Effects of Grafting on Organic Seedling Quality and Tomato Production in Greenhouse

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Grafting is used as an essential cultural technique to increase tolerance of vegetables against biotic and abiotic stresses. Since grafting contributes to sustainable agriculture by reducing the amount of agrochemicals used for soil disinfection, it is an important strategy in particular in organic production. However, grafted organic seedling production is not a common practice. Thus, this study was conducted in order to determine seedling growth and crop performance of organically grown grafted tomato plants in spring-summer season of 2016. Seeds of rootstocks namely 'Beaufort' and 'Sarafin' and scion cv. 'Melis' were sown in vermicompost:local peat (1:1.5 v/v) mixture and after germination in growth chamber, seedlings were moved to a greenhouse. When they had 3-4 fully developed leaf, they were grafted with tube grafting method. Self-grafted seedlings were used as control. Grafted seedlings were left in a healing unit for 10 days and placed again into greenhouse for adaptation. One week later, ten seedlings from each replicate were harvested to measure seedling performance and others were transplanted in greenhouse in order to determine their biomass, yield and fruit quality performance. The results confirmed that the use of rootstock affected root and stem length, stem diameter, shoot and root fresh and dry weights of seedlings; yield and plant growth of plants significantly. However, fruit quality did not change. The use of rootstocks increased the seedling quality, total and marketable yield and plant growth. Among the tested rootstocks 'Beaufort' was found more appropriate due to the highest performance on organic seedling growth and tomato production under greenhouse conditions.

Keywords: Rootstock, scion, biomass, yield, quality.

Introduction

Grafting of vegetable seedlings is a practical horticultural technology used for many years in the world. Grafted vegetables can improve the resistance to soil borne diseases and abiotic stress factors like drought, salinity, chilling etc. and enhance the resistance ability (King et al., 2008; Lee et al., 2010; Jianping, 2011). Since the root systems of rootstocks are usually much larger and more vigorous, they can absorb nutrients more efficiently as compared to non-grafted plants (Huang et al., 2015). Using rootstock also improve plant growth and yield, influence product quality (Ruiz et al., 1997; Khah, 2005; Martorana et al., 2007; Rouphael et al., 2010; Lee et al., 2010; Colla et al., 2011). The effects of rootstocks can be related to the synthesis and translocation of plant growth regulators, to water and nutrient uptake by vigorous roots of rootstock, as well as their assimilation (Lee et al., 2010).

Grafting is also a key technique for sustainable crop production to overcome the continuous cropping obstacle and to keep the vegetable products safe (Maršić and Osvald, 2004; Khah et al., 2006; Rivard and Louws, 2008; Louws et al., 2010; Schwarz et al., 2010). Thus, the use of grafting in greenhouses has become widespread because soil borne diseases such as Fusarium wilt, Verticillium wilt, bacteria wilt and root knot nematodes are more serious under protected cultivation compared to open fields. The second reason is that vegetables often suffer from low temperature and low light intensity (from late fall to early spring). In addition, the soil secondary salinization caused by excess fertilizer input and cover of protected facilities is also serious under protected cultivation. Through the selection of appropriate rootstock, the grafted plant tolerance to low temperature and salinity in greenhouses can be enhanced (Huang et al., 2010; Li et al., 2014; Huang et al., 2015).

In this regards, grafting could be used as a vital and clear strategy in order to avoid soil-borne diseases, especially in organic production under greenhouse (Oztekin and Tuzel, 2015). According to Council Regulation (EEC) No 2092/91 of 24 June 1991, seed and plant propagation material should be organically produced in organic farming (EUR-Lex, 1015) however, grafted organic seedling production is not a commercial practice due to necessity for intensive care, the requirement of knowledge and skill, availability of inputs (i.e.

organic seeds). The high cost of organically produced scion and rootstock seeds and also all other inputs (growing medium, fertilizers, pesticide, biological agents etc.), relatively lower amount of demand compared to conventional ones and lack of production protocols are other the most important drawbacks.

This study aimed to determine seedling growth and crop performance of organically grown grafted tomato plants in spring-summer season under greenhouse.

Materials and Methods

This research was conducted during the spring season of 2016 at the Department of Horticulture at Ege University in Izmir, Turkey (38°27’17”-N, 27°14’17”-E) in order to determine the performance of organically grown grafted tomato seedlings and plants grafted on different rootstocks.

Non-treated seeds of tomato (Lycopersicon esculentum) cultivar Melis (AG Seeds, Antalya-Turkey) were used as scion. Two interspecific tomato hybrid (L. esculentum × L. hirsutum) rootstocks namely Beaufort (De Ruiter, The Netherlands) and Sarafin (AG Seeds, Antalya-Turkey) were used for grafted tomato seedling production and compared with self-grafted plants used as control. The seeds of rootstocks ‘Sarafin’ were organically certified, but Beaufort seeds were washed before used since organic or non-chemical treated seeds were not available in the market.

Local peat (provided from Denizli peatlands from Aegean Region/Turkey) and vermicompost (Ekosol, Istanbul-Turkey) mixture (1:1.5 v/v) were used as organic growing medium (Tuzel et al., 2014) and Melis and Sarafin seeds were sown on the 13th of January 2016 while Beaufort seeds were sown 19th of January 2016 due to differences of seed germination period. Scion and rootstock seeds were sown into three trays having 128 plugs in each.

After sowing, trays were left in a germination room at 24/24°C and 80% RH under dark for 3 days and then moved to a climate controlled (24-26°C) greenhouse which is specialized for seedling production for 3 weeks. Seedlings were fertilized with liquid composted farmyard manure (Botanica, Camli Yem Besicilik, Izmir-Turkey) (30 L ha^{-1}) (Tuzel et al., 2014) every day with boom system. In this period, germination rate and germination period of seeds were noted. Germination rate was calculated by counting the number of germinated seeds in cells and expressed as %. Germination period was determined as the number of days required for 50% of seed emergence (Tuzel et al., 2014).

When seedlings had 3 to 4 fully developed leaf, tomato scions were grafted with tube grafting method at the date of 22.02.2016. Grafting was performed manually and then water was sprayed immediately and grafted seedlings were left in growing room for healing at 24-26°C, 80-90% RH, 16 h LED light/8 h dark for 10 days by covering each rack with PE cover (40 micron thickness, thermal curtain) in order to conserve humidity. PE cover was totally closed at the beginning and after 5 days it started to open half during the day.

Seedlings were placed again into greenhouse after healing for adaptation for a week. During this period they were fertilized as mentioned above. When seedlings were ready for planting, 10 seedlings were harvested from each replicate of treatments in order to measure root, rootstock and scion length, root and shoot diameter, shoot and root fresh and dry weights. Root length (cm) was determined with a ruler from the longest root. Rootstock length (cm) was measured from root collar to grafting point through ruler; scion length (cm) was the distance between grafting and growth point; rootstock and scion diameter (mm) was measured from the middle of seedling with a digital caliper. Roots were washed and cleaned from growing medium and separated from shoots. Root and shoot samples were weighed for fresh weight (g) and then dried in a thermo ventilated oven at 65°C and weighed for dry weight (g).

Seedlings were transferred into the greenhouse on 08th of March, 2016 with a plant density of 2.86 plants per m^2 with the randomized blocks experimental design with 8 replicates; each replicate include 14 plants. Organic growing standards prevailed throughout production. Sticky yellow traps (one per 15 m^2) were placed above the plant level and moved up as they grew. Pests and diseases were monitored weekly and bumblebees were used for pollination. Drip irrigation used and irrigation amount was based on Class-A-Pan. Irrigation intervals varied from 3 to 4 days. Plants were topped on the 6th trusses and removed on 27th of June, 2016.

Fruits harvest started on 23th of May and continued until 26th of June, 2016. Harvested fruits were weighed and counted to determine the total and marketable fruit yield (kg m^{-2}), fruit number (no m^{-2}) and average fruit weight (g). Fruits were separated in terms of diameters as ∅>5.5 cm, 4.5>∅<5.5cm, 3.5<∅<4.5 cm and ∅<3.5 cm (non marketable) and fruits in each group was counted. In order to determine fruit quality, ten fully ripened tomato fruits were taken from each grafting combinations with 4 replicates on 09th of June, 2016. Penetrometer (Nippon FHR-1) with a conical tip (base diameter 8 mm and length 10 mm) was used to measure rind strength (firmness) and the results were expressed in Newtons (N). The detailed surface colour of tomatoes was assessed with a colorimeter (Minolta, CR-300, Japan) by measuring opposite sides of randomly selected fruit using a 8 mm diameter viewing area. Measurements were recorded as L (lightness, from white = 100 to black = 0), a (red-green) and b (yellow-blue) CIE (Commission Internationale de l'Eclairage) colour co-ordinates. Fruit juice was extracted using a kitchen juicer and filtered. EC (dS m^{-1}) and pH values were measured with an EC and pH meter (Seven Easy, Mettler Toledo, Istanbul, Turkey). Total soluble solids (TSS, %) content was measured using a digital refractometer (Euromex RD 645, Arnhem, The Netherlands). Titratable acidity (TA) was determined by titration with 0.1 N NaOH to pH 8.1 and expressed as mval 100 ml^{-1}. The dry weight (DW, % of fresh weight) was determined by drying samples in an oven at 65°C until a constant weight was obtained. Nitrate (mg kg^{-1}) and vitamine C (mg 100 ml^{-1}) were determined according to Fresenius et al. (1998) and Pearson (1970), respectively. At the end of the growing cycles, measurements of plant height (cm) from soil to top with ruler; stem diameter (mm) with digital calipers; root, fruit and shoot fresh and dry weights (g $plant^{-1}$) at 65°C in a thermoventilated oven were also made on 4 plants in each treatment.

Data were subjected to analysis of variance to determine any statistically significant differences among rootstock by using the JMP statistical analysis package program (SAS Institute, USA). Tukey range test was conducted at 5% importance level ($P \leq 0.05$) in order to identify the differences between the means.

Results and Discussion

Seedling performance

Beaufort, Sarafin and Melis seeds completed 50% of emergence 2, 5 and 4 days after sowing, respectively. The germination rate was the highest in Beaufort (100%) compared with Sarafin (95%) and Melis (97%) (data not shown).

Effect of grafting on scion length and rootstock diameter; shoot fresh and dry weights and root dry weight were found statistically different, however, root and rootstock lengths did not change significantly in treatments. Scion stem length accepting as one of the most important seedling quality parameters was higher in Melis/Beaufort, followed by Melis/Sarafin and self-grafted plants. Beaufort gave the highest rootstock diameter while rootstock did not affect scion diameter at seedling stage. Rootstocks statistically affected root fresh and shoot fresh and dry weights. Melis/Beaufort gave the highest seedling biomass with the highest root and shoot fresh weights followed by Melis/Sarafin and self-grafted ones (Table 1).

Seedling quality has a vital role in the success of crop production and it is particularly related to well root and shoot development (Kubota et al., 2013). The results obtained from the study have shown that rootstocks had better performance than self-grafted seedlings especially in terms of root and shoot fresh weights, shoot dry weight and stem length and diameter. Although rootstocks showed more vigour and vegetative growth, it was depending on rootstock genotype. Among the tested rootstocks, the performance of Beaufort as a rootstock on seedling quality was obvious. This result showed similarity with our previous findings (Oztekin and Tuzel, 2016). Overall results confirms that Beaufort could be more appropriate for organic seedling production compared to other rootstocks due to its higher performance.

Table 1. Effects of treatments on organic tomato seedling growth (2 weeks after grafting)

Grafting Combinations	Root	Length (cm) Rootstock	Scion	Diameter (cm) Rootstock	Scion	Root ($g\ plant^{-1}$) Fresh weight	Dry weight	Shoot ($g\ plant^{-1}$) Fresh weight	Dry weight
Melis/Melis	9.26	3.42	10.04 c	2.33 b	3.11	0.20 b	0.02	2.68 b	0.29 b
Melis/Beaufort	10.21	3.32	19.15 a	3.03 a	2.93	0.46 a	0.05	5.44 a	0.52 a
Melis/Sarafin	9.79	2.88	15.94 b	2.61 b	2.85	0.27 ab	0.02	4.12 ab	0.38 ab

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).

Plant performance

Grafting combinations affected total and marketable yields significantly. Beaufort increased total and marketable yields by 12.7% and 13.6% respectively compared to self-grafted ones. Sarafin took a place between Beaufort and Melis and self-grafted plants had the lowest yield performance (Table 2).

Table 2. Effects of treatments on fruit yield of grafted organic tomato plants

Grafting Combinations	Total yield ($kg\ m^{-2}$)	Marketable yield ($kg\ m^{-2}$)	Mean fruit weight (g)	Total fruit number ($no\ plant^{-1}$)	Fruit diameter ratio (%) <3.5 cm	3.5-4.5 cm	4.5-5.5 cm	>5.5 cm
Melis/Melis	11.93 b	11.73 b	107.93	110.54	9.31	12.76	12.54	65.39
Melis/Beaufort	13.45 a	13.32 a	116.71	115.35	7.28	11.27	13.15	68.30
Melis/Sarafin	12.27 ab	12.08 ab	110.44	111.11	8.35	13.02	13.43	65.20

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).

Grafting did not affect fruit skin colour and lightness (Table 3) and also dry weight, firmness, titratable acidity, total soluble solids; nitrate and vitamine C content (Table 4). EC and pH values of fruit juice only changed within measured quality parameters. EC and pH was the lowest in fruit juices from self-grafted and Beaufort plants, respectively.

Table 3. Effect of different grafting combinations on fruit skin colour

Grafting Combinations	L	a*	b*	a/b	Hue°	Croma°
Melis/Melis	41.07	22.19	26.44	0.84	49.99	34.52
Melis/Beaufort	41.37	21.54	26.11	0.82	50.49	33.85
Melis/Sarafin	41.44	21.70	26.20	0.83	50.42	34.03

Table 4. Effect of different grafting combinations on some fruit quality parameters

Grafting Combinations	DW (%)	Firmness (N)	EC ($dS\ m^{-1}$)	pH	TA ($mval\ 100\ ml^{-1}$)	TSS (%)	NO_3-N ($mg\ kg^{-1}$)	Vitamin C ($mg\ 100\ ml^{-1}$)
Melis/Melis	7.89	48.95	5.40 b	4.77 a	4.93	5.08	28.00	24.90
Melis/Beaufort	6.45	49.10	5.67 ab	4.74 b	4.89	5.08	31.26	25.06
Melis/Sarafin	6.85	51.80	5.77 a	4.75 ab	4.92	5.28	35.64	26.04

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).
DW: dry weight, EC: electrical conductivity, pH: power of Hydrogen, TA: titratable acidity, TSS: total solube solids

Among plant biomass parameters scion diameter, vegetative, generative and root fresh and dry weights were affected from different rootstocks. Plants on Beaufort gave the highest stem diameter and fresh and dry weights. Rootstock Sarafin showed the same performance with self-grafted plants (Tables 5 and 6)

Tablo 5. Effets of rootstocks on grafted tomato plant growth

Grafting Combinations	Plant height (cm)	Root lenght (cm)	Stem diameters (mm)	
			Rootstock	Scion
Melis/Melis	206.4	29.7	17.5	12.8 c
Melis/Beaufort	193.6	29.7	19.1	15.8 a
Melis/Sarafin	198.6	32.1	18.5	14.2 b

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).

Table 6. Effect of different grafting combinations on fresh and dry weights of tomato plants

Grafting Combinations	Vegetative parts			Generative parts			Root		
	FW (g)	DW (g)	DW (%)	FW (g)	DW (g)	DW (%)	FW (g)	DW (g)	DW (%)
Melis/Melis	643.1 b	106.6 b	16.8	4069.6 b	268.9 b	6.6	30.9 b	8.2 b	26.5
Melis/Beaufort	1099.4 a	172.5 a	15.7	4775.6 a	313.2 a	6.6	55.3 a	16.2 a	28.7
Melis/Sarafin	678.4 b	108.0 b	15.9	4310.4 b	276.1 ab	6.4	31.5 b	8.2 b	26.0

Mean data followed by the same letter are not significantly different within each column (P≤0.05, Tukey test).
FW: fresh weight, DW: dry weight

Grafted plants have been used to improve plant growth and yield, provide earliness, extend harvesting period, influence product quality, improve water and nutrient use of efficiency etc. (Ruiz et al., 1997; Khah, 2005; Martorana et al., 2007; Rouphael et al., 2008; Rouphael et al., 2010; Colla et al., 2011). These may be caused by signals such as water, nutrients and especially hormones and nucleic acids that move through the graft union to affect scion growth (Perez Alfocea, 2015). When they are compared with the self-rooted plants, grafted plants have stronger and denser root structure, which increases internal plant hormones and as a result the rate of photosynthesis, thus inciting plant growth and development, and positively contributing to resistance against stress conditions (Ahn et al., 1999; Lee et al., 2010). Grafted plants reflect these advantageous properties more under stress conditions and against soil-borne disease problems. In this study there was no stress factor however especially total and marketable yields and plant stem diameter, fresh and dry weights of plant parts increased by the use of rootstock. This higher performance most probably came from the seedling stage due to increased seedling quality with higher fresh weight and stem diameter.
The response changed according to the rootstock genotype. As in the seedling phase, rootstock Beaufort showed better performance compared to Sarafin and Melis. These results are consistent with other studies showing that the grafting effect changed depending on the rootstock genotype (Santa Cruz et al., 2002; Lee and Oda, 2003; Abdelmageed et al., 2004; Khah, 2005; Öztekin, 2009).

Conclusions

Rootstocks showed more vigour in seedling stage, yield and vegetative growth in greenhouse condition. These parameters differed according to grafting combination; among the tested rootstocks Beaufort was found more appropriate for organic seedling and crop production compared to other rootstocks due to the higher performance. It was concluded that grafting could be used in organic agriculture providing organic inputs are supplied.

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