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Control of Fusarium Crown and Root Rot of Tomato, Caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici*, by Grafting onto Resistant Rootstocks

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Abstract: In this study, the efficacy of grafting two tomato cultivars onto two rootstocks was examined in growth chamber and in greenhouse conditions. The rootstock cultivars Beaufort F_1 and He-Man F_1, already known and confirmed as resistant to *Fusarium oxysporum* f. sp. *radicis-lycopersici*, were evaluated during two crop seasons under greenhouse heated with geothermal water in South Tunisia. The cv. Durintha F_1 showed the best plant growth, fruit yield and fruit quality when grafted onto Beaufort F_1; while cv. Bochra F_1 gave the best results when grafted onto He-Man F_1. This study demonstrated that grafting tomato cultivars onto Fusarium resistant rootstocks is one of the best alternatives for controlling Fusarium crown and root rot of tomato.

Key words: Tomato, rootstocks, disease resistance, yield, fruit quality

INTRODUCTION

Fusarium Crown and Root Rot of tomato (FCRR) induced by *Fusarium oxysporum* Schlect f. sp. *radicis-lycopersici* Jarvis and Shomaker (FORL) is one of the most damaging soil-borne diseases of tomato causing heavy economic losses on plant grown in sterilized soils (Rekah *et al*., 1999). This disease newly recorded in Tunisia, during 2000-2001 crop season (Hajlaoui *et al*., 2001; Hibar, 2002), caused heavy losses reaching 90% of plants in some geothermal greenhouses. The speed and extent with which the pathogen, including fungi, bacteria and nematodes, can disseminate and infect plants may explain why natural disease resistance is seldom monitored (Benhamou *et al*., 1997; Pharand *et al*., 2002). Although some cultivars with single dominant genes for resistance have been developed, control of FCRR is mainly restricted to eliminating the pathogen in soil by steaming or fumigating with chemicals and by planting pathogen-free seeds or transplants (Sivan *et al*., 1987). However, complete eradication of the fungus from soil has never been achieved, due in part to the appearance of fungicide-resistant strains in the pathogen population.

The difficulties in controlling FCRR have promoted scientists to search for other alternatives that are efficient, reliable and safe for environment (Caron *et al*., 1986; Sivan and Chet, 1993; Sivan *et al*., 1987).

Tomato grafting, used to be too expensive, is now widely used in various regions of the Mediterranean Basin. In Tunisia, grafting is popular for watermelon and melon; however, grafting tomatoes onto resistant rootstocks is only currently employed in a very low percentage, perhaps due mainly to the high labor cost for grafting.

In this study two rootstocks were evaluated under growth chamber and greenhouse conditions as potential sources for grafting commercial tomato hybrids to protect against Fusarium crown and root rot of tomato.

MATERIALS AND METHODS

Plant material: Two tomato (*Lycopersicon esculentum* Mill. 'Priscas') cultivars, Durintha F_1 and Bochra F_1, were used as scions and two commercial hybrids (Lycopersicon hirsutum×*L. esculentum*) Beaufort F_1 (De Ruiter) and He-Man F_1 (Syngenta) were used as rootstocks.

These two rootstocks were chosen because already known as resistant to Fusarium (Table 1). Tomato cultivars (Table 2) were used as scion because they represent 70% (10 ha) of the total covered area reserved to tomato culture in "5ème saison" exploitation located in Hammet Gabes, in South Tunisia, where Fusarium crown and root rot causes serious problems to tomato.

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Table 1: Characteristics of the two tomato rootstocks used in this study (Carrier, 2003)

Rootstocks	Resistance	Remarks
Beaufort F_1 (De Ruiter)	TmC_5KNVF_2Fr	Very used Non uniform germination
He-Man F_1 (Syngenta)	TmC_3NVF_2Fr	Tolerant to Corky root Less vigorous than Beaufort Germinates more uniform than Beaufort.

C_3: *Cladosporium fulvum* races A, B and C, C_5: *Cladosporium fulvum* races A, B, C, D and E, F_2: *Fusarium oxysporum* f.sp. *lycopercisi races* 1 and 2, Fr: *Fusarium oxysporum* f. sp. *radicis-lycopersici* (crown rot), K: *Pyrenochæta lycopersici* (Corky Root), N: Nematodes most common species, Tm: Tomato Mosaic Virus, V: *Verticillium* spp.

Table 2: Characteristics of the two tomato cultivars used as scions

Cultivar	Resistance	Fruit form	Growth
Bochra F_1	$TmVF_2$	Oblong	Indeterminate
Durintha F_1	$TmVF_2$	Round	Indeterminate

F_2: *Fusarium oxysporum* f.sp. *lycopercisi* races 1 and 2, Tm: Tomato Mosaic Virus, V: *Verticillium* spp.

Testing rootstock resistance to Fusarium crown and root rot of tomato under growth chamber conditions: The resistance to Fusarium of rootstock and scion cultivars utilised in this research were evaluated in growth chamber experiment on organic substrate (peat) artificially inoculated with FORL. The experiment was conduced in the glace house of the Horticultural High School of Chott-Mariem. Seeds were sown in seedling trays filled with previously sterilized peat. Young plants at the three-to four-true-leaf stage were removed from the peat; their roots were washed with sterilized water and then plants were transplanted into inoculated peat. The substrate (peat) was artificially inoculated with a 10^7 mL^{-1} spore suspension of one of four single-spore isolates (Fo2.01, Fo4.02, Fo1.03 or Fo1.04) of FORL 1 h before planting (Vakalounakis et Fragkiadakis, 1999). Plants were kept in growth chamber for 30 days at 23°C with a 12 h daylength (Woo *et al*., 1996), using a completely randomised design with 10 replicates per treatment. Tomato plants of susceptible cvs Durintha F_1 and Bochra F_1 treated similarly and transplanted into sterilized un-inoculated substrate served as controls. Plants were watered daily and no fertilizers were applied. The experiment was conducted twice.

Plants were classified either as resistant when they were healthy (no disease symptoms) or susceptible when they were dead or almost dead (Pavlou *et al*., 2002).

Rootstock evaluations for grafting tomatoes under greenhouse conditions: To investigate grafting compatibility (expressed as tomato plant growth, fruit yield and fruit quality) between cvs Bochra F_1 and Durintha F_1 and the two rootstocks Beaufort F_1 and He-Man F_1, one greenhouse experiment carried out in 2002-03 was repeated in 2003-04 crop season at the "5ème Saison" exploitation, situated in Hammet Gabes in South Tunisia where we have detected FORL for the first time in Tunisia (Hajlaoui *et al*., 2001; Hibar, 2002). The scion/rootstock combinations Durintha F_1/Beaufort F_1, Bochra F_1/Beaufort F_1, Durintha F_1/He-Man F_1, Bochra F_1/He-Man F_1 were evaluated with respect to plant growth, fruit production and fruit quality. Seeds of the four cultivars were sown in seedling trays filled with disinfected peat and kept in the growth chamber heated with geothermal water. Temperature fluctuated between 20 and 30°C and relative humidity between 80 and 90%. Seedlings of rootstock cultivars with 3-4 true leaves were cut over the cotyledons and immediately grafted with shoots of scion cultivars. Grafting clips were used to adhere the graft union (Santa-Cruz *et al*., 2002). Un-grafted plants of Bochra F_1 and Durintha F_1 were included as controls. Grafted and un-grafted seedlings were covered with a transparent plastic film to maintain high humidity level and to avoid leaf dehydration (Fernandez-Garcia *et al*., 2002).

Grafted plants were transferred from growth chamber to polyethylene covered greenhouses, heated with geothermal water. Soilless culture was adopted in sausage bags filled with perlite infested with FORL and using a drip irrigation system. The (scion/rootstock) combinations Durintha F_1/Beaufort, Bochra F_1/Beaufort F_1, Durintha F_1/He-Man F_1 and Bochra F_1/He-Man F_1 were planted in four greenhouses (one greenhouse for each combination) with a density of 2.5 stem m^{-2} (2 stems per plant), while un-grafted plants of Bochra F_1 and Durintha F_1 used as control were transplanted into sterilized perlite and grown in two other greenhouses. Plants in all greenhouses were treated similarly and grown according to local horticultural practices.

In 2003-04 crop season seeds of rootstocks, scions and un-grafted cultivars were sown on 10 August, 20 August and 30 August 2003, respectively, plants were transplanted to the greenhouses on 20 September 2003. In the following crop season sowing and plantation occurred 20 days earlier. From ten randomly selected plants per row (four rows per treatment) of each greenhouse, the following data were recorded: stem diameter (mm) and growth speed (cm j^{-1}) of plant, pH of fruit juice, soluble solids (°Brix), electric conductivity (EC) (ms), fruit weight (g/fruit), fruit caliber (mm) and total yield (kg/plant). All data were taken after seven months from the plantation date.

Variance analysis of the treatment effect on measured data was performed by using the general linear model procedure of SPSS (SPSS 10.0) with trials and replications treated as random effects and grafting combinations as fixed effects.

When F values were significant at p>0.05, differences among the treatments were determined by SNK (Student Newman Keuls) test.

RESULTS

Evaluation of rootstock resistance to Fusarium crown and root rot of tomato: Under growth chamber conditions, the two commercial rootstocks Beaufort F_1 and He-Man F_1 were found to be resistant to FORL as no disease symptoms were observed. In contrast, tomato cvs Bochra F_1 and Durintha F_1 were considered susceptible because all plants were dead (Table 3).

Rootstock resistance and effect on plant growth, fruit yield and quality of the used tomato cultivars: In both crop seasons, stem diameter and growth speed of plants; soluble solids concentration, EC, mean weight and size of fruit; total yield of cv Bochra F_1 grafted onto He-Man F_1 were higher respect to plants of cv Bochra F_1 grafted onto the rootstock Beaufort F_1, as well as of the un-grafted (self-rooted) plants cv Bochra F_1 (control) (Table 4a). The pH of fruit was lower for the same combination (Bochra F_1/He-Man F_1) and this was an additional criterion of fruit quality.

For cv Durintha F_1 the best values of all measured parameters, were obtained when grafted onto the rootstock Beaufort F_1. However; stem diameter, growth speed, soluble solids, EC, fruit weight, fruit size and yield of cv Durintha F_1 grafted onto the rootstock Beaufort F_1 were higher than those of cv Durintha F_1 grafted onto the rootstock He-man F_1, as well as of the un-grafted (self-rooted) plants cv Durintha F_1 (control) (Table 4b). The pH of fruit was lower for the same combination (DurinthaF_1/Beaufort F_1) which was also an additional criterion of fruit quality. These results were obtained in 2003-04 crop season and confirmed in the next crop season.

Table 3: Evaluation of the two rootstocks for resistance to four isolates of *Fusarium oxysporum* f. sp. *radicis-lycopersici* under growth chamber conditions

		Disease reaction[y]			
Commercial name	Species	Fo2.01	Fo4.02	Fo1.03	Fo1.04
Beaufort F_1	*Lycopersicon hirsutum* ×*L. esculentum*	R	R	R	R
He-Man F_1	*Lycopersicon hirsutum* ×*L. esculentum*	R	R	R	R
Bochra F_1 (control)	*Lycopersicon esculentum*	S	S	S	S
Durintha F_1 (control)	*Lycopersicon esculentum*	S	S	S	S

[y]R = Resistant reaction, healthy plants; S = Susceptible reaction, dead plants. The growth chamber experiment was conduced twice with 10 plants per tomato species and per Fusarium isolates

Table 4a: Growth parameters, fruit quality and total yield of tomato plants of the cv. Bochra F_1 grafted onto two rootstocks (Beaufort F_1 and He-Man F1) in two greenhouse experiments carried out during 2003-04 and 2004-05 crop season at the '5ème saison" exploitation in Hammet Gabes[y] in south Tunisia

	Bochra F_1/ Beaufort F_1	Bochra F_1/ He-Man F_1	Un-grafted Bochra F_1
2003-04 crop season			
Stem diameter (mm)[z]	7.230b	7.440b	6.290a
Growth speed (cm j^{-1})[z]	2.735ab	2.971b	2.511a
pH of fruit juice[z]	4.592b	4.171a	4.175a
Soluble solids (°Brix)[z]	5.210a	5.760b	5.340a
EC (ms)[z]	4.337b	4.393b	4.177a
Fruit weight (g/fruit)[z]	98.130b	102.100b	91.440a
Fruit caliber (mm)[z]	53.400a	55.400a	52.790a
Total yield (kg/plant)[z]	11.290b	11.760b	10.500a
2004-05 crop season			
Stem diameter (mm)[z]	7.250b	7.500c	6.320a
Growth speed (cm j^{-1})[z]	2.760b	2.952c	2.621a
pH of fruit juice[z]	4.576b	4.243a	4.325a
Soluble solids (°Brix)[z]	5.240a	5.810b	5.290a
EC (ms)[z]	4.326ab	4.421b	4.211a
Fruit weight (g/fruit)[z]	98.250b	101.800c	93.439a
Fruit caliber (mm)[z]	53.300a	55.340b	53.213a
Total yield (kg/plant)[z]	11.500b	11.850c	10.250a

[y]In the experiments of both crop seasons there were Four replicates (four rows per greenhouse) of ten plants per each treatment, [z]Within lines, means followed by the same letters are not significantly different (p = 0.05) according to SNK test

Table 4b: Growth parameters, fruit quality and total yield of tomato plants of the cv. Durintha F_1 grafted onto two rootstocks (Beaufort F_1 and He-Man F_1) in two greenhouse experiments carried out during 2003-04 and 2004-05 crop season at the '5ème saison" exploitation in Hammet Gabesy in south Tunisia

	Durintha F_1/ Beaufort F_1	Durintha F_1/ He-Man F_1	Un-grafted Durintha F_1
2003-04 crop season			
Stem diameter (mm)[z]	12.000b	10.200a	10.100a
Growth speed (cm j^{-1})[z]	3.070b	3.010b	2.880a
pH of fruit juice[z]	4.250a	4.380c	4.290b
Soluble solids (°Brix)[z]	4.360b	4.350b	4.290a
EC (ms)[z]	4.850a	4.840a	4.840a
Fruit weight (g/fruit)[z]	121.650b	115.950a	115.700a
Fruit caliber (mm)[z]	64.330a	60.290a	58.310a
Total yield (kg/plant)[z]	11.620c	10.650b	9.850a
2004-05 crop season			
Stem diameter (mm)[z]	11.850b	11.750b	10.120a
Growth speed (cm j^{-1})[z]	3.102b	2.980a	2.980a
pH of fruit juice[z]	4.185a	4.250b	4.180a
Soluble solids (°Brix)[z]	4.421c	4.380b	4.320a
EC (ms)[z]	4.954b	4.910a	4.890a
Fruit weight (g/fruit)[z]	122.250c	116.850b	112.950a
Fruit caliber (mm)[z]	64.250c	62.150b	59.120a
Total yield (kg/plant)[z]	11.950c	10.950b	10.150a

[y]In the experiments of both crop seasons there were four replicates (four rows per greenhouse) of ten plants per each treatment, [z]Within lines, means followed by the same letters are not significantly different (p = 0.05) according to SNK test

Basing on these results, the rootstock He-Man F_1 seemed to be more appropriate for tomato plants cv Bochra F_1; however, for tomato plants cv Durintha F_1, the rootstock Beaufort F_1 seems to be the best one.

Also, in both greenhouse experiments, none of the grafted plants were infected by FORL confirming the resistance of these rootstocks to this pathogen.

DISCUSSION

In the past, grafting tomato plants was considered too expensive but at present it is adopted at a commercial level in Tunisia and in many countries. Resistant rootstocks provide excellent control of many tomato soil borne pathogens and particularly of *F. oxysporum* f. sp. *lycopersici, F. oxysporum* f. sp. *radicis-lycopersici*, *Pyrenochaeta lycopersici* and *Meloidogyne* spp. In addition, tomato grafting gave others advantages such as plant growth promotion, yield increase, extension of yield period and improvement of fruit quality (Rivero *et al*., 2003).

Since Fusarium crown and root rot (*F. oxysporum* f. sp. *radicis-lycopersici*) is a new disease of tomato (Hajlaoui *et al*., 2001; Hibar, 2002), the possible use of grafting tomato onto resistant rootstocks to protect against this disease has not yet examined. In our growth chamber and greenhouse experiments, it was found that the two rootstocks, Beaufort F_1 and He-Man F_1 were resistant to FORL; therefore they could be used as rootstocks for grafting tomatoes to protect against Fusarium crown and root rot.

Data obtained from this study suggest that grafting the susceptible tomatoes cv Bochra F_1 and cv Durintha is an effective control measure against Fusarium crown and root rot. Similar results were reported by Trionfetti *et al.* (2002) and Miguel *et al.* (2004) on controlling Fusarium wilt by grafting two muskmelon cultivars and triploid watermelon respectively onto commercial rootstocks. Grafting was also effective in controlling some other pathogens such as sudden wilt in melons caused by *Monosporascus cannonballus* (Edelstein *et al*., 1999) and sclerotinia stem rot of soybean caused by *Sclerotinia sclerotiorum* (Vuong and Hartman, 2003).

Present results have shown that grafting susceptible tomatoes cvs Bochra F_1 and Durintha F_1 onto the rootstocks Beaufort F_1 and He-Man F_1 increase tomato growth, tomato yield and improve fruit quality. This increase in tomato yield through the use of grafted plants can be attributed mainly to disease control and secondly to better plant growth. Increased plant growth responses are a well-known phenomenon in grafted plants (Ibrahim *et al*., 2001; Santa-Cruz *et al*., 2002). In grafted plants, the rootstock's vigorous root system is often capable of absorbing water and nutrients more efficiently compared to the un-grafted plant and may serve as a good supplier of endogenous plant hormones (Fernandez-Garcia *et al*., 2002; Estan *et al*., 2005). However, the rootstock effect varies greatly with scion cultivar and growing season (Lee, 1994). An increased growth effect have observed by Pavlou *et al.* (2002) by grafting commercial Dutch type cucumber hybrids onto various resistant *Cucurbita* rootstocks; indeed, total fruit yield of cucumber plants cv Brunex F_1 grafted onto all rootstocks tested (A27, *Cucurbita fucifolia*, Patron, Peto 42.91, TS-1358 and TZ-148) was greater than that of the un-grafted (self-rooted) plants cv Brunex F_1 (control).

However the results shown by Trionfetti *et al.* (2002), by evaluating the potential of grafting for resistance to *F. oxysporum* f. sp. *melonis* on 13 commercial melon rootstocks and various *Cucurbitaceae* spp. and determining productivity and fruit quality characteristics of grafting on resistant rootstocks, suggested that yield and quality attributes of scion cultivars (Supermarket and Proteo) grafted on P360 and PGM 96-05 rootstocks were not improved relative to un-grafted controls.

CONCLUSIONS

On the basis of the results obtained in these experiments on tomato, grafting effectiveness seems to be determined not only by disease resistance of the rootstocks but also by their influence on both production and fruit quality. The rootstock Beaufort F_1, resistant to FORL, was also the best genotype capable of significantly improving the productivity and fruit quality of tomatoes cv. Durinth F_1; whereas, the rootstock He-Man F_1, also resistant to FORL, seemed to be more suitable for tomatoes cv Bochra F_1. Moreover and regardless of the used tomato cultivars, grafted plants have procreate the best results, concerning plant growth, fruit yield and fruit quality, compared to un-grafted (self-rooted) plants cv. Durintha F_1 and cv Bochra F_1 (controls).

However, grafting tomato cultivars onto resistant rootstocks are more expensive, since both scions and rootstocks are expensive hybrids. In addition, the development of grafted plants requires more time, materials, space, a high level of expertise, improved cultivation methods and expensive postgraft handling. But, actually in Tunisia, grafting is expected to increase significantly despite the high cost of labor and supplies, since it is one of the best alternative effective control methods found up to now for Fusarium.

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