

Evaluation of Four Local Colocynth Accessions and Four Hybrids, Used as Watermelon Rootstocks, for Resistance to Fusarium Wilt and Fusarium Crown and Root Rot

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ABSTRACT

Fusarium wilt and Fusarium crown and root rot, caused by *Fusarium oxysporum* f. sp. *niveum* and *F. solani* f. sp. *Cucurbitae*, respectively are damaging diseases of watermelon in Tunisia. While causing heavy losses to watermelon production, no or some effective disease control methods are available. In Tunisia, there are no approved fungicides to control these diseases. The use of tolerant or resistant rootstocks seems to be the most effective measure. Using local colocynth accessions seems to be a reliable solution for controlling Fusarium wilt and Fusarium crown and root rot. Indeed, the percent of diseased plants did not exceed 8.6%. Used as parental accessions and hybridized with *Citrullus lanatus* var. *citroides*, they generated resistant hybrids whose percentage of diseased plants was no more than 8.3%. These results suggest that colocynth accessions as well as the generated hybrids seem to be potential watermelon rootstock that can be used in Tunisian orchards where *Fusarium* species cause problems.

Keywords: *Fusarium oxysporum* f. sp. *niveum*, *F. solani* f. sp. *cucurbitae*, resistance hybrids, screening

INTRODUCTION

In Tunisia, watermelon culture is an important crop vegetable; indeed, the surface currently reserved for this culture is about 14,000 ha, representing 10% of the total surface reserved for vegetable cultures. However, this culture suffers from a number of serious fungal, bacterial, and viral diseases that reduce yield and quality (Bruton 1998). From these diseases, fusarium wilt caused by the soilborne fungus *Fusarium oxysporum* f.sp. *niveum* (*Fon*) and fusarium crown and root rot caused by *F. solani* f. sp. *cucurbitae* (*Fsc*) are the most damaging pathogens of this cucurbit (Purseglove 1987; Martyn 1996).

Fon has been separated into three pathogenic races: 0, 1, and 2 (Netzer 1976; Martyn 1987) while *Fsc* is represented only by one race, race 1. These pathogens reportedly cause heavy losses overall in the world such as North Africa, United states, Italy (Messiaen *et al.* 1991; Bruton *et al.* 1998) and also in Tunisia, especially on non-grafted plants.

Control of these diseases relies primarily on use of resistant cultivars and crop rotation. While many commercial cultivars have resistance to races 0 and 1 of *Fon*, the more aggressive race 2 overcomes all cultivars and has great potential for spread in watermelon production areas in Tunisia because it can be seedborne (Martyn and Netzer 1991; Hopkins *et al.* 1992; Bruton 1998; Boughalleb and El Mahjoub 2005). Resistance to the various races has been identified in plant accessions from Africa (Martyn 1987; Dane *et al.* 1998). A single dominant gene, designated *Fo-1*, confers resistance to race 1 of *Fon* in watermelon (Netzer and Weintall 1980), while resistance to race 2 in PI 296341-*FR* (*Citrullus lanatus* var. *citroides*) is thought to be conferred by a recessive gene with interactions with some minor genes (Martyn and Netzer 1991; Zhang and Rhodes 1993).

Grafting plants seemed to be the best solution to control

these pathogens. Indeed, this technique is used not only to control pathogen infections, but also to increase the yield production and the tolerance to abiotic stress such as Salinity (Blancard *et al.* 1991; Messiaen *et al.* 1991; Gignoux 1993; Jebari 1994; Aounallah-Chouka and Jebari 1999; Aounallah *et al.* 2002; Rivero *et al.* 2003; Edelstein *et al.* 2004; Tarchoun *et al.* 2005; Cohen *et al.* 2007).

Grafting watermelon onto resistant rootstocks is used especially to control fusarium wilt and fusarium crown and root rot (Cohen *et al.* 2002). Resistance mechanisms of the rootstocks can be explained by the fact that the root system synthesizes substances that inhibit the pathogen development and these are transported to the shoot through the xylem (Biles *et al.* 1989).

Several cucurbit rootstocks such as *Benincasa cerifera*, *Cucurbita maxima*, *Lagenaria leucantha* and *Lagenaria vulgaris* have been reportedly used for watermelon grafting (Messiaen *et al.* 1991; Gignoux 1993). In Tunisia, many hybrids (*Cucurbita moschata* x *Cucurbita maxima*) were used as watermelon rootstocks such RS841, Emphasis, Strong Toza (Blancard *et al.* 1991). Currently, another local rootstock (local of Mahdia), demonstrated to be resistant to *Fsc*, was recommended for watermelon grafting (Aounallah *et al.* 2002). Recently, Boughalleb *et al.* (2007) showed that grafting watermelon cv. 'Sugar pack' on the rootstocks Strong toza, TZ148, Emphasis, Achille and Ercole were resistant to *Fsc* and *Fon* isolates.

In this study, four colocynth accessions collected from different regions in Tunisia and four hybrids, obtained by crossing these accessions with *C. lanatus* var. *citroides*, were evaluated as potential sources for grafting commercial watermelon cultivars to protect against Fusarium wilt and Fusarium crown and root rot of watermelon.

Table 1 Colocynth accessions, their origins and hybrids used as watermelon rootstocks.

Colocynth (<i>C. colocynthis</i>) accessions	Origins of colocynth accessions	Hybrids
R 1	Sidi Bouzid (Center west of Tunisia)	H1 = R1 x <i>C. lanatus</i> var <i>ciroides</i>
R2	Kebeli (South of Tunisia)	H2 = R2 x <i>C. lanatus</i> var <i>ciroides</i>
R3	Sidi Bouzid (Center west of Tunisia)	H3 = R3 x <i>C. lanatus</i> var <i>ciroides</i>
R4	Sahel (East of Tunisia)	H4 = R4 x <i>C. lanatus</i> var <i>ciroides</i>

MATERIALS AND METHODS

Plant material

Four colocynth (*Citrullus colocynthis*) accessions were evaluated for resistance to Fusarium wilt and Fusarium crown and root rot of watermelon. They were collected from different regions of Tunisia (Table 1). After their evaluation for Fusarium resistance, during the crop season 2007-2008, and demonstrated resistance to two *Fusarium* species (*F. oxysporum* f. sp. *niveum* and *F. solani* f. sp. *cucurbitae*), these accessions were used in a hybridization program. Indeed, they were crossed with a citron watermelon (*C. lanatus* var. *ciroides*) and four hybrids were obtained (Table 1). These hybrids, used as watermelon rootstocks, were also evaluated for their resistance to Fusarium wilt and Fusarium crown and root rot of watermelon. Types of crossing and origins of colocynth accessions, used as rootstocks, are presented in Table 1.

Fungal isolates

Fusarium isolates used in this study were recovered from watermelon plants showing typical wilt or crown and root rot symptoms at different areas producing watermelon in Tunisia.

Fungal pathogen was isolated by planting root fragments (0.5 cm) of diseased plants (surface-disinfected with 1% sodium hypochlorite for 2 min) on PDA (potato dextrose agar) and incubating them at 25°C for 5 days (Katan *et al.* 1991). Isolates were identified as *F. oxysporum* or *F. solani* morphologically based on characteristics of the macroconidia, phialids, microconidia, chlamydospores, and colony growth traits (Leslie and Summerell 2006). The *forma specialis* of the pathogen was identified using pathogenicity tests (Boughalleb *et al.* 2005). Based on pathogenicity tests using differential cultivars, the more aggressive isolates of *F. oxysporum* f. sp. *niveum* classified in race 2, were selected for this study. The three isolates used in *in vivo* tests are presented in Table 2.

Resistance evaluation, of four local colocynth accessions and four hybrids, to Fusarium wilt and F. crown and root rot of watermelon under greenhouse conditions

The resistance to Fusarium wilt and Fusarium crown and root rot of colocynth accessions and hybrids used in this research were evaluated under greenhouse conditions on a 1: 1 mixture of peat and perlite artificially inoculated with *Fon* or *Fsc*. The experiment was conducted in a greenhouse in the Agronomic High Institute of Chott-Mariem during the 2007-2008 crop season.

Seeds were sown in seedling trays filled with previously sterilized peat. Young plants at the one-to-two-true-leaf stage were removed from the peat; their roots were washed with sterilized water, and then plants were transplanted into 20 cm diameter pots filled with infested substrate (peat+perlite) with one of the used Fusarium isolates. This substrate was artificially inoculated with a 10^7 ml⁻¹ spore suspension of one of three single-spore isolates (*Fon1*, *Fon2* and *Fsc*) 1 h before planting (Vakalounakis and Fragkiadakis 1999). Plants were kept in the greenhouse, in which temperature fluctuated between 20 and 30°C and relative humidity between 80 and 90% (Zhou and Everts 2007), using a completely randomised design with 10 replicates per treatment.

Watermelon plants of susceptible cv. 'Sugar Baby' treated similarly and transplanted into inoculated and sterilized un-inoculated substrate served as negative and positive controls, respectively. Plants were watered daily and fertilized twice a week using the nutritive solution presented by Fita *et al.* (2008). The experiment was conducted twice.

Basing on a scale described by Martyn and Mclaughlin (1983), plants were classified as very resistant, when the mortality level

Table 2 Fusarium isolates used for this study.

Isolates	Fusarium species	Host plant	Date of isolation
Fon1 (race 2)	<i>F. oxysporum</i> f. sp. <i>niveum</i>	Watermelon	2004
Fon2 (race 2)	<i>F. oxysporum</i> f. sp. <i>niveum</i>	Watermelon	2005
Fsc (race 2)	<i>F. solani</i> f. sp. <i>cucurbitae</i>	Watermelon	2005

Table 3 Percentage of rootstocks plants showing symptoms of wilting, caused by *F. oxysporum* f. sp. *niveum* (Fon1, Fon2), and crown and root rot caused by *F. solani* f. sp. *cucurbitae* (Fsc).

	Fon1	Fon2	Fsc
Colocynth accessions			
R1	0 a	0 a	0 a
R2	0 a	6.3 c	5.3 b
R3	5.3 c	8.3 d	0 a
R4	0 a	0 a	0 a
Hybrids			
H1	0 a	0 a	0 a
H2	8.6 d	4.3 b	8.3 c
H3	4.6 b	5.3 c	0 a
H4	0 a	0 a	0 a
Controls			
Un-inoculated Sugar Baby	0 a	0 a	0 a
Inoculated SugarBaby	82 e	83.3 e	80.6 d

Values represent the average of 10 plants per treatment. Within columns, means followed by the same letters are not significantly different (P=0.05) according to SNK test

was less than 20%, moderately resistant, if the percentage of infested plants varied between 21 and 50%, slightly resistant when diseased plants ranged from 51 to 80% and sensitive when percentage of wilted plants is more than 80%.

Statistical analysis

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 the S-N-K (Student-Newman-Keuls) test.

RESULTS

Resistance evaluation of local watermelon rootstocks to Fusarium wilt and Fusarium crown and root rot under greenhouse conditions

Under greenhouse conditions, resistance evaluation of local watermelon rootstocks (R1, R2, R3, R4, H1, H2, H3 and H4) to Fusarium wilt and Fusarium crown and root rot demonstrated that these either colocynth accessions or hybrids were found to be very resistant to the two *Fusarium* species. Indeed, for Fusarium wilt the percentage of diseased plants did not exceed 8.6% for all the used rootstocks and in many cases it was nil (Table 3). For Fusarium crown and root rot, only the rootstocks R2 and H2 exhibited few symptoms which did not exceed 8.3%. In this assay, we note also that the rootstocks R1, R4, H1 and H4 were 100% resistant to the two *Fusarium* species (Table 3).

By removing the substrate and by examining the root system, all used rootstocks (colocynth accessions and hybrids) appeared healthy and there were no symptoms of *Fusarium*.

Fig. 1, for example, illustrates the comparison between



Fig. 1 Comparison between a non-inoculated control cv. 'Sugar Baby' plant (A) and an inoculated hybrid H4 (B), 30 days after inoculation with *F. oxysporum* f. sp. *niveum* at about 28°C.

roots of the control cv. 'Sugar Baby' and hybrid H4, in which there were no symptoms of browning or crowning.

DISCUSSION

Grafting watermelon plants became the best solution since Fusarium wilt and F. crown and root rot became serious problems for producing watermelon in Tunisia and in many countries. Resistant rootstocks provide excellent control of many watermelon soilborne pathogens, particularly of *F. oxysporum* f. sp. *niveum*, *F. solani* f. sp. *cucurbitae*, *Monosporascus cannonballus* and *Meloidogyne* spp. In addition, watermelon grafting provides other advantages such as plant growth promotion, yield increase, extension of yield period and improvement of fruit quality (Rivero *et al.* 2003).

In Tunisia, grafting watermelon onto commercial rootstocks such as TZ 148, Ferro, GV 100 and Just seems to be the best solution for controlling Fusarium wilt and F; crown and root rot of watermelon. If some rootstocks (GV 100 and Just) were efficient in controlling these pathogens, some other rootstocks such as Ferro and TZ148 showed an intermediate behaviour and were moderately resistant to *F. oxysporum* f. sp. *niveum* (Boughalleb *et al.* 2008).

To ameliorate the genetic diversity and to search for other rootstocks more efficient in controlling soilborne pathogens, four colocynth accessions and four hybrids were used as watermelon rootstocks. Evaluation of these colocynth accessions and the obtained hybrids, under greenhouse conditions, demonstrate that both accessions and hybrids were classified as very resistant rootstocks to the two *Fusarium* species; therefore they could be used as rootstocks for grafting watermelon to protect against Fusarium wilt and Fusarium crown and root rot.

Data obtained from this study suggest that using resistant rootstocks is an effective control measure against *Fusarium* species. Similar results were reported by Trionfetti Nisini *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).

Grafting watermelon on resistant rootstocks can not only control pathogens but also increase yield production and fruit quality. This increase in watermelon 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 (Pavlou *et al.* 2002; Alexopoulos *et al.* 2007). 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 (Fernández-García *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* root-

stocks; indeed, total fruit yield of cucumber plants cv Brunex F₁ grafted onto all rootstocks tested (A27, *Cucurbita ficifolia*, Patron, Peto 42.91, TS-1358 and TZ-148) was greater than that of the un-grafted (self-rooted) plants cv. Brunex F₁ (control).

However the results shown by Trionfetti Nisini *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.

CONCLUSION

On the basis of the results obtained in these experiments on watermelon rootstocks, either colocynth accession or obtained hybrids can be used for grafting watermelon. Indeed, all the used rootstocks were classified as very resistant.

However, grafting watermelon cultivars onto resistant rootstocks are more expensive, since both scions and rootstocks are expensive. 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.

Finally, and to be more affirmative, experiments must be repeated in watermelon fields by using grafted plants to be sure of the compatibility, between scions and rootstocks, and the yield production.

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