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Evaluation of grafting effect on tomato crop yield and *Fusarium* crown and root rot disease

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Abstract

Tomato, *Lycospersicon esculentum*, is an important vegetable crop in Tunisia and many other Mediterranean countries. *Fusarium* crown and root rot of tomato are new diseases in the area, first reported during 2000-2001 crop season, threatening tomato production. Being a soil-borne pathogen, effective disease control methods of *Fusarium* crown and root rot are limited thus requiring the alternative measures for disease management. In this study the efficacy of grafting commercial Tomato cultivars Bochra and Amal, used as scions, onto a new rootstock Beaufort and Kemerit RZ was examined in controlled and natural conditions. Grafting was found, in this study, to be an effective method to attenuate the impact of *Fusarium* wilt, *Fusarium* crown and root rot. Moreover, grafting increased tomato growth parameters, yield and improved fruit quality.

Key words: Tomato, *Lycospersicon esculentum,* graft, *Fusarium* crown and root rot, grafting, Beaufort x Bochra, Beaufort x Amal, Kemerit x Bochra and Kemerit x Amal, rootstock

Introduction

Tomato, *Lycospersicon esculentum* Mill. (Solanaceae), is one of the world's most important vegetable crops with a worldwide fresh weight production of 80 million tons from a cropped area of about 3 millions hectares (FAO, 2005). The fruit improves the supply of vitamins and minerals in human nutrition (Sabongari and Aliero, 2004). *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*), is one of the most devastating diseases of tomato (Walker, 1971). *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*FORL*) which induced *Fusarium* crown and root rot of tomato is also an important and widespread vascular wilt pathogen of tomato (Jarvis, 1989; Jarvis and Shoemaker, 1978). Its epidemiology and geographical distribution have been studied extensively (Jarvis, 1977). In Tunisia, it was first reported during 2000-2001 crop season in some tomato geothermal greenhouses (Hajlaoui *et al.,* 2001). Although Fusarium wilt-resistant tomato cultivars have been developed, none of them have been widely used in disease control (Thibodeau and Simard, 1978).

For many years, control of Fusarium crown and root rot of tomato has been limited to the use of conventional soil sterilizing procedures together with the application of fungicides (Rowe and Farley, 1981). However, complete eradication of *FORL* from soil by steam-sterilization and fumigation with chemicals could not be achieved (Jarvis and Thorpe, 1980). Therefore, there is an urgent need to find alternatives that may protect plants from soilborne fungal pathogens, especially in covered crops. Tomato grafting is now widely used in various areas of the Mediterranean countries. In Tunisia, grafting is common for watermelon and muskmelon, but grafting tomatoes onto resistant rootstocks is very limited. The aim of the present study was to evaluate the resistance of tomato plants to *Fusarium* spp., so that they could be used as rootstocks for solanaceous plants.

Materials and methods

Plant material: Two tomato rootstocks, Beaufort and Kemerit RZ, as well as two tomato cultivars, Bochra and Amal, used as scions, were considered for this study (Table 1). The grafted plants tested were Beaufort x Bochra, Beaufort x Amal, Kemerit x Bochra and Kemerit x Amal.

Table 1. Characteristics of tomato cultivars (scions) and rootstocks used in this study

Cultivars or rootstocks	Resistance	Fruit form	Growth
Bochra	TMV, V, F_2	Oblong	Indeterminate
Amal	ASC, F_1, F_2, N, ST, V	Elongate	Vigorous
Beaufort (De Ruiter)	TMV, K, N, V, F_2, Fr	-	-
Kemerit RZ	TMV, V, F_2	-	-

TMV: Tomato mosaic virus, ASC: Alternaria stem canker, ST: Stemphilium, V: *Verticillium* spp., F_1, F_2: *Fusarium oxysporum* f. sp. *lycopersici* races 1 and 2. Fr: *Fusarium oxysporum* f. sp. *radicis-lycopersici*, K: *Pyrenochaeta lycopersici* (Corky root), N: Nematodes (most common species).

Fungal isolates: Two isolates of *Fusarium* were used to evaluate the reaction of tested tomato rootstocks and cultivars to *Fusarium* crown and root rot disease. Isolate of *F. oxysporum* f. sp. *lycopersici* named *FOL* and an isolate of *F. oxysporum* f. sp. *radicis-lycopersici* abbreviated as *FORL*.

Root-dip inoculation: Root-dip inoculation was performed with seedlings showing 3 to 5 expanded leaves. Regular surveys were carried out to detect the eventual presence of fungal diseases.

Tomato rootstocks and scions resistance to *Fusarium* wilt: Resistance of rootstocks and scion cultivars to *Fusarium* spp. affecting tomato, were evaluated upon artificial inoculation in growth chamber on peat substrate.

Evaluation of agronomic parameters in controlled conditions: To evaluate the effect of grafting on plant development, some parameters were measured to compare the behaviour of grafted and non-grafted plants: i) Number of leaves: this parameter was recorded on 10 seedlings in each kind of grafted plants and non-grafted ones. The counting of number of leaves was carried out after 15 days of grafting then this operation was repeated each week during one month. ii) Stem height: it was measured from the collar to until the apical bud of the seedlings. iii) Diameter of the stem: this parameter enabled to detect the effect of the grafting on the strength of the seedlings.

Evaluation of agronomic parameters in natural conditions: The grafted seedlings were planted in greenhouse in October in twinned lines, with mulching and on each line there were grafted seedlings on 1 arm, others on 2 arms and non grafted seedlings considered as controls. Agronomic parameters were evaluated to compare grafted and non grafted tomato plants as listed below: i) Tomato yield was recorded on selected plants in each line. Yield of 24 plants was evaluated for grafted plants with 2 arms and 24 plants for grafted plants with 1 arm and on the same number for control. The yield was estimated by weighing the fruits for each harvest. ii) The distance between the ground and the 1st flowers bunch, which is a very important parameter and this distance differs between grafted plant and non grafted ones. iii) The number of bouquets per seedling. iv) Diameter of the stems, this parameter indicates the effect of the grafting on growth of plants. Using a slide caliper the stems diameter of the grafted and non grafted plants was measured. v) Gauge of the fruits, the fruits were classified according to the gauges small, medium and large and vi) The gauges, they can be either homogeneous, or heterogeneous

Statistical analysis:. Variance analysis of the treatment effect was made using SPSS software. Means were compared by Duncan multiple test at 5% level.

Results

Evaluation of agronomic parameters in controlled conditions

Leaves number: Results showed significant differences between the number of leaves on grafted and non grafted plants. The mean number was more than 7 leaves per seedling for Kemerit RZ x Bochra plants, whereas it was around 6 for Beaufort x Bochra and Beaufort x Amal. For Kemerit RZ x Amal, this number was similar to non grafted plants (5 leaves plant^{-1}). This result shows the difference of affinity between scion and rootstock (Table 2).

Table 2. Evaluation of agronomic parameters of tomato cultivars (scions) and rootstocks used in this study in controlled conditions

Grafted tomato plant or cultivar	Leaves number seedling^{-1}	Stem height (cm)	Stem diameter (cm)
Beaufort x Bochra	6.2^{c}*	25^{b}	5^{c}
Beaufort x Amal	6.0^{c}	25^{b}	4.5^{b}
Kemerit RZ x Bochra	7.8^{d}	28^{c}	5.2^{d}
Kemerit RZ x Amal	5.2^{b}	20^{a}	4.5^{b}
Bochra	5.0^{a}	22^{a}	3.8^{a}
Amal	5.0^{a}	22^{a}	3.8^{a}

* Values with the same letter(s) are not significantly different at α=5% accor ding to Student-Newman-Keuls test

Stems height: These results revealed that grafted plants Kemerit RZ x Bochra showed the highest value of stems height (28 cm). However, for Kemerit RZ x Amal the stem height was of 20 cm (Table 2).

Stem diameter: Results showed a significant difference between grafted and non grafted plants according to the scion and the rootstock used. These results revealed that grafted plants Kemerit RZ x Bochra had the highest value of stems height (28 cm). However, for Kemerit RZ x Amal the stem height was of 20 cm (Table 2).

It seems that the grafting had a positive effect on stem diameter. Affinity and compatibility between the scion and the rootstock could influence the root system development and the rootstock strength and thus improves the vegetative growth. The two rootstocks used in this study (Kemerit RZ and Beaufort) showed the highest values of these three measured parameters (leaves number, stem height and diameter) when the scion was Bochra.

Resistance of grafted plants against *FOL* and *FORL* in controlled conditions: Results analysis of grafted plants inoculated by *FOL* and *FORL*, showed a highly significant difference according to the rootstocks used. The grafting of Amal cultivar on rootstock Beaufort seemed to improve its resistance to *Fusarium* spp. Moreover, this variety grafted on Kemerit RZ seemed to be highly resistant to *FOL* and *FORL* (0%). When using Beaufort as rootstock, seedlings appeared also to be very resistant to *FOL* and *FORL* (0%). The re-isolation of the pathogen from inoculated plants showing typical symptoms of the diseases confirmed that damages observed were due to the inoculation by the two *Fusarium* species used in this study, thus fulfilling the Koch postulate's (data not shown).

Evaluation of agronomic parameters in natural conditions

The distance between the ground and the 1st flower bunch: Statistical analysis showed a significant difference of the distance between the ground and the 1st flowers bunch during time. It was of 38 cm at the beginning of the growth cycle then decreased to 35 cm (Table 3). The variation of the distance between the ground and the 1st bunch was highly significant for the control compared to the grafted plants. However, this variation was not significant for grafted plants with 1 arm and 2 arms. This distance was of 30 cm for the grafted plants and of 47 cm for the control (Table 3).

Distance between 1st bunch and 2nd bunch: Statistical analysis revealed that the variation of the distance between 1st bunch and 2nd bunch during time was significant. This distance was of 22.5 cm in February and March then it decreased to 20.5 cm since the beginning of April (Table 3). Distance between 1st bunch and 2nd bunch was not significantly different between the different modes of culture. This distance was of 21.5 cm for the control plants and the grafted plants with 2 arms and it is of 22.5 cm for the grafted plants with 1 arm (Table 3).

Height of the plants: The variation of the height according to time and the mode of control of the plants was non significant. The height was 1.84 m for the control, 2.16 m for grafted plants with 1 arm and 2.02 m for the grafted plants with 2 arms (Table 3).

Diameter of the stems: Statistical analysis indicated that the variation in stem diameter over the period was non significant (data not shown). The variation of the diameter according to the mode of control of the seedlings was highly significant for the grafted plants with one arm. This diameter difference between the grafted plants with one arm (1.55 cm) and 1.15 cm for the control was significant (Table 3).

Table 3. Evaluation of agronomic parameters of tomato plants in natural conditions

Agronomic parameters	Time			Mode of control		
	February	March	April	Non grafted	Grafted (1 arm)	Grafted (2 arms)
Ground to 1st flower bunch (cm)	38^{b}*	35^{a}	35.44^{a}	47.44^{b}	30.22^{a}	30.22^{a}
1st to 2nd flower bunch (cm)	22.55^{b}	22.44^{b}	20.56^{a}	21.67^{a}	22.44^{b}	21.44^{a}
Height of plants (cm)	-	-	-	1.84^{a}	2.16^{b}	2.02^{b}
Diameter of stems (cm)	-	-	-	1.15^{a}	1.55^{b}	1.24^{a}
Number of bunches	10.77^{a}	11.33^{b}	10.44^{a}	7.44^{a}	9.67^{a}	15.44^{b}
Yield (kg m^{-2})	-	-	-	6.49^{a}	8.29^{a}	9.67^{b}

* Values with the same letter(s) are not significantly different at α=5% according to Student-Newman-Keuls test

Number of bunches: The variation of the number of bunches was non significant. The number of bunches increased in March (11 bunches) and decreased gradually (Table 3). The variation of the number of bunches according to the mode of control of the plants was, however, highly significant. We noted that this parameter did not differ between the control (8-10 bunches) and the grafted plants with 1 arm (8-10 bunches) but the difference was clear between grafted plants with 2 arms (15 bunches) and that of 1 arm (Table 3).

Yield: The variation of the yield was highly significant. Results showed that the total weight did not differ for the grafted plants with 1 arm (10.54kg) and the control plants (9.18kg). However, the difference was clear between the grafted plants with 2 arms (12.46kg) and those grafted with 1 arm (Table 3).

Fruit size and bunch homogeneity: We noticed that the fruits resulting from the grafted plants had a gauge better than those from the control plants. It is clear that the majority of bunches were homogeneous, for the grafted plants, from the point of view of gauges and stage of maturity (Fig. 1A, B, C).

Discussion

In Tunisia, *Fusarium* crown and root rot of tomato is a new disease identified since 2000-2001 crop season, causing serious damages reaching 90% in certain greenhouses (Hajlaoui *et al.,* 2001). *Fusarium oxysporum* f. sp. *radicis-lycopersici* is responsible for this problem. Similarly, Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* is also a serious disease affecting tomato cropped in greenhouse. While exhibited heavy losses on tomato production, no or some effective disease control methods are available and there is no approved fungicide to control. In the past, grafting tomato plants was considered too expensive but at present it is used at a commercial level in Tunisia and elsewhere. Resistant rootstocks provide excellent control of many tomato soilborne pathogens, particularly *F. oxysporum* f. sp. *radicis-lycopersici*, *F. oxysporum* f. sp. *lycopersici, Pyrenochaeta lycopersici* and *Meloidogyne* spp. Furthermore, tomato grafting gave other advantages such as plant growth promotion, yield increase, extension of yield period and improvement of fruit quality (Rivero *et al.,* 2003).

In our growth chamber experiments, it was found that the two rootstocks Beaufort and Kemerit RZ were resistant to *FORL* and *FOL*. So, they could be used as rootstocks for grafting tomatoes. Hibar *et al.* (2006) reported that Beaufort was effective against *FORL*. Data obtained from this study revealed that grafting can control *Fusarium* crown and root rot and *Fusarium* wilt. Similar results were shown by Trionfetti *et al.* (2002) and Miguel *et al.* (2004) on controlling *Fusarium* wilt by grafting two muskmelon cultivars and triploid watermelon onto commercial rootstocks, respectively. On Tomato, our results show that grafting of susceptible tomato cvs Bochra F_1 and Amal F_1 onto the rootstocks Beaufort and Kemeit RZ increased tomato growth parameters, tomato yield and improve fruit quality. This increase in tomato yield through the use of grafted plants could be attributed mainly to disease resistance and to better plant growth. In grafted plants, the rootstock's vigorous root system is often capable of absorbing water and nutrients more efficiently compared to non grafted plants and may serve as 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). This mechanism has not been intensively investigated. The disease tolerance in grafted seedlings may be entirely due to the tolerance of stock plant roots to such diseases.

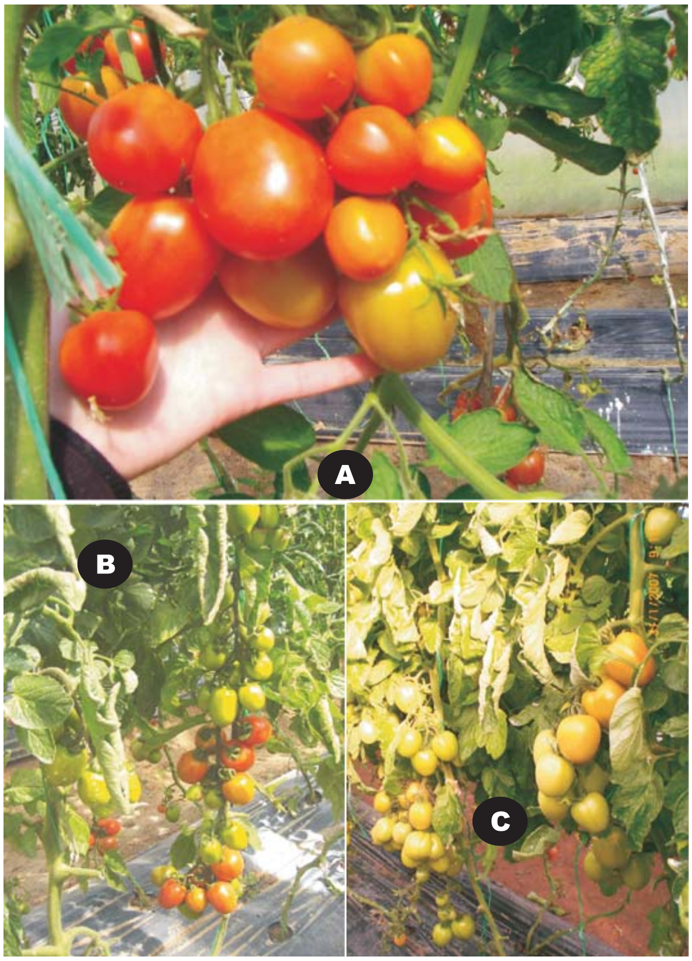

Fig. 1. A-Homogenous bunch from a single arm grafted plant. B-Fruits of similar size from a single arm grafted plant. C-A bunch of big sized fruit borne on a double arm grafted plant.

However, in actual plantings, adventitious rooting from the scion is very common (Lee, 1994). Plants having the root systems of the scion and rootstock are expected to be easily infected by soilborne diseases. However, seedlings having dual root systems often exhibit excellent disease resistance, almost comparable to those having only rootstock roots. This observation partially supports the previous report that substances associated with *Fusarium* tolerance are synthesized in the root and translocated to the scion through the xylem (Biles *et al.,* 1989). The activity of the substances related to disease resistance may vary during the development stages of the grafted plants (Heo, 1991).

On the basis of the results obtained in these experiments on tomato, grafting effectiveness seems to be determined not only by disease resistance but also by their impact on both production and fruit quality. The rootstocks Beaufort F_1 and Kemerit RZ, resistant to *FORL* and *FOL*, were also the best genotypes capable of significantly improving the productivity and fruit quality of tomatoes cultivars. Moreover, regardless of the used tomato cultivars, grafted plants gave the best results concerning plant growth, fruit yield and fruit quality, compared to non-grafted plants.

The tomato grafting proved to be an effective method to attenuate the impact of *Fusarium* wilt and *Fusarium* crown and root rot caused by *FOL and FORL*, respectively. Moreover, as grafted plants are expensive they could be trained in two arms to reduce seedlings number per unit area.

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