Evaluation of Local Watermelon and Melon Rootstocks Resistance to Six Soilborne Plant Pathogenic Fungi in Tunisia

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ABSTRACT

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Five melon (M16, M17, M12, M9.1, and V4R3) and two watermelon (P7 and P6.1) accessions were evaluated under greenhouse conditions for their resistance to *Fusarium oxysporum* f. sp. *melonis* (FOM), *F. solani* f. sp. *cucurbitae* (FSC), *F. oxysporum* f. sp. *niveum* (FON) *Monosporascus cannonballus*, *M. eutypoides*, and *Macrophomina phaseolina* based on disease severity index, leaf alteration index and shoot and root dry biomass. Student-Fisher test revealed significant difference in the susceptibility among the tested local germplasms. Separate statistical analyses of variance confirmed the Duncan test and revealed a significant effect of genotype × isolate interaction. For the four assessed traits, mean scores indicated that the resistance to the six soilborne pathogens varied from moderate to high. The local melon germplasm M9.1 was found to be the best accession showing the highest resistance. M9.1 and M17 accessions have showed important shoot and dry biomasses. For watermelon accessions, the lowest disease severity index and leaf alteration index were recorded for the combination germplasm, P6.1 and *M. eutypoides*. In the other hand, the presence of plant-pathogen interaction indicated that the mechanism controlling the resistance for each pathogen varied from one accession to another. The presence of several genetic sources of resistance to the six soilborne pathogens in the accessions assessed had two advantages, firstly the exploitation of the pool genes for further breeding program and secondly the limitation of emergence of new fungal adapted species.

Keywords: Local germplasms, melon, rootstock resistance, soilborne pathogens, watermelon

In Tunisia, watermelon (*Citrullus lanatus*) and melon (*Cucumis melo*) are crops of major importance. They cover about 21.300 ha, representing 12% of the vegetable area with a production of 498.000 tons in 2012 (40). However, important yield fluctuations were recorded from year to year and this is mainly due to several factors such as biotic and abiotic stresses. Many soilborne fungi such as *Fusarium oxysporum*, *F. solani* f. sp. *cucurbitae*, *Monosporascus cannonballus*, *M. eutypoides*, and *Macrophomina phaseolina* affecting cucurbits in Tunisia are responsible for collapse and vine decline and decrease both in yield and fruit quality (8, 12, 13, 16, 45).

Due to the persistent nature of these pathogens, the diseases are best managed with wilt-resistant cultivars (20). Rootstocks resistance to these soilborne fungi has been widely studied

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by many authors in Tunisia (9, 14, 15, 46) and in many other countries (4, 24, 66). Grafting has been used in Eastern Asia (51) and recently, it has been adopted on a large scale in the Western world (24). The ban of methyl bromide has resulted in the massive adoption of grafting technology in the Mediterranean basin and Europe (1, 2, 43, 52, 56, 57, 73). It was also adopted in North American (48) and Brazil (41). Grafting is effective in reducing the incidence of Fusarium wilt, Monosporascus sudden wilt and vine decline caused by *M. phaseolina* (20, 21, 23, 25, 37) and provides a fast and easy control approach to acute soilborne pathological problems, in contrast to long and expensive breeding programs (24). The efficacy of this method was not yet tested against *Macrophomina* [*Mm.*] *phaseolina* in Tunisia unlike *Fusarium* sp. (5, 6, 14, 15, 17, 45, 68) or *Monosporascus* [*Ms.*] *cannonballus* (9, 46). Root stocks were used to increase tolerance to low and high temperatures (63, 70), to enhance nutrient uptake (29), increase synthesis of endogenous hormones (34, 36), improve water use efficiency (64), reduce uptake of persistent organic pollutants from agricultural soils (59, 60), improve alkalinity tolerance (28), raise salt and flooding tolerance (26, 27, 74), and limit the negative effect of boron, copper, cadmium, and manganese toxicity (38, 39, 65, 67). Rootstock can exhibit excellent tolerance to serious soilborne pathogens such as *Fusarium* sp., *Verticillium* sp., *Phytophthora* sp., *Pseudomonas* sp., *Didymella bryoniae*, *Ms. cannonballus*, and nematodes (11, 18, 19, 22, 30, 31, 35, 58, 72) even though the degree of tolerance differs considerably with the rootstocks. Several attempts have been made to find sources for resistance (10, 42, 69, 70). However, in actual planting, adventitious rooting from the scion is common. Plants having the root systems of the scion and rootstock are expected to be easily infected by soilborne diseases (32, 33, 50, 51).

Tunisian plant flora is extremely rich in various species of *Cucurbita*; local melon and watermelon germplasm are a genetic makeup that varies from one region to another. The objective of this study was to evaluate local germplasm of wild selected local germplasm population melon and watermelon as rootstocks to control six major cucurbit soilborne fungi.

MATERIALS AND METHODS

Plant material and fungal isolates.

A total of 7 selected local germplasm population of cucurbitaceous 5 *Cucumis melo* and 2 *Citrullus lanatus* (as citron or preserving melon [*C. lanatus* var. *citroides*] collected from the south of Tunisia (oasis)) at 2010/11. The vegetative and fruit characteristics of the local melon and watermelon germplasms studied are presented below (Fig. 1; Tables 1 and 2).

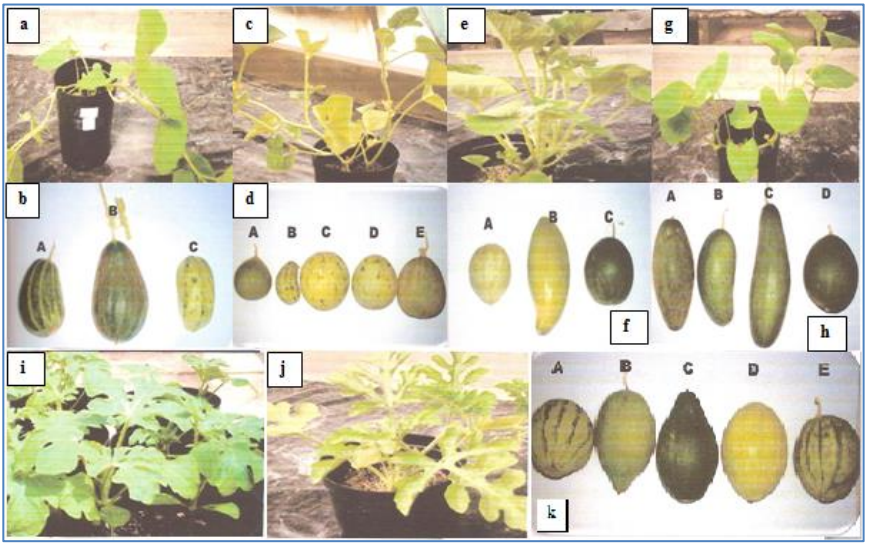

Fig.1: Morphological characterization of melon and watermelon local germplasms. a, c, e and g: melon plant and leaves; i and j: watermelon plant and leaves; b, d, f and h: melon fruits; k: watermelon fruits.

Table 1. UPOV descriptors used for morphological characterization in melon M12/M16/M17/M9.1/V4R3

Traits		Description	Local germplasm code
Seedling	Length of hypocotyl	Short Medium Long	M12/M16 M12/M17/V4R3 M9.1
	Size of cotyledon	Small Medium Large	M16 M12/M17 M9.1/V4R3
Plant	Leaf blade: intensity of color	Light Medium Dark	M16 M17/M9.1/V4R3 M12
	Leaf blade: blistering	Weak Medium Strong	M12/M16/M17/M9.1/V4R3
	Leaf blade: undulation of margin	Weak Medium Strong	M16/M17 M12 M9.1/V4R3
	Leaf blade: width	Narrow Medium Broad	M12/M16/M17/M9.1/V4R3
	Petiole: length	Short Medium Long	M17 M12/M16/M9.1/V4R3
Fruit	Shape of longitudinal section	Round Broad elliptic Elliptic Cylindrical	M16/M17/M9.1 M16 M12/M17/V4R3 M12/M17/M9.1
	Ground color of skin	White Yellow Green	 M12/M16/M17/M9.1/V4R3 M12/M16/M17/M9.1/V4R4

Table 2. UPOV descriptors used for morphological characterization in watermelon P6.1/P7

Traits		Description	Local germplasm code
Seedling	Shape of cotyledon	Narrow elliptic Elliptic Broad elliptic	P6.1/P7
	Size of cotyledon	Small Medium Large	P6.1/P7
Plants	Leaf blade: ratio length/width	Small Medium Large	P6.1/P7
	Leaf blade: intensity of color	Light Medium Dark	P6.1 P7
	Leaf: degree of lobing (beyond first flower)	Weak Medium Strong	P6.1/P7
	Petiole: length	Short Medium Long	P6.1/P7
Fruit	Shape of longitudinal section	Round Broad elliptic Elliptic Cylindrical	P6.1/P7 P6.1/P7
	Ground color of skin	White Yellow Green	P6.1/P7

They were evaluated for resistance to *F. oxysporum* f. sp. *melonis* (FOM) (melon Afamia; Beja (2010)), *F. oxysporum* f. sp. *niveum* (FON) (watermelon Crimson sweet; Kairouan (2010)), *F. solani* f. sp. *cucurbitae* (FSC) (melon Afamia; Sfax (2010)), *Ms. cannonballus* (MC) and *Mn. phaseolina* (watermelon Sentinel; Chott-Mariem (2011)), and *Ms. eutypoides* (watermelon Dumara; Sfax (2010)).

FOM, FON, and FSC were grown in Potato Dextrose Broth (PDB) on a rotary shaker for 10 days at room temperature. Microconidia were harvested by filtration through an autoclaved nylon mesh. Spore concentration was determined using a hemocytometer and adjusted to 10^6 conidia/ml. For *Monosporascus* spp. and *Mm. phaseolina*, the inoculum was prepared using the method described by Ben Salem *et al.* (9). Fungal cultures were first grown on PDA for 6 days at 26°C. Inoculum was produced on bread wheat (*Triticum estivum*) seeds, which were soaked for twelve hours in distilled water and then air dried. Seeds were transferred to 1-litre flasks, which were subsequently autoclaved on 3 successive days at 120ºC during 1 h. Two fungal discs of each isolate, previously grown on PDA at 25ºC, were placed aseptically in separate flasks. The flasks were incubated at 28ºC for four weeks, and shaken once a week to avoid clustering of inoculum.

Inoculation and experimental design.

Ten-day-old pre-germinated local melon and watermelon germplasm

seedlings were transferred to plastic pots containing peat and vermiculite (v:v), 10 ml of the conidial suspension (10^6conidia/ml) of the three species of *Fusarium*, were added to each pot. The pots were then placed in a greenhouse at 26/22°C (day/night). Plants were kept under observation for 30 days; seedlings were irrigated and fertilized in order to favorite a normal growth. At the end of this period, the presence of any Fusarium wilt symptom or Fusarium root and collar rot were noted in order to classify each plant as susceptible or resistant. For the two *Monosporascus* species and for *Mm. phaseolina*, seedlings were placed in pots containing the inoculated substrate (200 g of infected wheat/pot at the root zone of each plant). Each pot contains 40 g of inoculated wheat. Thus, 200 g of infected wheat seed were mixed with 1 kg of peat, and distributed into five pots (25 cm in diameter), and cucurbit seedlings were planted separately (one plant per pot). The pots were spaced apart and carefully irrigated to prevent soil splashing. The inoculated plants were then kept in the greenhouse for 45 days. Control treatment consisted of 40 g of non-infested sterile wheat seed per pot.

The evaluation of the resistance was performed in a randomized complete block, conducted under greenhouse conditions with 10 plants per replicate (3 replicates) for each individual treatment. This assay consists of the artificial inoculation of the 7 local germplasm by each fungus and treatment was distributed randomly. This essay was conducted twice, the isolates of *Fusarium* sp., were inoculated by drenching the substratum per pot (near the roots) and *Monosporascus* spp., and *Mm. phaseolina* isolates were inoculated using the infected wheat mixed with the substratum.

Measurements and analysis.

Disease severity index (DSI).

Response of watermelon and melon rootstocks to Fusarium wilt and collar rot. Thirty days after inoculation, inoculated plants of different local germplasm with the different *Fusarium* species were assessed for typical symptoms incited by each fungus. Severity of symptoms induced by FOM on melon accessions was assessed based on an arbitrary 1 to 5 scale (1: no symptoms; 2: beginning of wilting or yellowing symptoms on leaves; 3: leaves heavily affected; 4: all leaves completely wilted, stem standing; 5: dead plants) (3). FON-incited disease severity on watermelon accessions was assessed using the 0 to 4 scale adopted by Boughalleb *et al.* (12) where 0: healthy; 1: healthy hypocotyl with a slight discoloration of roots; 2: healthy hypocotyl with 10% of necrotic roots; 3: hypocotyl slightly infected with 30% of necrotic roots; 4: hypocotyl infected with 70% of the primary and secondary necrotic roots. Severity of FSC-induced symptoms on watermelon and melon accessions were assessed using the 0 to 3 scale adopted by Boughalleb *et al.* (12) where 0: healthy; 1: slight yellowing of leaves with slight rot pivot and lateral roots and crown rot; 2: significant yellowing in leaves with or without wilting, stunting of plants, severe rot at the pivot and lateral roots, significant rot and discoloration of vessels in the stem; 3: death of the plant. Plants scored with 1 or 2 were ranked as resistant whereas plants scored with 3, 4 or 5 were classified as susceptible (3).

Response of watermelon and accessions to *Ms. cannonballus* and *Ms. eutypoides*. All plants were carefully extracted from the pots 45 days after planting. Their roots were carefully

submerged in a container of clean water using a fine mesh strainer to allow all sand to wash away. Clean roots were then rated based on an arbitrary 1 to 5 scale where 1: no apparent necrosis, healthy roots; 2: slight necrosis of fine roots, few tan lesions; 3: slight necrosis of all roots, moderate tan lesions; 4: severe necrosis of all roots, few remaining fine roots, extensive tan lesions; 5: only tap root remaining, necrotic and completely tan to brown (31).

Response of watermelon and accessions to *Mm. phaseolina*. Disease severity index (DSI) was assessed using the scale described by Ambrosio (4), where 0: symptomless, 1: 1 to 3% of shoot tissues infected, 2: 10% of shoot tissues infected, 3: 25% of shoot tissues infected, 4: 50% of shoot tissues infected and 5: more than 75% of shoot tissues infected.

Leaf alteration index (LAI).

Foliar symptoms (leaf alterations) for all treatments were evaluated twice a week, 15 days after inoculation. Symptoms were recorded using the leaf alteration index expressing the progress and the severity of the disease (7, 22, 53). Leaf alteration index was evaluated using an arbitrary0 to 4 scale where 0: healthy leaves; 1: discoloration of leaves; 2: yellowing of leaves; 3: necrotic leaves; 4: leaves wilted and died.

Re-isolation of soilborne fungi.

To verify the Koch postulates, small root fragments were surface-disinfected for 1 min in a sodium hypochlorite solution (1.5% active chlorine) and washed twice with sterile water. Root fragments from discolored tissues were transferred onto Potato Dextrose Agar (PDA) (Biokar-Diagnostics, Zac de Ther, France) containing streptomycin sulfate (Sigma-Aldrich, Madrid, Spain) (PDAS) at 0.5 mg/ml and incubated in darkness at 25°C. For all samples, 21 root fragments per plant (3 Petri dishes containing 7 root fragments each) were prepared. Plates were examined daily for fungal growth during 7 days. The developed colonies were selected, purified and identified.

Shoot and root dry biomass.

For the evaluation of growth parameters, fifteen plants were harvested and graded for disease using the top and root dry biomass rates, weighing the shoot and roots separately before and after drying for 48 h at a temperature of 70°C for each treatment. The percentage of dry biomass DB (%) is determined for the shoot and roots of plants following formula: DB (%) = (DW/FW) × 100, with DB: dry biomass, DW: dry weight and FW: fresh weight of shoot or roots.

Data analysis

Disease severity (DSI) and leaf alteration (LAI) indexes were analyzed with the GENMOD procedure using the multinomial distribution and the cumulative logit as link function, and means of the values were separated by χ^2 test at $P < 0.05$ using SAS program (SAS Institute, Cary, NG). The other variables: SDW and RDW, were compared by analysis of variance (ANOVA) and means of the values were separated with Student´s least significant difference (LSD) test at $P < 0.05$ using SPSS 20.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

According to the GLM analysis data of IDS, LAI, SDW and RDW, local melon and watermelon germplasms differed significantly among all combination with tested pathogens ($P < 0.05$) (Table 3).

Table 3. Analysis of variance for the effects of pathogens and crops on disease severity index (DSI), leaf alteration index (LAI), and Shoot (SDW) and root (RDW) dry biomass (%)

Parameters		Pathogens	Local germplasms	Pathogens ×Local germplasms
DSI	df[a]	5	6	30
	MS[b]	2.476	0.824	1.145
	P>F[c]	< 0.05	< 0.05	< 0.05
LAI	MS[b]	1.606	0.482	1.083
	P>F[c]	< 0.05	< 0.05	< 0.05
SDW	df[a]	6	6	36
	MS[b]	43.089	89.939	11.902
	P>F[c]	< 0.05	< 0.05	< 0.05
RDW	MS[b]	572.202	8168.527	543.003
	P>F[c]	< 0.05	< 0.05	< 0.05

[a] Degrees of freedom.
[b] Mean square.
[c] Probabilities associated with individual F tests.

Disease severity index (DSI).

Disease severity index data of the different local germplasms inoculated with the soilborne plant pathogenic fungi are presented in Table 2. Local melon germplasms showed high resistance to FOM where DSI ranged from 0.67 (M9.1) to 0.89 (M12), with an exception of M17, for which a DSI of 1.1 was recorded. Local watermelon germplam P7 was resistant to FON with 0.33 as DSI. All local germplasm showed high resistance to FSC recording the lowest values ranging between 0.56 and 0.89. Germplasms M9.1 and V4R3 with P7 behaved as resistant against *Mm. phaseolina* with 0.33 and 0.11, respectively. M9.1 was resistant to *Ms. cannonballus* (0.54) but susceptible to *Ms. eutypoides* (1.11), P6-1 and P7 were both resistant to both species (Table 4).

Table 4. Disease severity index noted on five melon (M16, M17, M12, M9.1 and V4R3) and two watermelon (P6-1 and P7) local germplasms after their inoculation with *Fusarium* sp. FOM, FON, FSC, *Macrophomina phaseolina* (MP), *Monosporascus cannonballus* (MC),and *Ms. eutypoides* (ME)

Local germplasms	Disease severity index[a]						
	Control	*FOM*	*FON*	*FSC*	*MP*	*MC*	*ME*
M16	0	0.78[b]±0.01ab	-	0.68±0.01a	0.89±0.04ab	0.67±0.01a	0.56±0.01bc
M17	0	1.112±0.01a	-	0.66±0.01a	1.33±0.02a	0.56±0.01a	0.78±0.01ab
M12	0	0.89±0.05ab	-	0.67±0.02a	0.56±0.01bc	0.56±0.02a	0.56±0.04bc
M9.1	0	0.67±0.01b	-	0.56±0.02a	0.33±0.01bc	0.54±0.02a	1.11±0.04a
V4R3	0	0.78±0.02ab	-	0.63±0.02a	0.33±0.01bc	0.67±0.02a	0.56±0.04bc
P6-1	0	-	1.11a	0.89±0.01a	1.22±0.02a	0.67±0.01a	0.11±0.01c
P7	0	-	0.33±0.01b	0.89±0.01a	0.11±0.02c	0.67±0.01a	0.22±0.01c
***P* values**	-	< 0.05	< 0.05	0.445	< 0.05	0.886	< 0.05

[a] Disease severity index per each fungus (three plants/replication).
[b] Means ± standard error in the column followed by the same letter are not significantly different according to χ^2 test at $P < 0.05$.
FOM: *Fusarium oxysporum* f. sp. *melonis*
FSC: *F. solani* f. sp. *cucurbitae*
FON: *F. oxysporum* f. sp. *niveum*

Leaf alteration index (LAI).

The lowest leaf alteration index was recorded on melon local germplasm M9.1 inoculated with FOM (0.66) and on local watermelon germplasm P7 inoculated with FON with a LAI of 0.55. Additionally, three local melon germplasms, namely M12, M9.1, and V4P3, showed low susceptibility to FSC with a LAI of 0.55. Local germplasm exhibiting resistance to *Mm. phaseolina* were V4P3 and P7 with 0.11 and 0.18 as LAI values, respectively. Both M17 and M12 inoculated with *Ms. cannonballus* recorded the lowest leaf alteration index with 0.29 and 0.33, respectively. For the second *Monosporascus* species, P6-1 showed resistance to *Ms. eutypoides* with 0.18 (Table 5).

Table 5. Leaf alteration index of the five melon (M16, M17, M12, M9.1 and V4R3) and two watermelon local germplasms (P6-1 and P7) after inoculation with *Fusarium* sp. FOM, FON, FSC, *Macrophomina phaseolina* (MP), *Monosporascus cannonballus* (MC), and *M. eutypoides* (ME)

Local germplasms	Leaf alteration index (LAI)[a]						
	Control	***FOM***	***FON***	***FSC***	***MP***	***MC***	***ME***
M16	0	0.85[b]±0.01ab	-	0.63±0.01ab	0.62±0.02bc	0.66±0.01ab	0.59±0.02b
M17	0	0.7±0.04ab	-	0.85±0.01ab	0.88±0.02ab	0.29±0.01b	0.44±0.02bc
M12	0	0.99±0.04a	-	0.52±0.01b	0.26±0.04d	0.33±0.01b	0.40±0.02bc
M9.1	0	0.66±0.05b	-	0.55±0.02b	0.33±0.04cd	0.37±0.01b	1.03±0.02a
V4R3	0	0.81±0.05ab	-	0.55±0.02b	0.11±0.01d	0.62±0.01ab	0.44±0.01bc
P6-1	0	-	1.03±0.05a	0.92±0.02a	1.14±0.05a	0.88±0.01a	0.18±0.01c
P7	0	-	0.55±0.01b	0.92±0.02a	0.18±0.01d	0.88±0.01a	0.41±0.01bc
***P* values**	-	< 0.05	< 0.05	0.0507	< 0.05	< 0.05	< 0.05

[a] Leaf alteration index per each fungus (three plants/replication).
Means ± standard error in the column followed by the same letter are not significantly different according to χ^2 test at $P < 0.05$.
FOM: *Fusarium oxysporum* f. sp. *melonis*
FSC: *F. solani* f. sp. *cucurbitae*
FON: *F. oxysporum* f. sp. *niveum*

Shoot and root dry biomass rates.

Shoot dry biomass.

Obtained results showed a low susceptibility of both local melon germplasms M16 and M17 to FSC with SDW of 15.34 and 11.31%, respectively. The highest SDW values were recorded on the majority of melon local germplasms inoculated with FOM where recorded values were comprised between 8.09 and 11.21%. Local watermelon germplasm P7 inoculated with FON showed the highest SDW value about10.42%. Both M9.1 and V4P3 local melon germplasms inoculated with all fungi species presented the lowest values of shoot dry biomass with values comprised between 7.02 and 9.09% (Table 6).

Table 6. Shoot dry biomass of the five melon (M16, M17, M12, M9.1 and V4R3) and two watermelon (P6-1 and P7) local germplasms after inoculation with *Fusarium* sp. FOM, FON, FSC, *Macrophomina phaseolina* (MP), *Monosporascus cannonballus* (MC), and *Ms. eutypoides* (ME)

Local germplasms	Shoot dry biomass (%)[a]						
	Control	*FOM*	*FON*	FSC	MP	*MC*	*ME*
M16	11.54[b]±0.8a	13.7±1.2a	10.56±0.8a	15.34±1.24a	9.42±0.04b	9.11±0.01b	7.22±0.02d
M17	9.18±0.12b	10.40±0.8b	10.76±0.8a	11.31±1.07b	10.82±0.04a	10.21±0.01a	10.5±0.01b
M12	9.25±0.02b	11.21±0.9b	10.29±0.9ab	9.55±0.09c	9.89±0.01b	8±0.01c	9.21±0.01b
M9.1	9.09±0.01b	8.09±0.07c	7.27±0.06c	7.7±0.01d	7.02±0.02d	7.3±0.02c	7.25±0.01d
V4R3	9.03±0.01b	8.98±0.07c	7.21±0.06c	8.62±0.01cd	7.94±0.02c	8.1±0.02c	8.53±0.02bc
P6-1	10.12±0.01b	10.42±0.9b	7.19±0.06c	8.59±0.01cd	8.23±0.04c	7.49±0.02c	8.12±0.02cd
P7	9.18±0.01b	10.42±0.9b	9.48±0.7b	8.59±0.01cd	8.16±0.04c	7.49±0.02c	8.09±0.02d
***P* values**	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

[a] Shoot dry biomass to each fungus (three plants/rep).
[b]Means ± standard error within a columnfollowed by the same letter are not significantly different according to χ^2test at $P < 0.05$.
FOM: *Fusarium oxysporum* f. sp. *melonis*
FSC: *F. solani* f. sp. *cucurbitae*
FON: *F. oxysporum* f. sp. *niveum*

Root dry biomass.

The highest root dry biomass values were noted on two local melon germoplasms M17 and M12 among all treatments, M17 inoculated with FON and MP with 53.5 and 53.32%, respectively, and M12 inoculated with ME, FSC and FON with values comprised between 42.32 and 45.19%. Concerning local watermelon germoplasm P6-1 inoculated with MC and MP, RDW values ranged between 12.4 and 11.75%, respectively, compared to 8.02% noted in control plants (Table 7).

DISCUSSION

Intraspecific grafting, in which the rootstock and the scion belong to the same species, is common for tomato, for which a large collection of tomato rootstocks that vary in specific traits is available (61). In cucurbits, however, interspecific grafting is common. Intraspecific grafting that is grafting melon on melon rootstocks (18) or watermelon on watermelon rootstocks (33, 49), could prevent the fruit-quality problems resulting from interspecific grafting. However, developing such rootstocks requires finding sets of resistance that are absent or unknown in commercial watermelon cultivars and breeding the multi-resistant rootstocks. In the survey for resistant melon and watermelon germplasm, some important pathogens were screened for their interactions with local melon and watermelon germplasms. The two local melon (*C. melo*) germoplasms, M9.1 and V4R3, were highly resistant. According to disease severity index (DSI) and leaf alteration index (LAI) data, all these inoculated local germplasms showed a high resistance to the six fungal pathogens where these parameters ranged from 0.37 (P7) to 0.76 (M17) and from 0.42 (M12 and V4R3) to 0.69 (P6.1), respectively. The importance of disease severity index in the discrimination between resistant and susceptible genotypes was reported in many

researches (24, 62). The variation in leaf alteration scores depending upon crops and the pathogen indicated that the mechanisms involved in the control of these fungal diseases were not similar and confirmed the significant interaction between local germplasms and pathogens ($P < 0.05$).

Table 7. Root dry biomass of the five melon (M16, M17, M12, M9.1 and V4R3) and two watermelon (P6-1 and P7) local germplasms after inoculation with *Fusarium* sp. FOM, FON, FSC, *Macrophomina phaseolina* (MP), *Monosporascus cannonballus* (MC), and *Ms. eutypoides* (ME)

Local germplasms	Root dry biomass (%)[a]						
	Control	*FOM*	*FON*	*FSC*	*MP*	*MC*	*ME*
M16	17.17[b]±0.21c	24.15±1.05[b]	27.51±0.05b	33.73±0.04c	20.25±2.02c	19.11±0.03b	11.11±0.8c
M17	25.18±0.01b	37.23±1.33a	53.5±3.05a	47.83±0.05a	53.32±3.02a	29±1.02a	33.15±0.8b
M12	26.97±0.01b	22.51±0.05b	27.31±0.44b	44.53±0.05ab	35.5±0.02b	29.39±1.02a	45.19±3.6a
M9.1	28.64±0.02b	25.37±0.06b	29.16±0.01b	24.02±0.05d	36±0.02b	32.72±2.56ab	32.22±2.01b
V4R3	38.16±0.04a	18.47±0.06b	13.77±0.01c	38.33±0.06bc	10.38±0.02d	17.27±1b	16.72±0.2c
P6-1	8.02±0.04d	8.41±0.05c	8.86±0.02c	9.09±0.01e	11.75±0.01d	12.4±0.9b	9.85±0.05c
P7	9.74±0.04d	8.41±0.05c	11.11±0.02c	9.09±0.01e	9.47±0.01d	12.4±0.9b	8.78±0.05c
***P* values**	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

[a] Root dry biomass to each fungus (three plants/rep).
[b]Means ± standard error within a columnfollowed by the same letter are not significantly different according to χ^2test at $P < 0.05$.
FOM: *Fusarium oxysporum* f. sp. *melonis*
FSC: *F. solani* f. sp. *cucurbitae*
FON: *F. oxysporum* f. sp. *niveum*

At the end of the assay, most of the accessions showed symptoms to all pathogens, however, FOM and *Mm. phaseolina* were the most re-isolated. The roots are not immune to the pathogen; this latter penetrates through the roots and could be found in root extracts, similar to the response of *Cucurbita* rootstock (40). Previous studies suggested that the vigor root system induced the plant development even in the presence of soilborne pathogens (21, 33). Wild *Cucumis* species, which belong to the subgenus *Melo*, have been reported to possess resistance to some melon (*C. melo*) diseases such as Fusarium wilt (3, 55, 71). The most alteration of leaves was observed on local watermelon germplasm P6-1 inoculated with *Mm. phaseolina* (1.14) whereas the lowest value was noted on local melon germplasm V3R4 inoculated by the same pathogen. Ambrosio *et al.* (4) reported the resistance of seven *C. melo* accessions against *Mm. phaseolina*, one cantaloup from PTO, one common accession from Korea, two wild *agrestis* and one *acidulus* from Africa and two *dudaim* accessions from Middle East. The screened 22 exotic watermelon resistance against Fusarium wilt caused by FON, Fusarium crown rot caused by *F. oxysporum* f. sp. *radicis-cucumerinum*, *Mm. phaseolina* and *Ms. cannonballus* exhibited various responses to the tested pathogens indicating high levels of resistance and no negative effect on fruit quality (24). In addition, phytopathological data on such a

germplasm collection could serve as a tool for studying the resistance mechanisms and the genetics of disease resistance (24). In Tunisia, most of watermelon and melon seedlings are grafted onto *Cucurbita* rootstocks, namely *Cucurbita moschata*, *C. maxima*, *C. pepo*, *Benincasa hispida*, *Lagenaria siceraria*, and *Sicyos angulatus* (49, 50). Jebari *et al.* (46) showed that grafting Pancha and Protéo on the rootstocks Strongtosa and TZ-148 enhanced plant growth and increased early and total yield as well as weight of fruits, compared to control treatments. However, grafted plants wilted towards the end of the culture. On the other hand, most of the plants grafted on the rootstock Emphasis wilted after plantation, probably due to the attack by *Pythium* spp. (46). Aounallah *et al.* (6) found rootstocks showing resistance to FSC and were recommended for the grafting. Other rootstocks like Strongtoza, TZ148, Emphasis, Polifemo and Ercole (14) and GV100 and Just (15) ascendants of *Citrullus colocynthis* and hybrids (17) showed resistance to FSC and FON. An assessment of eight *Cucurbita* hybrid rootstocks resistance to *Ms. cannonballus* was conducted in a greenhouse experiment. Kasuko F1, Carnivor F1 and Citrus F1 appeared to be resistant to *Ms. cannonballus* (9).

To our knowledge, this work is the first screening of local cucurbit germplasms for resistance to soilborne fungi. Horticultural traits such as shoot and root dry biomass (SDW and RDW) showed a significant variation between local melon and watermelon germplasms. In fact, the highest values of SDW and RDW were found for M16/FSC and M17/FON combinations, respectively. However, the lowest values were recorded on the two local watermelon germplasms. Grafting watermelon on watermelon rootstocks has been studied and the examined exotic watermelon accessions did not adversely affect fruit quality and can be used as a basic germplasm for watermelon rootstock breeding (39).

Cohen *et al.* (20) studied *Mm. phaseolina* management using grafted plants or soil application of fungicides to non-grafted melons during the growing season, two Ananas-type melons cv. 6405 and Eyal, were grafted onto interspesific F1 *Cucurbita* rootstock TZ-148. None of the tested melon cultivars was immune to all the soilborne plant pathogenic fungi as *Ms. cannonballus*, *Mm. phaseolina* and *Rhizoctonia solani*. However, Salari *et al.* (66) reported that two melon cultivars were moderately resistant to all the three fungi under greenhouse conditions. The disease management achieved from tolerant rootstocks could be less consistent due to environmental factors or high inoculum pressure challenging tolerance (19, 33). Unfortunately, not all rootstocks have resistance to every target pathogen. For example, some rootstocks for watermelon are resistant to FON, but rootstocks used for the management of *Mm. cannonballus*, *Phytophthora capsici* and *Verticillium dahliae* in watermelon are only tolerant to these pathogens (33). However, the use of tolerant rootstocks in combination with additional cultural practices or pesticides can provide high levels of efficacy (44, 74).

To conclude, the presence of several genetic sources of resistance to the six soilborne pathogens in the accessions assessed had two advantages. Firstly the exploitation of the pool genes for further breeding program and secondly the limitation of the possibility of creation of new fungal adapted species.

RESUME

Boughalleb-M'Hamdi N., Ben Salem I., Bnejdi F. et M'Hamdi M. 2016. Évaluation de la résistance des porte-greffes locaux de melon et de la pastèque vis-à-vis de six champignons phytopathogènes telluriques en Tunisie. Tunisian Journal of Plant Protection 11: 191-206.

Cinq germoplasmes locauxde melon (M16, M17, M12, M9.1 et V4R3) et deux de pastèque (P7 et P6.1) ont été évalués sous serre pour leur résistance à *Fusarium oxysporum* f. sp. *melonis*, *F. solani* f. sp. *cucurbitae*, *F. oxysporum* f. sp. *niveum*, *Monosporascus cannonballus*, *M. eutypoides* et *Macophomina phaseolina* en mesurant l'indice de sévérité des dégâts racinaires (IDS), l'indice d'altération foliaire (IAF) et le taux de réduction de la biomasse sèche de la tige et des racines. Le test Student-Fisher a révélé une différence significative entre les sept germoplasmes locaux. Les analyses statistiques de la variance ont confirmé l'effet significatif de l'interaction germoplasmes locaux × pathogènes. Selon les quatre paramètres mesurés, la résistance aux six agents pathogènes était variable. Le germoplasme local de melon M9.1 était le plus résistant. Les accessions M6.1 et M17 avaient enregistré une réduction faible de la biomasse sèche de la tige et des racines. Pour la pastèque, les plus faibles valeurs de IDS et IAF ont été enregistrées au niveau de la combinaison P6.1/*M. eutypoides*. En revanche, la présence de l'interaction plante-pathogène a indiqué que le mécanisme de résistance vis-à-vis de chaque agent pathogène varie entre les accessions. La présence de plusieurs sources génétiques de résistance aux cinq pathogènes au niveau des accessions évaluées a deux avantages : l'exploitation des gènes de résistance pour les programmes de sélection des porte-greffes potentiels et la limitation de la possibilité de créer de nouvelles espèces fongiques adaptées.

Mots clés: Germoplasmes locaux, melon, pastèque, pathogènes telluriques, porte-greffes résistants

ملخص

بوغلاب-محمدي نعيمة وابتسام بن سالم وفتحي بناجدي ومحمود محمدي. 2016. تقييم مقاومة الأصول الوراثية المحلية من البطيخ و الدلاع لفطريات التربة. Tunisian Journal of Plant Protection 11: 191-206.

في نطاق البحوث التي قمنا بها تم اختبار مدى مقاومة خمسة أصول وراثية من البطيخ (M16 وM17 و M12 و M9.1 و V4R3) واثنين من الدلاع (P6.1 و P7) في الزراعة المحمية ضد *Fusarium oxysporum* f. sp. *melonis* و *Fusarium solani* f. sp. *cucurbitae* و *Fusarium oxysporum* f. sp. *niveum* و *Monosporascus cannonballus* و *Monosporascus eutypoides* و *Macophomina phaseolina* اعتمادا على مؤشرمرض الجذور ومؤشر تغيير الورقة ونسبة التخفيض للوزن الجاف من الأجزاء الهوائية والجذور. لوحظ وجود فرق معنوي بين الأصول الوراثية المحلية. وأكدت التحليلات الإحصائية تفاعل أصول وراثية × فطريات تربة. ومن بين النتائج كانت هناك درجات متفاوتة بينهما وفقا لتقييم المعايير الأربعة. تبين أن الأصل الوراثي المحلي للبطيخ M9.1 هو الأكثر مقاومة. أما M17 وM6.1 فسجلا انخفاضا طفيفا للكتلة الجافة من الأجزاء الهوائية والجذور. بالنسبة إلى الأصول الوراثية المحلية للدلاع، فإن أقل المعدلات لمؤشرات مرض الجذور وتغيير الورقة سجلت في تركيبة *eutypoides*/*M.* P6.1 إلا أن وجود تفاعل بين النبات وفطريات التربة بيّن أن آلية المقاومة تجاه مسببات الأمراض تتفاوت حسب الأصول الوراثية المحلية. إن وجود مصادر وراثية متعددة لهذه المقاومة لمسببات الأمراض لها مزيتين: استغلال الجينات في برامج تربية الأصول المقاومة المحتملة والحد من إمكانية ظهور أنواع فطريات جديدة متأقلمة.

كلمات مفتاحية: أصول مقاومة، أصول وراثية محلية، بطيخ، دلاع، فطريات تربة، أصول مقاومة

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