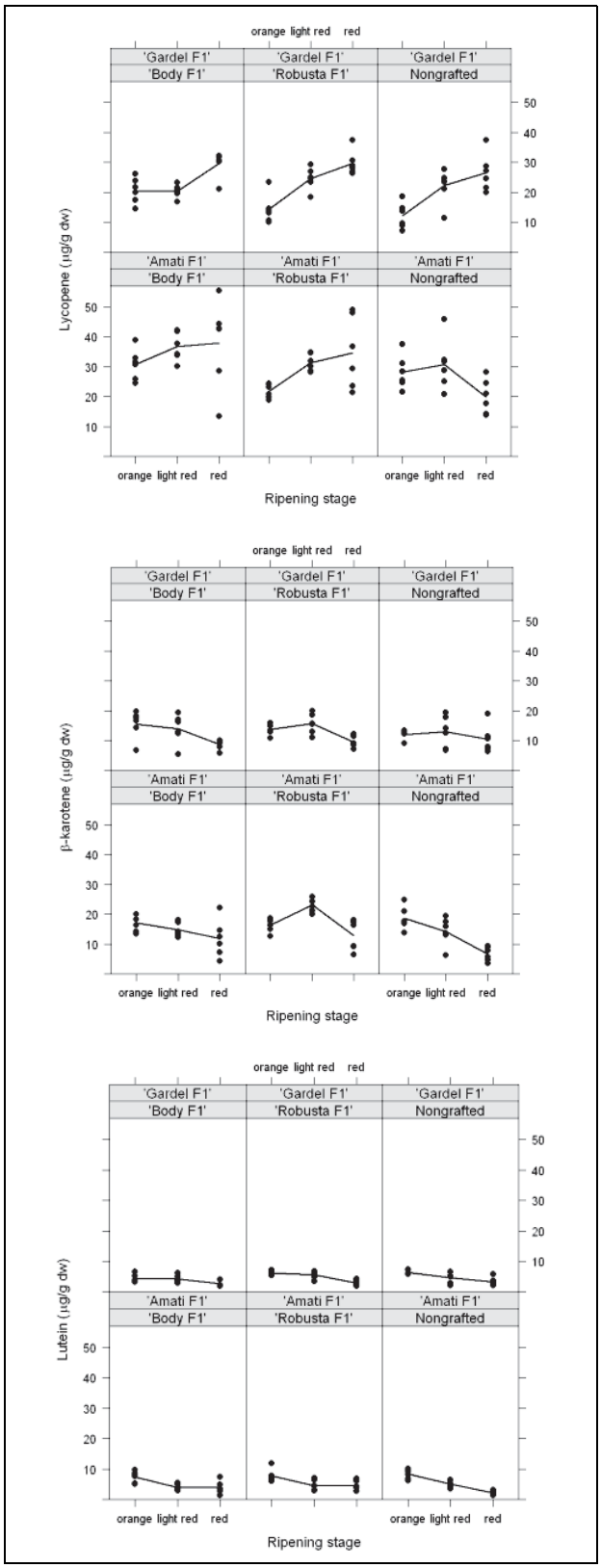

Fig. 4. Results of the measurements of three carotenoid components: lycopene, β-carotene and lutein. Black lines connect averages of six replications of each treatment determined with tomato scion ('Amati F1', 'Gardel F1'), ripening stage (orange, light red, red) and grafting combination (nongrafted, 'Body F1' rootstock, 'Robusta F1' rootstock).

analysis of the structure of the main carotenoids, the relationship between lycopene*β-carotene and lutein has been analysed (ilr_2 coordinate) (Table 4). The results of compositional data analysis showed statistically significant interactions of ripening stage and grafting combination on log-ratio between the amount of lycopene*β-carotene and lutein (ilr_2) for both cultivars. For 'Amati F1' scion, the ripening stages had a significant impact on this log-ratio, which was revealed in grafted plants on both rootstocks as a significant decrease in the relative amount of lutein detected in orange fruits (13 and 17 % total carotenoid) compared to other two ripening stages, where the relative amount of lutein ranged between 7 % in the light red stage and 9 % in the red stage. In fruits harvested from non-grafted plants, the relative amounts of lutein significantly decreased from orange stage (15 % total carotenoid) to 8 % in the red stage. A similar trend was observed in fruit of 'Gardel F1' cultivar where different impact of the ripening stages on the log-ratio lycopene*β-carotene/lutein has been confirmed. In fruits from plants grafted on 'Robusta F1' rootstock, the relative lutein content varied significantly through the ripening stages, from 18 % in orange fruits to 12 % in light red and 7 % in red fruits (Table 4). In fruits harvested from non-grafted plants the relative lutein content was significantly lower in light red and red fruits (11 and 8 %) compared to orange fruits (21 %). On the other hand, in fruits from plants grafted on 'Body F1' rootstock, significantly lower relative lutein content (7 % total carotenoids) has been detected in red fruits compared to light red and orange fruits (11 and 10 % total carotenoids, respectively). A decrease of lutein content during fruit ripening is a consequence of complex biochemical and developmental processes characteristic for the development of tomato fruits (Hirschberg 2001; Kuti and Konuru 2005). Although these processes are genetically regulated (Fraser et al. 1994; Hirschberg 2001; Stigliani et al. 2011) and several studies deal with the genetic modification impacting carotenoid content levels of fruits (Ronen et al. 1999; Rosati et al. 2000; Bramley 2002), our results show, that the levels and composition of the main carotenoids can also be affected by scion/rootstock regulation and this impact is different among cultivars and ripening stages.

Conclusions

The present study was based on the fact that the organoleptic and health-promoting quality attributes of tomato are not only genetically regulated, but are also closely linked to the developmental and ripening changes of fruits. Tomato plant grafting presents an important advance in tomato production, decreasing the susceptibility of plants to biotic and abiotic stresses, as well as increasing fruit yield through increased plant vigour. In our study we were able to demonstrate that some of the evaluated scion/rootstock combinations had a significant impact on fruit colouration, while fruit firmness was more affected by the scion cultivar

and the ripening stage than by the scion/rootstock combination. Our results also indicate that the impact of the scion/rootstock combination on the carotenoid composition was significant in fruits of 'Amati F1' scion, which was revealed in a significant increase of lycopene and a decrease of β-carotene, when the fruits changed from orange to red colour. No significant increase/decrease in the relative amount of lycopene/β-carotene during the ripening period has been confirmed in the other scion ('Gardel F1'). Compositional data analysis which was performed for the detail analysis of the structure of the main carotenoids showed that the relative amount of lutein detected in fruits of some of the scion/rootstock combinations, decreased significantly during fruit ripening. It can therefore be inferred that the scion/rootstock combination could influence the carotenoid composition when tomatoes are at technological ripening stages, which is important in terms of human health and also in terms of tomato growers, who require additional information on the usefulness of the scion/rootstock combinations. From that point of view, further investigation should be done, in order to find the compatible scion/rootstock combinations, which include commercially interesting scion and rootstock cultivars, such as 'Beaufort F1', 'Maxifort F1', 'Optifort F1', 'Heman F1 (De Ruiter Seeds 2011; Syngenta seeds 2011) and other rootstocks. These should not only be tested for the quantity of fruit yield but additional analysis investigating the content and composition of carotenoids in tomato fruits should be performed.

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