Fruit Quality of Grafted Watermelon (*Citrullus lanatus*): Relationship between Rootstock, Soil Disinfection and Plant Stand

Merav ZAAROOR [1, 3], Sharon ALKALAI-TUVIA [1], Daniel CHALUPOWICZ [1], Yohanan ZUTAHY [1], Marina BENICHES [2], Abraham GAMLIEL [2], Elazar FALLIK [1(✉)]

Summary

Grafting of vegetable transplants is a unique horticultural technology, which was adapted from the practice in perennial crops. However, rootstock/scion combinations may affect and alter the final size, yield, and quality of fruits of grafted plants, both immediately postharvest and during prolonged storage. We evaluated the effect of two rootstocks [TZ148 and Nurit (commercial *Cucurbita* spp. hybrids)] grafted on one scion (seedless watermelon cv. 1262) in two plant stands (2500 and 5000 plant ha^{-1}), on plant viability, number of marketable fruits (fruits weight above 5 kg) and fruit quality after one week storage at 20°C, in non- or disinfested soil. Soil disinfection significantly improved the viability of non-grafted plants. All grafted plants significantly performed better vine vigor, with no wilt or vine decline symptoms, in either disinfested or non-treated soil, regardless of the type of the rootstock. Plant stand did not affect plant viability. The number of marketable watermelon fruits per m^2 was 75 to 700% higher in grafted plants than in non-grafted. Grafting on Nurit produced significantly more marketable fruits than grafting on TZ148. The quality of fruits harvested from grafted plants was significantly better than non-grafted fruit in both plant stands and soils. Watermelons harvested from Nurit-grafted plants had better taste and texture and almost seedless compared to control and TZ-148-grafted plant.

Key words

Grafting; plant stand; plant density; postharvest; quality

[1] Department of Postharvest Science of Fresh Produce, ARO-the Volcani Center, Bet Dagan 50250, Israel
✉ e-mail: efallik@volcani.agri.gov.il
[2] Department of Growing, Production and Environmental Engineering ARO-the Volcani Center, Bet Dagan 50250, Israel
[3] Robert H. Smith, Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

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Introduction

The commercial use of grafted vegetable transplants has been practiced for over 50 years in East Asia to overcome crop limitations associated with intensive cultivation on limited and challenging arable land (Kubota et al., 2008). The major advantage of this practice is that the grafted stem is protected from soilborne pathogens and pests (Louws et al., 2010). These pathogens and pests were eradicated using soil fumigants such as methyl bromide, but most of soil fumigants have been banned to be used under commercial agricultural practices, leading to an extensive search for alternatives that fall in with the new integrated pest management (IPM) systems (Louws et al., 2010).

Grafting can protect vegetables against soil-borne diseases and nematodes, against abiotic stresses such as high/low temperatures, salinity, drought or excessive soil-water content, and against elevated soil concentrations of heavy metals and organic pollutants (Colla et al., 2010; Savvas et al., 2010; Sánchez-Rodríguez et al., 2011). In addition, the grafted plant takes up water and nutrients from the soil more efficiently and retains its vitality for longer periods during the growing season (Schwarz et al., 2010). However, rootstock/scion combinations may affect and alter the final size, yield, and quality of fruits from grafted plants, both immediately postharvest and during prolonged storage. These alterations may be attributed in part to differing production environments and methods, the type of rootstock/scion combinations used, and harvest date (Bekhradi et al., 2011; Kyriacou and Soteriou, 2012).

One of the most remarkable characteristics of the grafted plants when using these rootstocks is their greater vigor, so it becomes necessary to determine a plant stand adequate to this system, in order to avoid a decrease of the production and maintain the quality level; this principle has been applied in every place in the world where the use of grafted plants has become popular (Ricárdez-Salinas et al., 2010). High plant densities in the field in the cucurbit crops affect melon and watermelon production seriously, because of the low effectiveness of the pollinating insects. Low plant densities cause low productivity and perhaps the size of the fruits harvested would not be suitable for the market (Kultur et al., 2001). Yet, the information about the postharvest fruit quality in relation to plant stand in the field is very little.

Watermelon (*Citrullus lanatus* L.) fruits in the Mediterranean basin are usually handled and stored after harvest under nonrefrigerated conditions for up to two weeks at 10–15°C, depending on the cultivar and agricultural practices (Kyriacou and Soteriou, 2012). However, the quality and shelf life of grafted watermelon fruits vary among cultivars and appear to depend upon both the rootstock and the scion (Kyriacou and Soteriou, 2015; Xu et al., 2015). Hence, grafting can improve or reduce the fruit's external and/or internal quality, depending on the specific rootstock/scion combination (Alexopoulos et al., 2007; Donas-Ucles et al., 2015). In addition, the inconsistencies in reported fruit quality and shelf life can be attributed to differences in production environments, and optimal harvest timing.

The main objective of the present study was to evaluate the effect of grafting, soil disinfestations and plant stand on pre- and postharvest external and internal quality during marketing simulation.

Materials and methods

Plant materials and growth

Seedless watermelon cv. 1262 (oval shape, green skin with red flesh; Gadot Agro, Israel) was used in this study. The experiment was conducted in an open field in loessial (sierozem) soil at the Eden experimental station, which is located in Syrian African rift, in the southern part of the Bet-She'an Valley. This cultivar is commercially grown in the area for local market during the early spring to early summer in Israel.

The field had a 10-year history of cropping cucurbits and infested with *Macrophomina phaseolina*, the causal agent of charcoal rot and vine decline in several cucurbits and other vegetables and field crops. The experiments consisted of three-bed-wide plots (bed width 1.93 m). All three beds were used for data collection. The fumigation treatments were arranged in a randomized complete block design with five replications per treatment and conducted at the end of August. A wide, impermeable Ozgard plastic sheet (Ginegar, Kibbutz Ginegar, Israel) was manually laid over the three beds. Metam sodium (MS) was injected at a rate of 60 g m^{-2} through polyethylene irrigation drip lines, which were placed under the plastic prior to mulching. The MS was applied in the irrigation water (30 liter m^{-2}) 2 weeks after the plastic mulch had been laid. The plastic film was kept on the mulched plot for an additional 3 weeks, and then manually removed.

Seedlings of watermelon cv. 1262 were grafted on rootstocks of one of the two commercial *Cucurbita* spp. hybrids: 'TZ-148' (*Cucurbita maxima* Duchesne x *Cucurbita moschata* Duchesne, Tezier, France) and 'Nurit' which is a local nursery-selected rootstock (Hishtil Ltd., Nehalim, Israel). The seedlings were grafted by the "hole insertion grafting" method. Non-grafted '1262' transplants were used as control.

Grafted and non-grafted transplants were planted in a regular plant stand (2500 plant ha^{-1}) and in a double stand (5000 plant ha^{-1}) in the indicated plots at the end of January for a total of 12 combinations of grafting/rootstock/stand treatments. Each treatment consisted of plots that were 3 beds wide and 15 m long. The experiment was set up in a factorial split-plot design with five replicates for each treatment. The plants were drip-irrigated and farmed as per recommendations for commercial watermelon production in the region.

In the middle of May, vine decline was determined by counting the number of collapsing vines. The watermelon fruits were manually harvested, counted and weighed according to market grade (over 5 kg). Market quality fruits (over 5 kg) were transferred to the Department of Postharvest Science in Bet Dagan, Israel, within 8 h of harvest for postharvest quality assessments. After 7 days at 20°C (market simulation of watermelon in Israel), the quality parameters of 7 uniform fruits in size, shape, and rind color, from each treatment (rootstock/scion/stand combination) were analyzed.

Evaluation of external and internal fruit quality

Each fruit was cut in half along the polar plane and the following quality parameters were evaluated: Skin color was scored on a scale of 1–3, where 1 = light green, 2 = green, 3 = dark green; Rind thickness was measured at 2 points on each fruit cross section using an electronic caliper; Flesh color was scored on a scale of 1–3, where 1 = pink, 2 = red, 3 = dark red; Appearance of seeds was evaluated on a scale of 0–3, where 0 = no seeds, 1 = some white seeds, 2 = mostly white seeds and a few black seeds, 3 = mostly black seeds; Total soluble solids (TSS) content was determined with an Atago (Atago Inc., Tokyo, Japan) digital refractometer by squeezing about 2 × 2 cm^2 of flesh tissue that was taken near the fruit rind (outer flesh) and from the heart of the fruit (inner flesh). Results were obtained as percentage Brix (TSS).

Sensory analyses (taste and texture)

The flesh of the watermelon fruit (a 3 × 3 cm section from the heart of the fruit) was evaluated by six trained tasters as follows: Overall taste was scored on a scale of 1–3, where 1 = very bad taste with severe bitterness or off-flavor, 2 = reasonable taste, 3 = excellent taste, sweet, no off-flavor or bitterness; Texture was scored on a scale of 1–3, where 1 = very soft and mealy, or gummy, 2 = fine, 3 = very crispy and firm.

Statistical analysis

Data on disease incidence and watermelon yield were subjected to analysis of variance (ANOVA) to test for possible interactions among the main effects, followed by mean separation using Tukey's honestly significant difference (HSD) test. Data on fruit quality were from 7 fruit of uniform size and shape per treatment. Since no significant differences were found between plant stand (density), all data were subjected to one-way or two-way (grafting and soil disinfection) statistical analysis with statistical significance set at $P = 0.05$ using the JMP10 Statistical Analysis Software Program (SAS Institute Inc. Cary, NC, USA) (Sall et al., 2001).

Results

Vine viability (decline) and marketable fruit

A 100% plant wilt was evident in the non-grafted plants grown in non-treated soils, in the two plant densities (Table 1). Soil disinfection significantly improved the viability of non-grafted plants, in both plant densities, compared to the same plants grown in nontreated soil (as reflected by percent vine decline. Plants which were grafted on 'Nurit' or 'TZ' rootstocks showed 100% vine vigor with no visible disease symptoms or vine decline in either disinfested or nontreated soils (Table 1).

Analysis of variance showed that grafting significantly increased the number of marketable fruit per m^2 ($P = 0.0001$) in both plant densities, while soil disinfection moderately increased marketable fruit per m^2 ($P = 0.01$). Grafting significantly affected the number of marketable fruit over 5 kg/m^2 ($P = 0.0001$) in both regular and double plant stand. The higher of fruit number was found in Nurit-grafted plant (Table 2). Soil disinfection was also affected number of marketable fruit per m^2 ($P = 0.01$). However, no interaction was found between grafting and soil disinfection (Table 2).

Table 1. Influence of rootstock and soil disinfection in relation to plant stand (density per hectar), on vine decline (due to *Macrophomina phaseolina).*

Treatment/rootstock	Vine decline (%)			
	Regular plant stand		Double plant stand	
	No disinfection	Soil disinfection	No disinfection	Soil disinfection
Non-grafted	100	58	100	50
Nurit	0	0	0	0
TZ148	0	0	0	0

Table 2. Influence of rootstock and soil disinfection in relation to plant stand (density per hectare), on number of marketable fruit over 5 kg m^{-2} and number of marketable fruit per m^2.

Treatment/rootstock	Number of fruits over 5 kg (Marketable fruit)/m^2			
	Regular plant stand		Double plant stand	
	No disinfection	Soil disinfection	No disinfection	Soil disinfection
Non-grafted	0.4 Bb^Z	0.8 Ba	0.3 Cb	0.5 Ba
Nurit	1.4 Aa	1.4 Aa	1.2 Aa	1.5 Aa
TZ148	1.0 ABb	1.5 Aa	0.7 Ba	1.3 Aa
LSD	0.25	0.17	0.06	1.17
	Analysis of Variance (P-value)			
Grafting (G)	****		****	
Soil disinfection (D)	**		***	
G x D	NS		NS	

^{Z}Values followed by different letters are significantly different among rootstocks/treatments (uppercase letters) or soil treatments (lowercase letters) at 5%. Analysis was conducted separately for regular and double plant stand; *, **, ***, and **** = significant at the 0.05, 0.01, 0.001 and 0.0001 levels, respectively. NS – no significant

Table 3. Influence of rootstock and soil disinfection in relation to plant stand (density per hectare), on marketable fruit weight per m^2.

Treatment/rootstock	Weight of fruit over 5 kg m^{-2}			
	Regular plant stand		Double plant stand	
	No disinfection	Soil disinfection	No disinfection	Soil disinfection
Non-grafted	2.8 Bb^Z	6.0 Ba	2.3 Ba	4.0 Ba
Nurit	11.6 Aa	13.0 Aa	9.3 Aa	10.7 Aa
TZ148	7.9 ABa	10.9 Aa	7.1 Aa	10.0 Aa
LSD	2.11	0.96	0.61	1.4
	Analysis of Variance (P-value)			
Grafting (G)	****		****	
Soil disinfection (D)	**		**	
G x D	NS		NS	

^{Z}Values followed by different letters are significantly different among rootstocks/treatments (uppercase letters) or soil treatments (lowercase letters) at 5%. Analysis was conducted separately for regular or double plant stand; *, **, ***, and **** = significant at the 0.05, 0.01, 0.001 and 0.0001 levels, respectively. NS – no significant

Table 4. Influence of rootstock and soil disinfection on external quality parameters of watermelon fruit after 7 days at 20°C in 2014.

Treatment	Rind color (1-3)				Rind thickness (mm)				Seeds (1-3)				Flesh color (1-3)			
	RP		DP		RP		DP		RP		DP		RP		DP	
	ND	SD	ND	SD	ND	SD	ND	SD	ND	SD	ND	SD	ND	SD	ND	SD
Non-grafted	2.1	2.1	2.1	2.0	11.3	12.2	11.0	11.7	1.3	1.2	1.4	1.4	2.3	1.9	2.1	2.1
	Aa^Z	Ba	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Ba	Ba	Ba	Aa	Ba	Aa	Aa
Nurit	2.0	2.2	1.9	2.2	11.5	12.0	12.4	11.8	1.4	1.3	1.3	1.4	2.2	2.2	2.1	2.2
	Aa	Aba	Ab	Aa	Aa	Aa	Aa	Aa	Aa	Ba	Ba	Ba	Aa	Aba	Aa	Aa
TZ-148	2.2	2.3	2.1	2.2	10.2	10.5	11.3	12.2	1.5	1.9	2.1	2.0	2.3	2.3	2.3	2.1
	Ab	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Ab
LSD	0.07	0.08	0.77	0.09	0.73	1.13	0.62	1.04	0.15	0.14	0.18	0.18	0.12	0.12	0.10	0.15
	Analysis of Variance (P-value)															
G	**		NS		NS		NS		****		****		NS		NS	
S	NS		**		NS		NS		NS		NS		NS		NS	
G x S	NS		NS		NS		NS		**		NS		**		NS	

RP – regular plant stand , DP – double plant stand , ND – no soil disinfection, SD – soil disinfection, G – grafting, S – Soil disinfection, NS – no significance; ^{Z}Values followed by different letters are significantly different among rootstocks/treatments (uppercase letters) or soil treatments (lowercase letters) at 5%. Analysis was conducted separately for RP and DP; *, **, ***, and **** = significant at the 0.05, 0.01, 0.001 and 0.0001 levels, respectively.

Similar results were obtained for an average of marketable fruit weight in grafted and non-grafted plant (Table 3). Analysis of variance showed that grafting significantly increased marketable fruit weight ($P = 0.0001$) in both plant densities, while soil disinfection moderately increased marketable fruit weight ($P = 0.01$). Fruit weight harvested from Nurit plant was significant higher, compared with non-grafted fruit, particularly in regular plant stand. No interaction between grafting and soil disinfection was found regarding marketable fruit weight (Table 3).

Fruit quality parameters

Table 4 shows the influence of grafting, soil treatment and plant stand on fruit rind color and thickness, seeds appearance and flesh color. From analysis of variance (Table 4), grafting moderately affected rind color in regular plant stand, in disinfested soil ($P = 0.01$) and highly affected seeds appearance in both regular and double plant stand ($P = 0.0001$). Fruit harvested from TZ rootstock had a significant more black seeds compared with fruits harvested from Nurit rootstock or from non-grafted plant in double plant stand (2-2.1 compared with 1.3-1.4, respectively). A moderate effect of soil disinfection was observed in rind color in Nurit-harvested fruit, in double plant stand grown with or without soil disinfection. A moderate interaction between grafting and soil disinfection was found in seed appearance and flesh color in fruit grown in a regular plant stand (Table 4).

The parameters that reflect fruit sensory are shown in Table 5. Grafting had a moderate to a very strong effect on the side-TSS and fruit texture in both plant densities (Table 5). Grafting also had a strong effect on fruit taste in double plant stand. Fruit harvested from Nurit rootstock had a significant better taste in double plant stand, but in regular plant stand, fruit taste was similar to harvested from TZ rootstock. Soil sterilization influenced fruit texture in double plant stand. Interaction between grafting x soil sterilization was observed in TSS-side and fruit texture ($P = 0.001$ and $P = 0.01$, respectively) (Table 5).

Table 5. Influence of rootstock and soil disinfection on sugar content, texture and taste of watermelon fruit after 7 days at 20°C in 2014.

Treatment	TSS - side (%)				TSS - heart (%)				Texture (1-3)				Taste (1-3)			
	RP		DP		RP		DP		RP		DP		RP		DP	
	ND	SD	ND	SD	ND	SD	ND	SD	ND	SD	ND	SD	ND	SD	ND	SD
Non-grafted	7.9	8.0	9.4	8.5	11.3	11.2	11.4	10.2	1.8	1.8	1.3	2.0	1.8	1.9	2.0	1.9
	Aa[Z]	Aa	Aa	Aa	Aa	Aa	Aa	Bb	Ba	Aa	Bb	Aa	Aa	Aa	Aa	Ba
Nurit	8.1	8.5	8.3	8.7	10.7	11.2	11.6	11.8	2.2	2.0	2.1	2.3	2.1	2.0	2.2	2.3
	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Aa
TZ-148	8.6	6.8	6.9	7.0	11.3	9.8	10.0	10.1	2.3	2.1	2.2	2.2	2.0	2.0	2.1	2.0
	Aa	Bb	Ba	Ba	Aa	Ab	Ba	Ba	Aa	Aa	Aa	Aa	Aa	Aa	Aa	Ba
LSD	0.42	0.48	0.64	0.49	0.72	0.53	0.84	0.63	0.13	0.19	0.17	0.15	0.12	0.17	0.10	0.13
	Analysis of Variance (P-value)															
G	**		****		NS		**		***		****		NS		***	
S	NS		NS		NS		NS		NS		***		NS		NS	
G x S	***		NS		NS		NS		NS		**		NS		NS	

RP – regular plant stand , DP – double plant stand , ND – no soil disinfection, SD – soil disinfection, G – grafting, S – soil disinfection, NS – no significance; [Z]Values followed by different letters are significantly different among rootstocks/treatments (uppercase letters) or soil treatments (lowercase letters) at 5%. Analysis was conducted separately for RP and DP; *, **, ***, and **** = significant at the 0.05, 0.01, 0.001 and 0.0001 levels, respectively.

Discussion

One of the major problems of watermelon production is the decrease of fruit yield and quality caused by soil diseases. Soil disinfection with methyl bromide has been used to prevent fungus attacks; however, its use is being restricted because this substance damages the ozone layer. Various approaches are used to prevent the infection of soil pathogens to plants, including crop rotation, genetic improvement, and soil fumigation, however, each of these practices has limitations and downsides inconveniences (Fallik and Ilić, 2014).

Grafting vegetables has been adopted for several reasons and objectives. However, the major drive is the strong tolerance or resistance of rootstocks to some soil diseases and nematodes (Fallik and Ilić, 2014). Grafting with resistant rootstocks offers one of the best ways to avoid soil diseases and improves growth under stress conditions (Cohen et al., 2014; Wimer et al., 2015). In addition, grafting improves yield and fruit quality, by improving plant growth (Turhan et al., 2012; Wimer et al., 2015). We have also found significantly better vigor of the grafted vs. non-grafted plants, resulting in higher fruit yield and better fruit quality as evaluated by weight and number of marketable fruit. Although yield and quality of fruit from the same scion grafted on different rootstocks can differ (Petropoulos et al., 2012), we did not find significant differences in fruit weight or number of marketable fruit per m^2 between the 'Nurit' and 'TZ' rootstocks, although 'Nurit' rootstock provided better marketable fruit quality.

Harvested watermelon fruit quality can benefit from grafting (Fallik and Ilic', 2014; Kyriacou and Soteriou 2015; Wimer et al., 2015). We found significant differences in external and fruit taste and texture between grafted and non-grafted plants. Watermelon fruit harvested from plants grafted on 'Nurit' were tastier and had a better flesh texture than fruit harvested from 'TZ'-grafted plants. But the results were not significant, except in double plant stand. With the use of grafted watermelon plants, planting stand may be reduced by 50%, obtaining higher yields than those obtained from non-grafted plants grown on fumigated soil (Huitron-Ramirez et al., 2009). Therefore, based on our findings, low plant stand in the field can maintain marketable fruit quality and postharvest quality.

Most of the fruit quality parameters, evaluated at postharvest in this work, were not significantly affected by the rootstocks or by soil disinfection. Yet, it seems that the 'Nurit' rootstock adds several advantages over TZ-148 rootstock and therefore, improves fruit quality. It may result from better water uptake and mineral content in the fruit due to the physical characteristics of its root system, including lateral and vertical development in the soil in this region (Martínez-Ballesta et al. 2010). It is also possible that the 'Nurit' rootstock provides better and more balanced conditions for ripening rate than the TZ-148 rootstock and non-grafted plants, as reported for watermelon in which grafting retarded ripening and therefore enhanced fruit quality, especially sweetness and firmness (Soteriou et al. 2014; Xu et al. 2015).

Conclusions

Fruits harvested from Nurit rootstock showed better quality in marketable and postharvest parameters compared to the fruit harvested from TZ-148 rootstock. However, plant stand did not affect fruit quality.

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