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Resistance Evaluation of Grafted Watermelon (*Citrullus lanatus* L.) Against Fusarium Wilt and Fusarium Crown and Root Rot*

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Abstract: The tolerance of different *Cucurbita* spp. rootstocks to *Fusarium* spp. affecting watermelon crop was evaluated under greenhouse conditions. Grafted plants on TZ148, Ferro, GV 100 and Just rootstocks were inoculated with two isolates of *Fusarium oxysporum* f. sp. *niveum* and one isolate of *F. solani* f. sp. *cucurbitae*. The survey was undertaken at three stage of plant development: vegetative growth, flowering and fructification. Grafted plants revealed to be more resistant to tested *Fusarium* isolates compared to non-grafted ones. However, it is important to mention that the tolerance of plants depends on the development stages of plants. At young stage of plant development, no significant difference between all tested rootstocks was noted. At later stages (flowering and fructification), the level of resistance depends on isolates and vary between very to moderately resistant. For example, GV 100 and Just remained at high level of resistance. These rootstocks could be used in watermelon grafting to control the disease caused by two *Fusarium* species affecting watermelon.

Key words: Grafting, *Citrullus lanatus*, *Fusarium oxysporum* f. sp. *niveum*, *F. solani* f. sp. *cucurbitae*

INTRODUCTION

The cultivation of grafted plants gradually increased in Tunisia the last years. This technique was intended to reduce damage caused by soilborne pathogens, to boost plant growth and development, to control wilt caused pathogens and to increase the tolerance to biotic stresses such as viral, fungal and bacteria infection (Rivero *et al*., 2003; Edelstein *et al*., 2004; Cohen *et al*., 2007). In melons and watermelons, resistant *Cucurbita* rootstocks were used for different purposes. Intraspecific grafting is mainly used to ovoid damage due to wilt pathogens, such as *Fusarium oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *niveum* for which resistance genes exists in certain melon varieties. This approach provides complete protection from the disease with no reduction in fruit quality and quantity (Cohen *et al*., 2002). Mechanisms of diseases tolerance in grafted plants may be due to the resistance of the rootstocks as it is accepted that the root system synthesizes substances resistant to pathogen attack and these are transported to the shoot through the xylem (Biles *et al*., 1989). The activity of these substances, related to disease resistance can vary during the development stages of grafted plants (Heo, 1991).

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Fusarium wilt and Fusarium crown and root rot of watermelon induced by *Fusarium oxysporum* Schlechtend.: Fr. f. sp. *niveum* (E.F.Sm.) W.C. Snyder and H.N. Hans. (*Fon*) and *Fusarium solani* (Mart.) Sacc. f. sp. *cucurbitae* W.C. Snyder and H.N. Hans. (*Fsc*) race1, respectively, were probably the most damaging soilborne diseases of this cucurbit causing heavy economic losses (Bruton *et al*., 1998; Martyn and Bruton, 1989). These diseases were serious in many areas of the world such as Northern Africa, Italy, United States and Israel (Messiaen *et al*., 1991). In Tunisia, the problem becomes more and more important and the races of these fungi were frequently identified in the majority of watermelon cropping areas (Boughalleb, 2003; Boughalleb and El Mahjoub, 2005; Boughalleb *et al*., 2005).

The watermelon grafting onto cucurbit rootstocks is a best alternative to control soilborne diseases and an agronomic interest for plant vigour and production (Blancard *et al*., 1991; Messiaen *et al*., 1991; Gignoux, 1993; Jebari, 1994; Aounallah-Chouka and Jebari, 1999; Aounallah *et al*., 2002; Tarchoun *et al*., 2005). *Benincasa cerifera*, *Cucurbita maxima*, *Lagenaria vulgaris* and *Lagenaria leucantha* were reported by many authors as best watermelon rootstocks (Messiaen *et al*., 1991; Gignoux, 1993). In Tunisia, the hybrid RS 841 has often been used for melon grafting (Aounallah-Chouka and Jebari, 1999). This rootstock is known resistant to Fusarium wilt and to nematodes (Blancard *et al*., 1991). Since several years, many rootstocks such RS841, Emphasis, Strong Toza, originated from the hybrid *Cucurbita moschata* × *Cucurbita maxima* were used for cucurbit grafting (Blancard *et al*., 1991). Currently, several rootstocks were subscribed and recommended for watermelon grafting. Aounallah *et al*. (2002) showed that, among the three watermelon rootstocks tested (*Lagenaria siceraria*, local of Mahdia and *RS 841*), *L. siceraria* is resistant to *Fon* isolated but susceptible to *Fsc*. However, local of Mahdia is resistant to *Fsc* and *RS 841* proved to be resistant to *Fon* and moderately resistant to *Fsc*. In the same way, the variety Sugar Baby used like control revealed to susceptible *Fon* and *Fsc*.

Recently, Boughalleb *et al*. (2007) showed that the use of grafted seedlings of sugar pack cultivar on Strong toza, TZ148, Emphasis, Achille and Ercole rootstocks were resistant to *Fsc* and *Fon* isolates. Authors concluded that these rootstocks could be interesting in grafting of watermelon to resolve the problem of two *Fusarium* species affecting watermelon.

Rootstocks-scion interaction showed against soilborne pathogens tolerance was indicated (Cohen *et al*., 2007). It is interesting to enlarge the game of available rootstocks tested in our conditions for each cropping watermelon cultivar.

The aim of this study is to evaluate resistance level of some new cucurbit rootstocks against *Fon* and *Fsc* isolates under controlled conditions as potential sources for grafting of commercial watermelon varieties.

MATERIALS AND METHODS

Plant Material

The cultivar Delta grafted on the four rootstocks was tested in this research: TZ 148 as a positive control, Ferro, GV 100 and Just as new testing rootstocks. Non-grafted cv. Delta inoculated and none-inoculated were used as negative control. This study was conducted at the laboratory of Higher Institute of Agronomy in Chott Mariem, Tunisia in 2007.

Growth Conditions and Testing Rootstocks Resistance to Fusarium Wilt and Crown and Root Rot of Watermelon

Grafted plants were transplanted into pots containing previously sterilized peat. Plants were kept at growth chamber for 30 days at 23°C with a 16 h day length. A completely randomised design with 3 replicates was used. For each treatment, 30 plants were evaluated. Plants were watered daily and no fertilizers were applied.

Two *Fusarium* species were used for evaluation for resistance to *F. solani* f. sp. *cucurbitae* with two isolates (*Fsc*) and *F. oxysporum*. f. sp. *niveum* (*Fon*1 and *Fon*2) collected from different regions in Tunisia and preserved in glycerol (50%) to 4°C.

The method of inoculation used for resistance evaluation of plants was similar to that developed by Latin and Snell (1986).

Spore suspensions were prepared from cultures grown on PDB on a rotary shaker at room temperature (22°C) for 14 days and adjusted at a concentration of 1×10^6 conidia mL^{-1} with a hematocymeter. When the first true leaf was evident, the plants were uprooted and the roots washed under a stream of gently flowing water. Seedlings were root-dipped into the respective inocula for 15-20 sec, swirled several times and transplanted into 7.5 cm diameter pots (three seedlings per pot containing vermiculite) and five pots per isolate. Thus, ten plants per isolate were tested. Controls were prepared by root-dipping the plants into sterile distilled water. All plants were maintained in the greenhouse. The average air temperature, during the experiment, was about 27°C. The experiment was conducted three times.

Plants were classified as very resistant, when the level of mortality is ranged between 0 to 15%, resistant, if the percent of infested plants varied from 15 and 30% and were considered as susceptible when presented more than 30% of diseased plants. The survey of inoculated plants was conducted regularly at three stages of plant development: vegetative growth, flowering and fructification.

Statistical Analysis

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

RESULTS AND DISCUSSION

Evaluation of Plant Resistance at Vegetative Growth Stage

The results showed a meaningful difference between the rootstocks to the three isolates of *Fusarium* spp. The rootstocks Ferro and Just seemed to be very resistant to all isolates of *Fusarium* spp. tested in this study. Grafted plants on TZ 148 demonstrated low percent of wilted plants for *Fsc* and *Fon* isolates (6.66%). Besides, Ferro, very resistant to *Fon*1 and *Fon*2, proved to be moderately susceptible to *Fsc* (Table 1). It revealed that at vegetative growth stage, all rootstocks could be efficient to resolve the problem of wilting and crown and root rot of commercial varieties.

Evaluation Plant Resistance at Flowering Stage

The results showed a meaningful difference between the rootstocks to the tested isolates. It revealed that only rootstock GV 100 remained very resistant only to *Fsc* (Table 2). Ferro and GV 100 showed a partial resistance to some isolates of *Fon* with percent of infested plants of 12%. TZ148 proved to be resistant to *Fon*2 (6.66%) but moderately susceptible to *Fon*1 and *Fsc* (Table 2).

Table 1: Percentage of plants showing symptoms of wilting, crown and root rot of the grafted plants inoculated by *F. solani* f. sp. *cucurbitae* (*Fsc*) and *F. oxysporum* f. sp. *niveum*, at vegetative growth stage (*Fon*1 and *Fon*2)

	Fsc	*Fon*1	*Fon*2
Non-grafted Delta (none-inoculated)	0.00^a	0.00^a	0.00^a
Non-grafted Delta (inoculated)	26.00^c	6.66^b	0.00^a
Grafted plants			
Delta/TZ148	6.66^b	6.66^b	6.66^b
Delta/Ferro	0.00^a	6.66^b	0.00^a
Delta/GV 100	0.00^a	0.00^a	0.00^a
Delta/Just	0.00^a	0.00^a	0.00^a

The values followed by the same letter(s) are not significantly different at p<5%. The reading of the results has been done after 15 on 20 days of the inoculation

Table 2: Percentage of plants showing symptoms of wilting, crown and root rot of the grafted plants inoculated by *F. solani* f. sp. *cucurbitae* (*Fsc*) and *F. oxysporum* f. sp. *niveum*, at flowering stage (*Fon* 1 and *Fon* 2)

	Fsc	*Fon*1	*Fon*2
Non-grafted Delta (none-inoculated)	0[a]	0[a]	0.00[a]
Non-grafted Delta (inoculated)	35[c]	30[c]	26.00[c]
Grafted plants			
Delta/TZ148	20[c]	25[c]	6.66[b]
Delta/Ferro	26[c]	20[c]	12.00[b]
Delta/GV 100	0[a]	20	12.00
Delta/Just	0[a]	0[a]	0.00[a]

The values followed by the same letter(s) are not significantly different at p<5%. The reading of the results has been done after 15 on 20 days of the inoculation

Table 3: Percentage of plants showing symptoms of wilting, crown and root rot of the grafted plants inoculated by *F. solani* f. sp. *cucurbitae* (*Fsc*) and *F. oxysporum* f. sp. *niveum*, at fructification stage (*Fon*1 and *Fon*2)

	Fsc	*Fon*1	*Fon*2
Non-grafted Delta (none-inoculated)	0[a]	0[a]	0[a]
Non-grafted Delta (inoculated)	0[a]	0[a]	8[b]
Grafted plants			
Delta/TZ148	0[a]	0[a]	6[b]
Delta/Ferro	0[a]	0[a]	6[b]
Delta/GV 100	0[a]	0[a]	8[b]
Delta/Just	0[a]	0[a]	0[a]

The values followed by the same letter(s) are not significantly different at p<5%. The reading of the results has been done after 15 on 20 days of the inoculation

Evaluation Plant Resistance at Fructification Stage

The results of rootstocks behaviour, at fructification stage, against *Fsc* and *Fon* are shown in Table 3. At the end of the assay, it appeared that the rootstocks Ferro, GV 100 and Just were very good included to the two species of *Fusarium* spp. affecting watermelon and are considered like very resistant or resistant. However, Ferro, TZ148 and GV 100 showed an intermediate behaviour and were moderately resistant to *Fon*2 (Table 3).

DISCUSSION

Data obtained from this study suggest that grafting the susceptible watermelon cv. Delta is an effective control measure against of Fusarium wilt and Fusarium crown and root rot affecting watermelon. 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 effective in controlling some other pathogens such as melon sudden wilt caused by *Monosporascus cannonballus* (Edelstein *et al*., 1999). Resistant rootstocks provide excellent control of many watermelon soilborne pathogens and particularly *F. oxysporum* f. sp. *niveum*, *F. solani* f. sp. *cucurbitae*, *Monosporascus cannoballus* and nematodes. In addition, watermelon grafting gave others advantages such as plant growth promotion, yield increase, extension of yield period and improvement of fruit quality (Tarchoun *et al*., 2005). Many rootstocks were evaluated for their resistance level to be introduced in Tunisia and used for resolving the problem of *Fusarium* spp. affecting watermelon (Boughalleb *et al*., 2007). However, it is recognized that grafting watermelon 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 post graft 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* spp. Lee (1994) reported that the tolerance disease exhibited by grafted plants could be explained by their vigorous roots. In fact, high and significant positive correlations were found between plant fresh weights and mortality rates in grafted plants developed during two different cropping seasons (Edelstein *et al.*, 2004).

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. However, in actual plantings, adventitious rooting from the scion is very common (Lee and Oda, 2003). 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).

CONCLUSION

The watermelon grafting proved to be an effective method to attenuate the impact of Fusarium wilt and Fusarium crown and root rot of watermelon caused by *Fon* and *Fsc*, respectively. The present study permitted to enlarge the game of rootstocks used for watermelon grafting. In fact, the three new rootstocks could be kept from this work.

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