

Grafting as a Tool to Improve TYLCV-Tolerance in Tomato

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Abstract: The purpose of this study was to investigate possible positive effects of grafting and use of different TYLCV-resistant rootstocks on the tolerance/resistance level and tomato fruit yield and quality. Tomato cvs used as scions were TYLCV-susceptible cv. Castlerock and TYLCV-tolerant hybrid cv. TH99806 (Nirouz). The rootstocks were TYLCV-resistant accessions *Solanum chilense* LA2779, *S. habrochaites* LA1777 and *S. pennellii* LA716 and TYLCV-susceptible *S. lycopersicum* CGN14330 cv. Moneymaker. Cleft grafting method was applied. The following characteristics of grafted and non-grafted plants were recorded: TYLCV mean score, number of days to symptoms appearance, fruit set percentage, average fruit weight, early and total yield, fruit contents of TSS, vitamin C and titratable acidity and pH value. Results showed that grafting increased TYLCV-tolerance in susceptible plants, expressed as delay in the appearance of TYLCV symptoms and an increase of yield components compared to non-grafted plants. Grafting TYLCV-tolerant scion 'TH99806' onto wild species *S. chilense*, *S. habrochaites* and *S. pennellii* remarkably lowered early and total yield in comparison to grafting onto other rootstocks due to a reduction in fruit set percentage. The related species *S. pimpinellifolium* is considered a suitable tolerant rootstock for tomato grafting to improve TYLCV-tolerance, fruit yield and quality.

Key words: Tomato • Grafting • TYLCV • TSS • pH • Yield

INTRODUCTION

Tomato yellow leaf curl disease (TYLCD) is one of the most devastating diseases of cultivated tomatoes in tropical and subtropical regions, including Egypt. yield losses ranged between 28.4% to 92.3% and reached 100% in some reports, according to the age of the plant at the time of infection and environmental conditions [1-2]. The management of TYLCD in tomato is difficult, expensive and with limited options. The best way to reduce yield losses inflicted by TYLCD and to reduce the spread of the virus is by the use of virus-resistant tomato cvs, as their use is perhaps the easiest, safest, most practical and best environment-friendly method for controlling this viral disease [2 - 4]. No resistance to TYLCV was found in cultivated *S. lycopersicum* [2, 5] but was identified in some accessions of wild species, viz. *S. galapagense*, *S. chilense*, *S. habrochaites*, *S. pennellii*, *S. peruvianum* and *S. pimpinellifolium* [6]. Efforts to introduce this resistance to commercial cultivars through conventional plant breeding techniques are underway, but progress in breeding has been relatively slow, due to the complicated genetics of resistance to TYLCV,

difficulties in interspecific crosses between wild species, especially between each of *S. chilense* and *S. peruvianum* and cultivated tomato and agronomic traits that must be recovered from susceptible tomato cultivars to satisfy consumer preferences and industrial demand. Therefore, short term alternative methods for deploying this virus resistance in tomato are desirable. Grafting of commercial tomato cvs onto selected resistant rootstocks could be a promising tool as a rapid alternative to the relatively slow breeding methods intended for improving resistance and tomato fruit yield and quality.

Grafting early purpose was to manage the soilborne disease fusarium wilt (*Fusarium oxysporum*) on watermelon but the reasons for grafting, as well as the kinds of vegetables grafted, have increased dramatically [7]. Grafting is now common in many parts of the world not only to manage soil-borne diseases but also to improve fruit quality and to improve crop response to abiotic stresses such as salinity, drought, flooding and heat and cold stress [8 - 11]. The use of grafting in vegetable production systems has expanded to manage a broad range of pathogens including fungi, oomycetes, bacteria, nematodes and viruses [12 - 15]. Few studies

have shown potential for management of viruses through the use of vigorous and resistant rootstocks. Jenns and Kuc [16] found that systemic resistance of tobacco necrosis virus (TNV) was transmitted by grafting on resistant rootstock. Wang *et al.* [17] reported an increased tolerance in seedless watermelons to virus complexes, presumed to include cucumber mosaic virus (CMV), watermelon mosaic virus II (WMV-II), papaya ringspot virus (PRSV), or zucchini yellows mosaic virus (ZYMV). Increased tolerance to TYLCV has also been reported on grafted tomato plants [15]. It is presumed in each case that the resistance is the result of increased vigor provided by the rootstock, which allows the scion to continue to grow in the presence of the virus. Even the scion infection with certain viruses such as TMV races could be markedly influenced by virus resistant rootstocks depending upon the level of resistance in scion and rootstocks [9].

Many researchers reported that an interaction between rootstocks and scions exists resulting in high vigor of the root system and greater water and mineral uptake leading to increased yield and fruit enhancement [18 - 22]. Chung and Lee [23] found that tomato marketable yield increased by up to 54% with 'kagemusia' and 51% with 'Helper' rootstocks and also abnormal fruits significantly decreased. Qaryouti *et al.* [24] reported that tomato fruit yield of cv. Cecilia grafted on 'He-Man' and 'Spirit' increased by 12-27% in soilless culture and by 16-38% in soil culture, respectively. Also, Ibrahim *et al.* [25] stated that the total yield of grafted plants was higher by 11.9-12.41% than non-grafted plants. Pogonyi *et al.* [21] reported that the increase of yield of grafted plants was caused mainly by higher average fruit weight. On the contrary, Romano and Paratore [26] stated that vegetable grafting does not improve the yield when the selection of the rootstock is not suitable. For example, the self-grafted cv. Rita had a lower yield than the non-grafted plants. Also, Ntatsi *et al.* [27] found that grafting 'Kommeet' onto cold-tolerant *S. habrochaites* LA1777 significantly suppressed fruit yield as a result of reduced fruit number per plant.

There are some contradictory results about how grafting affects fruit quality traits. For example, Traka-Mavrona *et al.* [28] reported that the solutes associated with fruit quality are translocated in the scion through the xylem, whereas Lee [18] reported that quality traits (fruit shape, skin color, skin or rind smoothness, flesh texture and color and soluble solids concentration) are influenced by the rootstock. Grafting on the cultivated tomato and wild tomato *S. cheesmaniae* rootstocks

improved soluble solids concentration and titratable acidity [29]. In contrast, other researchers showed that grafting did not affect fruit quality [26]. Khah *et al.* [30] found that soluble solids, titratable acidity and lycopene contents and pH value in hybrid tomato fruits were not affected by grafting. Pogonyi *et al.* [21] reported that grafting non-significantly decreased soluble solids concentration, titratable acidity and carbohydrate contents of fruit. Also, Qaryouti *et al.* [24] reported that total soluble solids, antioxidant capacities, vitamin C, lycopene and β -carotene contents in tomato fruit were reduced or slightly improved by grafting. However, fruit size and shelf life were not affected and fruit firmness improved by grafting. Neocleous [31] observed that grafting did not affect yield, leaf nutrient status, or fruit quality. These findings are of great importance because they show that grafting is a rapid and efficient mean for improving fruit quality.

The influence of grafting on TYLCV-resistance in tomato plants has not yet been studied in detail. The purpose of this study was to investigate possible positive effects of grafting and use of different TYLCV-resistant rootstocks on the tolerance/resistance level, fruit yield and quality traits of tomato plants.

MATERIALS AND METHODS

Plant Materials: The experiment was conducted during the 2012 and 2013 fall plantings at private land in El-Khalidiya village, Ibshiwiaa and vegetable nursery at Com-Oshim, El-Fayoum Governorate, Egypt. Four TYLCV-resistant tomato wild accessions, *viz.* *S. habrochaites* LA1777, *S. pennellii* LA716, *S. chilense* LA2779 (Tomato Genetic Resources Center - TGRC, University of California, Davis, USA) and *S. pimpinellifolium* PI211840 (USDA-ARS, Plant Introduction Station, Ames, Iowa, USA) [6]. In addition to TYLCV-susceptible *S. lycopersicum* CGN14330 cv. Moneymaker (Centre for Genetic Resources the Netherlands – CGN, Wageningen University, the Netherlands) were used as rootstocks, while, TYLCV-susceptible tomato cv. Castlerock and TYLCV-tolerant hybrid TH99806 (Nirouz, Syngenta) were used as scions.

Grafting Methods: Due to uneven emergence and seedling development, wild rootstock seeds were sown on the 1st of August, 10-20 days earlier than scion seeds and followed 5-10 days later with sowing seeds of rootstock 'Moneymaker' in speedling trays (84 cells) filled with a mixture of peat-moss and vermiculate (volume 1:1)

enriched with macro and micro elements. Seeds of scions were sown on the second half of August in speedling trays (209 cells) filled with the same previous mixture. The trays were placed in anti-virus greenhouse until full germination. Seedlings were fertilized every 2 days using commercial compound fertilizer 20:20:20 (N:P:K). Due to a limitation in the number of seeds of rootstock *S. pennellii* LA716 in the second season, stem cuttings were planted 10 days earlier than sowed scion seeds.

In anti-virus greenhouse, tongue graft was applied when the seedlings started to initiate their third true leaf as indicated by Oda [19]. The scion and the rootstock were cut off with a razor blade just above the cotyledons at a maximum of 2 cm above the medial line. Scion and rootstock were connected using a silicon-grafting clip. After grafting, both graft combinations were placed immediately into a clear, closed shady plastic tunnel of 30 cm height with inside relative humidity of nearly 100%. The exposure to direct sunlight was prevented. From day 5, the tunnel was gradually ventilated daily and the light intensity was increased till the morning of day 7 when the plastic was removed. When wilting was observed, foliar spraying of grafted plants with water was effective in improving survival. Grafts were treated for another 5 days to ensure full recovery and to inoculate with TYLCV.

The grafts were field-transplanted in a randomized complete block design (RCBD) with three replicates. Each experimental unit (EU) consisted of 2 rows, 1 m wide × 4 m long (EU area = 8 m²). Plants were set 50 cm apart and subjected to the common agricultural practices.

Inoculation and Evaluation of TYLCV: Virus infection was enhanced by natural viruliferous whitefly infestation in the nursery and in field plots. No insecticides were applied to encourage heavy infestation.

Days to observe the first TYLCD symptoms on grafted and non-grafted plants were recorded. Data on TYLCV resistance was recorded for individual plants 3 months after transplanting on a 1-5 scale, depending on the severity of TYLCV symptoms as follows: 1: no symptoms appearing on the plant, 2: slight symptoms on plant top, 3: moderate symptoms, 4: severe symptoms on the entire plant and 5: severe symptoms and plant stunting. Individual plant ratings of each accession were added and divided by the number of evaluated plants to obtain the corresponding mean disease score.

Evaluated Traits: Flower and fruit numbers per cluster (the second cluster to the fifth), fruit set percentage, average fruit weight (average weight of 15 fruits), early

yield (the first three harvests) and total yield (all the collected fruits) per plant were determined. Samples of 10 ripe fruits (from the third and fourth trusses) representing each EU were picked for analysis of fruit quality traits; viz. total soluble solids (TSS), vitamin C, titratable acidity (TA) and pH of fruit juice. An extract was obtained by blending and filtering flesh of each fruit sample. TSS was determined using a hand refractometer. TA was ascertained using 0.1 N NaOH solution and phenolphthalein as indicator [32]. Vitamin C (ascorbic acid) was measured using 2,6 dichlorophenol indophenol dye [32]. pH value was determined by immersing the glass electrode of a pH meter into juice extracted from a 200 g fruit sample per plot.

Statistical Analysis: Data were statistically analyzed using MSTAT-C v. 2.1 (Michigan State University, Michigan, USA) and mean comparisons were based on the least significant difference (LSD) test Maxwell and Delaney [33].

RESULTS AND DISCUSSION

Grafting induced significant effect on TYLCV-tolerance level in TYLCV-susceptible scion 'Castlerock', while in TYLCV-tolerant scion 'TH99806', grafting was affected on TYLCV mean score in only the second season (Table 1). Rootstock *S. pennellii* LA716 showed low TYLCV mean score with 'Castlerock' scion followed, in an ascending order, by rootstocks *S. habrochaites* LA1777, *S. chilense* LA2779 and *S. pimpinellifolium* PI211804. Non-grafted 'Castlerock' showed high TYLCV mean score followed by 'Castlerock' grafted on TYLCV-susceptible rootstock 'Moneymaker' (Table 1).

TYLCV symptoms appear 2-4 weeks after inoculation and become fully developed after a period of up to 2 months [34 - 35]. Effect of grafting on TYLCV-tolerance in scion was evident by delaying the appearance of symptoms. No. of days to appear TYLCV-symptoms was high with 'Castlerock' and 'TH99806' grafted on TYLCV-resistant rootstocks, especially *S. habrochaites* and *S. pennellii* (Table 1). Delaying the appearance of TYLCV-symptoms probably depends on reducing the viral replication rate in grafted plants on resistant rootstock.

Significant differences were observed among grafts and between grafts and the control cv. in yield component traits (Table 1). Grafting increased fruit set percentage under TYLCV-infection conditions for susceptible scion on resistant rootstock. In both seasons, 'Castlerock' grafts

Table 1: TYLCV mean score and yield components of various scion-graft combinations in the 2012 and 2013 fall plantings.

Scion	Rootstock	TYLCV mean score ^z	No. of days to appear the symptoms	Fruit set (%)	Average fruit weight (g)	Early yield (kg/plant)	Total yield (kg/plant)
Season 2012							
Castlerock	<i>S. chilense</i> LA2779	3.11	53.00	58.09	87.53	0.50	2.41
	<i>S. habrochaites</i> LA1777	3.04	58.33	55.77	87.23	0.42	2.11
	<i>S. pennellii</i> LA716	2.46	58.33	51.96	88.50	0.37	1.99
	<i>S. pimpinellifolium</i> PI211804	3.26	47.00	70.88	90.29	0.79	3.13
	<i>S. lycopersicum</i> cv. Moneymaker	3.80	31.33	37.99	84.44	0.26	1.34
Castlerock (non-graft -Control)		4.49	26.67	22.10	87.51	0.28	0.87
	LSD _{0.05}	0.44	3.38	9.67	5.67	0.13	0.34
TH99806	<i>S. chilense</i> LA2779	1.24	61.67	58.34	117.2	1.44	4.10
	<i>S. habrochaites</i> LA1777	1.23	65.67	64.12	120.9	1.68	4.84
	<i>S. pennellii</i> LA716	1.22	63.67	65.28	119.3	1.30	4.68
	<i>S. pimpinellifolium</i> PI211804	1.22	58.33	97.53	119.6	1.89	7.77
	<i>S. lycopersicum</i> cv. Moneymaker	1.4	37.67	76.41	120.5	0.98	5.52
TH99806 (non-graft - Control)		1.36	45.33	96.04	120.5	1.35	7.33
	LSD _{0.05}	NS	7.15	7.36	NS	0.18	0.54
Season 2013							
Castlerock	<i>S. chilense</i> LA2779	3.12	53.67	54.39	88.57	0.49	2.36
	<i>S. habrochaites</i> LA1777	2.86	58.33	54.69	86.80	0.48	2.10
	<i>S. pennellii</i> LA716	2.28	58.33	50.98	89.77	0.38	2.02
	<i>S. pimpinellifolium</i> PI211804	2.99	47.00	65.36	88.52	0.77	3.23
	<i>S. lycopersicum</i> cv. Moneymaker	3.74	31.33	39.22	82.49	0.24	1.37
Castlerock (non-graft - control)		4.60	26.67	23.52	87.76	0.28	0.94
	LSD _{0.05}	0.37	3.38	8.87	4.65	0.12	0.36
TH99806	<i>S. chilense</i> LA2779	1.22	61.67	60.33	118.9	1.42	4.36
	<i>S. habrochaites</i> LA1777	1.18	65.67	64.56	120.5	1.66	4.93
	<i>S. pennellii</i> LA716	1.17	63.67	66.67	121.3	1.37	4.85
	<i>S. pimpinellifolium</i> PI211804	1.16	58.33	98.77	119.2	1.97	7.75
	<i>S. lycopersicum</i> cv. Moneymaker	1.40	37.67	79.55	120.2	0.94	5.81
TH99806 (non-graft - control)		1.42	45.33	96.10	120.5	1.38	7.43
	LSD _{0.05}	0.01	7.15	8.01	NS	0.163	0.51

^zDisase scores: 1, symptomless; 2, slight; 3: moderate; 4: severe symptoms and 5: very severe symptoms.

on *S. pimpinellifolium* gave the highest significant percentage of fruit set followed by grafts on *S. chilense*, *S. habrochaites* and *S. pennellii* (Table1). Meanwhile, with TYLCV-tolerant scion 'TH99806', grafting did not exert a significant effect on fruit set percentage. Grafts on *S. pimpinellifolium* gave the highest percentage, but it was not significantly different from the non-grafted control 'TH99806' (Table 1).

Grafting had a limited impact on average fruit weight of susceptible scion, while there were no significant differences between the grafted and non-grafted tolerant scion (Table 1). The early and total yield were affected bygrafting, especially with susceptible scion, while with resistant scion, grafting on some of wild accessions was reduced the early and total yield as a result of reduced fruit set percentage with not increasing in average fruit weight (Table 1). Generally, grafting of 'Castlerock' and

'TH99806' on rootstock *S. pimpinellifolium* had the highest values of fruit set percentage and early and total yield (Table 1).

Accordingly, grafting, especially on wild TYLCV-resistant rootstocks, increased TYLCV-tolerance in susceptible plants. This is in agreement with findings of Rivero *et al.* [15]. It is presumed in each case that tolerance is the result of increased vigor provided by the rootstock, which allows the scion to continue to grow in the presence of the virus. Therefore, the increase of TYLCV-tolerance in susceptible scion shows in delayed appearance of TYLCV symptoms and an increase in yield components as compared with non-grafted plants. With tolerant-scion, grafting didn't affect TYLCV-tolerance level and didn't increase yield components. Rather, grafting decreased early and total yield of grafted plants compared with the non-grafted plants (Table 1).

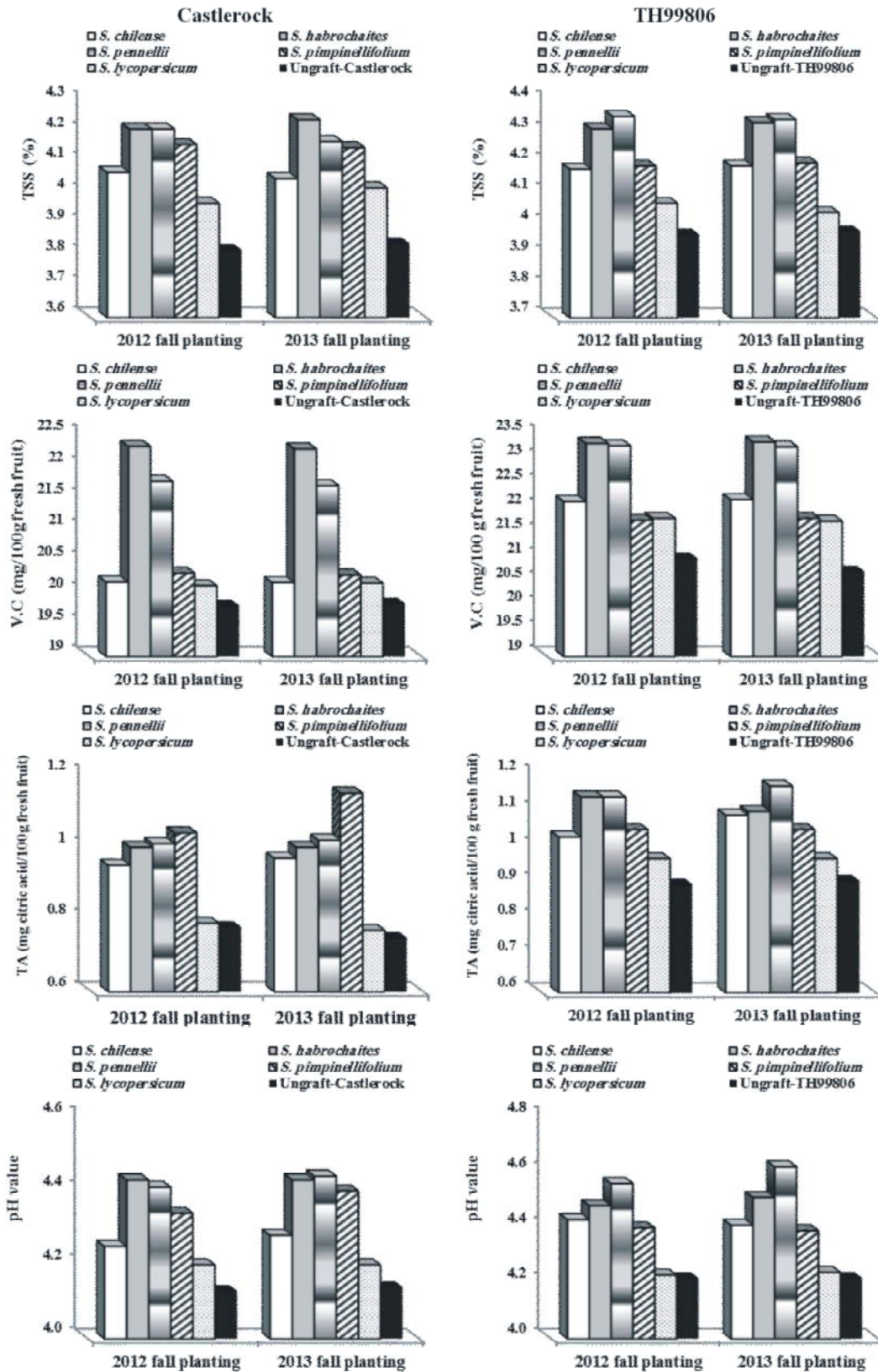


Fig. 1: Fruit quality characters of various scion-graft combinations in the 2012 and 2013 fall plantings.

Therefore, the wild resistant rootstocks *S. chilense* LA2779, *S. habrochaites* LA1777 and *S. pennellii* LA716 are not suitable rootstocks for tomato grafting. These results are in agreement with those obtained by Romano and Paratore [26]. The decrease in yield components of grafted tolerant scion on those wild rootstocks was due to the influence of the rootstock on fruit set percentage compared with fruit set in non-grafted plants. These results coincided with those obtained by Ntatsi *et al.* [27] who reported that grafting on *S. habrochaites* LA1777 rootstock significantly suppressed fruit yield of scion as a result of reducing fruit number per plant while not influencing flower number per plant and mean fruit mass. Negative effect of wild rootstocks on fruit setting points to impairment of pollen fertility indicating signals originating from the root.

Grafting improved fruit content of TSS, vitamin C and titratable acidity and pH value (Fig. 1). Generally, grafting on wild accessions of *S. habrochaites* and *S. pennellii* gave the highest values of TSS, vitamin C, titratable acidity and pH, while grafting on related tomato *S. pimpinellifolium* gave acceptable results of fruit quality than in case of non-grafted plants. These results confirm previous report by Flores *et al.* [29] concerning the improvement of fruit quality by grafting on wild species *S. cheemaniae*.

Several reports mentioned that an interaction between rootstocks and scions exists resulting in high vigor of the root system and greater water and mineral uptake leading to increased yield and fruit enhancement [18 - 22]. But our results showed a reduction yield (Table 1). Therefore, high vigor of grafted plants leads to increasing fruit quality (Fig. 1).

CONCLUSION

Overall, it is concluded that grafting on resistant rootstocks improved TYLCV-tolerance in susceptible tomato scions and delayed appearance of TYLCV symptoms. However, yield components were negatively affected by grafting on wild accessions of *S. chilense*, *S. habrochaites* and *S. pennellii*, as they decreased fruit set percentage but without affecting average fruit weight. Fruit quality traits were also affected by grafting. *S. pimpinellifolium* is considered a suitable tolerant-rootstock for used in tomato grafting to improving tolerance and yield.

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