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

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Abstract: From 3 watermelon cultivars Sentinel, Charleston Gray and Sugar Pack tested against some Tunisian isolates of *Fusarium solani* f. sp. *cucurbitae* and *Fusarium oxysporum* f. sp. *niveum*, Sugar Pack revealed to be the most susceptible cultivar and was used for grafting to evaluate the resistance of 9 cucurbit rootstocks. Strong toza, TZ148, Emphasis, Polifemo and Ercole were resistant to highly resistant to five *Fusarium* isolates tested. Rootstocks of Ferro RZ, Macis and S.Camel showed a partial resistance to some isolates of *Fsc* and *Fon*. Achille revealed to be highly resistant to *Fsc*, but susceptible to some isolates of *Fon*. Seedlings grafted Strong toza × Sugar Pack, TZ 148 × Sugar Pack, Emphasis × Sugar Pack, Achille × Sugar Pack and Ercole × Sugar Pack were resistant to *Fsc* and *Fon* isolates. These rootstocks could be used in grafting of watermelon to resolve the problem of two *Fusarium* species affecting watermelon.

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

Introduction

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*) race 1, respectively, were 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 of 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 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 good watermelon rootstocks (Messiaen *et al*., 1991; Gignoux, 1993). In Tunisia, the hybrid RS 841 has often been used for melon grafting (Aounallah and Jebari, 1999). This rootstock is known as 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* x *Cucurbita maxima* were used for cucurbit grafting (Blancard *et al*., 1991). Currently, several

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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 be susceptible to *Fon* and *Fsc*.

The objective 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

Nine rootstocks, hybrids of *Cucurbita maxima* x *Cucurbita moschata*, were tested in this work: Strong toza, TZ 148, Ferro RZ, Polifemo, S. Camel, Macis, Achille, Ercole and Emphasis. Three watermelon cultivars (Sentinel, Charleston Gray and Sugar Pack) were also tested in the same conditions (Table 1).

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

Seeds of rootstocks were sown in seedling trays filled with sterilised peat five days before those of scion variety. Young plants, at one-two true leaf or one true leaf stage, for rootstocks and scion, respectively, were transplanted into pots containing previously sterilized peat. Seedlings of rootstock cultivars were cut over the cotyledons and immediately grafted with shoots of scion cultivars. We adopted the method described by Taussing *et al*. (1996). The scion/rootstock combinations Sugar Pack × Strong toza, Sugar Pack × TZ 148, Sugar Pack × Ferro RZ, Sugar Pack × Polifemo, Sugar Pack × S. Camel, Sugar Pack × Macis, Sugar Pack × Achille, Sugar Pack × Ercole and Sugar Pack × Emphasis were evaluated.

Plants were kept at growth chamber for 30 days at 23°C with a 12 h day length (Woo *et al*., 1966), using a completely randomised design with 10 replicates per treatment. Watermelon plants of susceptible cv. Sugar pack treated similarly and transplanted into sterilized substrate served as controls. Plants were watered daily and no fertilizers were applied. The treatment was conducted twice.

Two *Fusarium* species were used for plants evaluation resistance *F. solani* f. sp. *cucurbitae* with two isolates (*Fsc*1 and *Fsc*2) and *F. oxysporum*. f. sp. *niveum* (*Fon*1, *Fon*2 and *Fon*3) 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).

Table 1: Cultivars characteristics used for grafting evaluation against *Fusarium* sp.

	Cultivars	Resistance
Commercial cultivars	Sentinel	Susceptible
	Charleston gray	Susceptible
	Sugar pack	Very susceptible
Rootstocks	Strong toza	*Fon*
	TZ 148	*Fon*
	Ferro RZ	*Fon*
	Polifemo	*Fon*
	S. Camel	*Fon*
	Macis	*Fon*
	Achille	*Fon*
	Ercole	*Fon*
	Emphasis	*Fon*

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, fifteen 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.

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.

Statistical Analysis

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

Results and Discussion

Evaluation of Scion Resistance

The inoculation of the 3 cultivars used as scion revealed a meaningful difference against isolates of *Fusarium* sp. tested in this study.

Sugar Pack inoculated by *Fsc*1 showed the highest percent of infected plants (73%), while the lowest percent was found with *Fsc*2 (33%). Reaction difference of these isolates is due to their variability aggressiveness. Charleston Gray is the most sensitive to *Fsc*2 (43%). Sentinel showed to be the most resistant to *Fsc*1 having 42% of diseased plants (Table 2).

For *Fon*, we concluded, that Sugar Pack is the most susceptible to the three isolates with level varying from 54 to 72%, whereas Sentinel and Charleston Gray showed a variable sensitivity according to isolates. The comparison of susceptibility of the 3 cultivars showed that Sentinel and Charleston Gray, seems to be moderately resistant to *Fsc* and resistant to *Fon*3 with rates varying from 15 to 17%, whereas they appear very susceptible to *Fon*1. On the other hand, Sentinel and Charleston Gray revealed to be resistant to *Fon*3 but susceptible to the other isolates with 67% level for *Fon*1, which appeared to be the most virulent. Sugar Pack (type Sugar Baby) was susceptible to all isolates of *Fusarium* sp., infested plants level varying from 33 to 73%. These results confirmed those of Aounallah *et al*. (2002) who found that Sugar Baby is susceptible to *Fon* and *Fsc* although the plant material type is different. For this reason, Sugar Pack was used as scion to evaluate the resistance of the nine rootstocks tested in this study.

Evaluation of Rootstocks Resistance

The results showed a meaningful difference between the nine rootstocks to the five isolates of *Fusarium* sp. The rootstocks Polifemo, Ercole and Emphasis seemed to be very resistant (0 to 8%) to all isolates of *Fusarium* sp. tested in this study (Table 2). Strong toza was resistant to *Fsc* (12%) and very resistant to *Fon* (4%). TZ 148 was very resistant to *Fsc* (0%) and resistant to *Fon* (0 to 16%), while Ferro RZ appeared to be very resistant to *Fon* (0%) but moderately resistant to *Fsc* (24%). The rootstocks S. Camel and Macis were the most susceptible to *Fsc* and relatively resistant to *Fon*. Besides, Achille, very resistant to *Fsc*, proved to be moderately susceptible to *Fon* and particularly to isolate *Fon*3.

It revealed that only rootstocks Strong toza, TZ148, Emphasis, Polifemo and Ercole were very resistant to the five isolates of *Fusarium*. Ferro RZS, Macis and S. Camel showed a partial resistance

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

		*Fsc*1	*Fsc*2	*Fon*1	*Fon*2	*Fon*3
Commercial cultivars	Sentinel	42^{a}	38^{b}	67^{b}	39^{a}	15^{a}
	Charleston Gray	48^{b}	43^{c}	63^{a}	36^{a}	17^{a}
	Sugar Pack	73^{c}	33^{a}	72^{c}	54^{b}	56^{b}
Rootstocks	Strong toza	11^{c}	0^{a}	0^{a}	3^{a}	0^{a}
	TZ 148	0^{a}	0^{a}	0^{a}	16^{b}	0^{a}
	Ferro RZ	23^{d}	0^{a}	0^{a}	0^{a}	0^{a}
	Polifemo	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	S. Camel	0^{a}	38^{c}	10^{c}	3^{a}	0^{a}
	Macis	31^{e}	23^{c}	3^{a}	0^{a}	23^{b}
	Achille	0^{a}	0^{a}	0^{a}	0^{a}	25^{b}
	Ercole	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}
	Emphasis	3^{b}	0^{a}	8^{c}	0^{a}	0^{a}
Grafted plants	Strong toza × Sugar Pack	0^{a}	0^{a}	15^{bc}	0^{a}	0^{a}
	TZ 148 × Sugar Pack	0^{a}	0^{a}	10^{b}	0^{a}	0^{a}
	Ferro RZ × Sugar Pack	22^{b}	10^{b}	0^{a}	0^{a}	0^{a}
	Polifemo × Sugar Pack	0^{a}	0^{a}	20^{c}	10^{b}	0^{a}
	S. Camel × Sugar Pack	0^{a}	20^{c}	25^{c}	25^{c}	0^{a}
	Macis × Sugar Pack	45^{d}	50^{d}	30^{d}	60^{d}	30^{b}
	Achille × Sugar Pack	0^{a}	0^{a}	10^{b}	0^{a}	0^{a}
	Ercole × Sugar Pack	0^{a}	0^{a}	15^{bc}	0^{a}	0^{a}
	Emphasis × Sugar Pack	0^{a}	17^{c}	10^{b}	10^{b}	0^{a}

The values followed of the same letter are not meaningfully different to the doorstep 5%. The reading of the results has been done after 15 on 20 days of the inoculation

Table 3: Behaviour of different rootstocks and grafted plants inoculated by *Fsc* and *Fon*

		Fsc	*Fon*
Rootstocks	Polifemo	VR	VR
	Ercole	VR	VR
	Emphasis	VR	VR
	Strong toza	VR	VR
	TZ 148	VR	R
	Ferro RZ	MR	VR
	S. Camel	S	VR
	Macis	S	MS
	Achille	VR	MR
Grafted plants	Polifemo × Sugar Pack	VR	R
	Ercole × Sugar Pack	VR	R
	Emphasis × Sugar Pack	R	R
	Strong toza × Sugar Pack	VR	R
	TZ 148 × Sugar Pack	VR	R
	Ferro RZ × Sugar Pack	R	VR
	S. Camel × Sugar Pack	S	S
	Macis × Sugar Pack	S	S
	Achille × Sugar Pack	VR	R

VR: Very Resistant, S: Sensible, R: Resistant, MR: Moderately Résistant

to some isolates of *Fsc* with percent of infested plants ranged from 0 to 38% and those of *Fon* (0 to 23%). Achille proved to be very resistant to *Fsc* (0%) but susceptible to some isolates of *Fon* (0 to 25%) (Table 2).

The results of rootstocks behaviour against *Fsc* and *Fon* are presented in Table 3. The first five rootstocks were very good included to the two species of *Fusarium* sp. affecting watermelon and are considered like very resistant or resistant. Ferro RZ showed an intermediate behaviour and was moderately resistant to *Fsc*. Rootstocks S. Camel and Macis proved to be susceptible to *Fsc*, resistant to moderately resistant to *Fon*. On the other hand, Achille appeared very resistant to *Fsc* and moderately resistant to *Fon*.

Evaluation of Resistance Combinations Scion/rootstocks

The percentage of grafted success plants ranged from 40 to 88% according to combination scion/rootstock. The best level was of 88% with the rootstocks S. Camel and Macis, whereas the lower (40%) with Ferro RZ.

The grafting of Sugar Pack (scion) on the 9 rootstocks revealed highly meaningful differences (Table 2). It revealed that the combinations Strong toza x Sugar Pack, TZ 148 x Sugar Pack, Emphasis x Sugar Pack, Achille x Sugar Pack and Ercole x Sugar Pack were resistant to *Fsc* and *Fon* isolates. The grafting of Sugar Pack on Ferro RZ showed plants very resistant to *Fon*, but resistant to *Fsc* (22%). The grafting on Macis and S.Camel seemed susceptible to *Fsc* and *Fon* isolates (Table 2 and 3).

Discussion

In the past, grafting of watermelon 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 watermelon soilborne pathogens and particularly *F. oxysporum* f. sp. *niveum*, *F. solani* f. sp. *cucurbitae*, *Monosporascus cannoballus* and nematods. 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).

Data obtained from this study suggest that grafting the susceptible watermelon cv. Sugar Pack 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). However, 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 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 bet alternative effective control methods found up to now for *Fusarium*.

Conclusions

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 grafting of watermelon. In fact, five rootstocks could be kept from this work: Strong toza, TZ 148, Emphasis, Achille and Ercole.

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