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Full Length Research Paper

Reaction of melon (*Cucumis melo* L.) cultivars to soil-borne plant pathogenic fungi in Iran

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The fungi *Macrophomina phaseolina* (Tassi), *Monosporascus cannonballus* (Pollack and Uecker) and *Rhizoctonia solani* (Kuhn) are responsible for significant destruction and melon crop losses in the Sistan region of Iran. In this study, eighteen melon cultivars were screened for resistance to these pathogens under greenhouse conditions twice. The melon cultivars were grown in pots and inoculated with each pathogen individually in three different experiments. None of the tested melon cultivars was immune to all the soil-borne plant pathogenic fungi. However, two cultivars, namely 'Sfidak khatdar' and 'Sfidak bekhat' were moderately resistant to all the three fungi. These melon cultivars are promising sources of resistance to *M. phaseolina*, *M. cannonballus* and *R. solani*, and should be the preferred choice for melon grown in infested areas. This study is the first report on screening of melon cultivars in Iran for resistance to *M. phaseolina*, *M. cannonballus* and *R. solani* and it reports the resistance of melon cultivars to three important soil-borne plant pathogens found worldwide.

Key words: Melon, fungal resistance, *Macrophomina phaseolina*, *Monosporascus cannonballus*, *Rhizoctonia solani*.

INTRODUCTION

Melon is an important dessert fruit in the Sistan region of Iran, but its cultivation is threatened by attacks of *Macrophomina phaseolina* (Tassi), *Monosporascus cannonballus* (Pollack and Uecker) and *Rhizoctonia solani* (Kuhn) (Safarnezhad, 2004). Melon death induced by these soil-borne plant pathogenic fungi has become increasingly severe in many intensively cultivated fields in the Sistan region.

M. phaseolina is a destructive pathogen that causes charcoal rot of melon and other dicotyledonous crops. Chemical management is not feasible in subsistence farming conditions, and the plurivorous nature of the fungus limits the effectiveness of some cultural methods of control. Identification of melon cultivars that are resistant or tolerant to *M. phaseolina* is the most efficient control measure, but no attempt has been made to find out resistance to *M. phaseolina* in melon. Thus, tolerant or resistant melon cultivars are yet be known.

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Monosporascus root rot is an important disease affecting melons worldwide (Martyn and Miller, 1996), and it is now a serious problem in the Sistan region. Specific losses vary annually, but constitute about 10 to 30% of the crop. It is not uncommon for individual fields to suffer complete (100%) loss (Safarnezhad, 2004). The use of cultivars resistant to plant diseases is one of the best control measures, but there are currently no commercially available Monosporascus-resistant cultivars (Cohen et al., 2000). In one study, 'Deltex', an Ananas-type melon, was found to be more tolerant to *M. cannonballus* than commonly used commercial varieties of cantaloupe such as 'Caravelle', a western shipper type. Though chemical control of *M. cannonballus* is possible (Mertely et al., 1991, 1993a), most available chemicals are expensive. Screening experiments have identified several sources of intermediate resistance to *M. cannonballus* (Crosby et al., 2000; Crosby, 2000). Crosby (2001) screened germplasm accessions of the melon (*Cucumis melo* L. var. agrestis), along with commercial melons, for resistance to *M. cannonballus*. Three accessions, 20608, 20747 and 20826, demonstrated high resistance or immunity to *M. cannonballus*.

The Rhizoctonia canker caused by *R. solani* Kühn can damage different parts of the melon plant, causing seed, root and fruit rots, damping-off and stem canker. All these diseases lead either to premature plant death and/or decreased yield (Bruton, 1998; Garciá-Jiménez et al., 1999). *R. solani* control is extremely difficult given that it is a soil-borne pathogen that combines high saprophytic competitive ability with a wide host range (Blancard et al., 1991; Bruton, 1996). To avoid the disease, farmers often abandon infested areas and migrate to non-infested fields. This practice causes large economic losses, due both to the devaluation of the abandoned areas and to the need for reinstalling the production infrastructure in new fields. In this context, the use of resistant cultivars is a strategic measure that forms part of the integrated management of Rhizoctonia canker. Michereff et al. (2008) tested twenty melon genotypes with *R. solani* and reported that the genotypes Sancho, AF-1805, Athenas, AF-682, Torreon and Galileo were highly resistant to two *R. solani* isolates.

In this study, we identified sources of resistance to *M. phaseolina*, *M. cannonballus* and *R. solani* isolated from the Sistan region of Iran among a collection of Iranian melon cultivars.

MATERIALS AND METHODS

In 2010, eighteen melon cultivars, including 'Gandah', 'Sfidak khatda', 'Sfidak bekhat', 'Mollamosi', 'Nabijani', 'Shadegan', 'Zard evanaki', 'Moshi', 'Sooski', 'Jajrood', 'Hajmolashahi' and 'Khaghani' were obtained from the growers (land races) and were collected from several regions of Iran to determine their resistance to *M. phaseolina*, *M. cannonballus* and *R. solani*. The most aggressive isolates of *M. phaseolina*, *M. cannonballus* and *R. solani* are deposited in the Culture Collection of the University of Zabol, and these were used for this study. The fungi were grown on potato dextrose agar (PDA) medium.

Screening for *M. phaseolina* resistance

Sandy-clay soil was autoclaved for 45 min at 80°C, on five consecutive days (Crosby, 2001), then sterilized sandy-clay soil was transferred to pots (20 × 20 cm) and the melon cultivars were sown immediately at a density of 8 seeds per pot. For inoculation, 10-day-old culture discs (5 mm in diameter) of each fungus were placed on the crowns of plants that were 20 to 30 cm in length. The inoculated plants were kept in a greenhouse, with the air temperature ranging from 31 to 33°C.

The experiment was performed with a completely randomized design (CRD) using three replications. Four weeks after inoculation, disease severity was assessed using the scale described by Ravf and Ahmad, (1998), 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.

The average disease severity was calculated for each cultivar and was used to cluster the cultivars in five reaction classes: 0 = immune (SI); 0.1 to 1.0 = highly resistant (HR); 1.1 to 2.0 = moderately resistant (MR); 2.1 to 4.0 = susceptible (SU) and 4.1 to 5.0 = highly susceptible (HS).

Screening for *M. cannonballus* resistance

Inoculum was grown on a mixture of washed sea sand and ground oat hulls, combined at a rate of 45 g of oat hulls to 500 cm^3 of sand. In 1-L flasks, 100 ml of water was combined with 500 cm^3 of this medium and autoclaved twice for 60 min with a 1-day interval. The medium was inoculated with two to three 1-cm^2 pieces of colonized agar cut from a PDA culture (Aegerter et al., 2000). The flasks were kept at room temperature under 12 h of fluorescent light/day for 5 weeks, and yielded 60 colony forming units (CFUs) of *M. cannonballus* per gram of the sand medium (Aegerter et al., 2000; Bruton et al., 1995). Thereafter, 20 × 20 cm pots were filled with 200 g of the sand medium with the inoculum and placed into each 15 cm deep pot. Three replicates of both control and inoculated pots were sown with eighteen melon cultivars. The inoculated plants were kept in a greenhouse at an air temperature of 30°C for up to 50 days. The experiment was performed using a CRD. Seeds were watered and germination was observed. Fifty days after sowing, all plants were carefully extracted from the pots. 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 on a scale of 1 to 5: 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 (Crosby, 2001). The average disease severity was calculated for each cultivar and was used to cluster the cultivars in five reaction classes: 1 = similar to immune (SI); 1.1 to 2.0 = highly resistant (HR); 2.1 to 3.0 = moderately resistant (MR); 3.1 to 4.0 = susceptible (SU) and 4.1 to 5.0 = highly susceptible (HS).

Screening for *R. solani* resistance

R. solani was grown on sterilized rice grains (50 g) in Erlenmeyer flasks that were then kept for ten days in an incubator at 25°C with constant luminosity (Michereff et al., 2008). The colonized substrate was placed in paper bags and dried for 48 h at 30°C with constant luminosity. Later, the substrate was ground in a blender for five minutes and weighed to prepare aliquots for incorporation into the soil. Sandy-clay soil was autoclaved for 45 min at 80°C, on five consecutive days (Crosby, 2001), then sterilized sandy-clay soil was transferred to pots (20 × 20 cm) after infestation with *R. solani* (50 mg of colonized substrate per kg of soil). Melon seeds were sown immediately after soil infestation at a density of 8 seeds per pot. The control treatment consisted of seeds sown in non-infested soil. The plants were kept in a greenhouse at an air temperature ranging from 27 to 35°C. The experiment was performed using a CRD with three replications. Cultivars were evaluated daily for emergence, and 15 days after sowing, disease severity was assessed using the following scale (Noronha et al., 1995) adapted for melon roots: 0 = symptomless; 1 = small lesions on the hypocotyls; 2 = large lesions on the hypocotyls, but no constriction; 3 = full hypocotyl constriction, showing damping-off; and 4 = non-emerged seeds and/or plantlets. The average disease severity was calculated for each cultivar and was used to cluster the cultivars into five reaction classes: 0 = similar to immune (SI); 0.1 to 1.0 = highly resistant (HR); 1.1 to 2.0 = moderately resistant (MR); 2.1 to 3.0 = susceptible (SU); and 3.1 to 4.0 = highly susceptible (HS).

The second screening

In 2011, the most susceptible and resistant cultivars selected after

Table 1. Reaction of melon cultivars to *M. phaseolina*, *M. cannonballus* and *R. solani* in the first screening.

Cultivar	Charcoal rot		Monosporascus root rot		Rhizoctonia	
	Average	Reaction	Average	Reaction	Average	Reaction
Jajrood	4.273[az]	HS	3.663[b]	SU	2.330[c]	SU
Termeh	4.213[a]	HS	3.619[b]	SU	3.010[b]	SU
Soosky	4.163[a]	HS	3.440[bc]	SU	2.997[b]	SU
Janati	4.163[a]	HS	4.439[a]	HS	3.047[b]	SU
Shadgan	4.163[a]	HS	3.000[cd]	MR	2.320[c]	SU
Sadri	4.163[a]	HS	4.390[a]	HS	3.500[a]	HS
Hajmashallahi	4.163[a]	HS	2.943[de]	MR	1.777[d]	MR
Ahmady	4.137[a]	HS	3.629[b]	SU	1.77[d]	MR
Zard evanaki	3.940[a]	SU	4.330[a]	HS	2.497[c]	SU
Chappat	3.901[a]	SU	3.008[cd]	MR	2.384[c]	SU
Khaghani	3.720[a]	SU	4.553[a]	HS	3.053[b]	HS
Zaboly	3.615[a]	SU	3.439[bc]	SU	2.403[c]	SU
Moshi	3.607[a]	SU	3.607[b]	SU	3.500[a]	HS
Mollamosai	2.497[b]	SU	2.607[def]	MR	1.217[e]	MR
Ghandak	2.493[b]	SU	2.720[def]	MR	1.000[e]	HR
Nabijani	2.107[b]	SU	2.273[f]	MR	2.320[c]	SU
Sfidak khatdar	1.940[b]	MR	2.553[def]	MR	1.000[e]	HR
Sfidak bekhat	1.940[b]	MR	2.440[ef]	MR	1.212[e]	MR

[z]Means within a column followed by the same letter are not significantly different at $P = 0.01$ according to the Duncan's multiple range test. HS, Highly susceptible; SU, susceptible; MR, moderately resistant.

Figure 1. *M. phaseolina* inoculated shoots of 'Jajrood' (left) and 'Sfidak khatdar' (right), demonstrating high rot of shoot tissues of plant of 'Jajrood' and less rot of shoot tissues of plant of 'Sfidak khatdar'.

the 2010 trial were screened against the three pathogens, following the method described above for the first trial.

Statistical analysis

All data were subjected to ANOVA and mean separations were assessed by Duncan's multiple range test (DMRT) using MSTAT-C software v.11.0; a *P* value of 0.01 was considered to be significant.

RESULTS

The first screening

Immunity to all the soil-borne plant pathogenic fungi tested, namely *M. phaseolina*, *M. cannonballus* and *R. solani*, was not recorded for any of the cultivars studied (Table 1). Cultivars 'Sfidak khatdar' and 'Sfidak bekhat' were moderately resistant to *M. phaseolina*, while cultivars 'Nabijani', 'Ghandak', 'Mollamosai', 'Moshi', 'Khaghani', 'Zard evanaki', 'Zaboly' and 'Chappat' were susceptible, and cultivars 'Hajmashallahi', 'Shadgan', 'Sooski', 'Jajrood', 'Termeh', 'Janati', 'Sadri' and 'Ahmadi' were highly susceptible. Cultivars 'Sfidak khatdar' and 'Sfidak bekhat' had the lowest levels of disease severity (Table 1). Figure 1 demonstrates the condition of shoot tissues after inoculation of 'Sfidak khatdar' (most resistant cultivar) with *M. phaseolina*, as compared to 'Jajrood' (most susceptible cultivar). Percentage of infected shoot tissues was higher for 'Jajrood' (60%) than for 'Sfidak

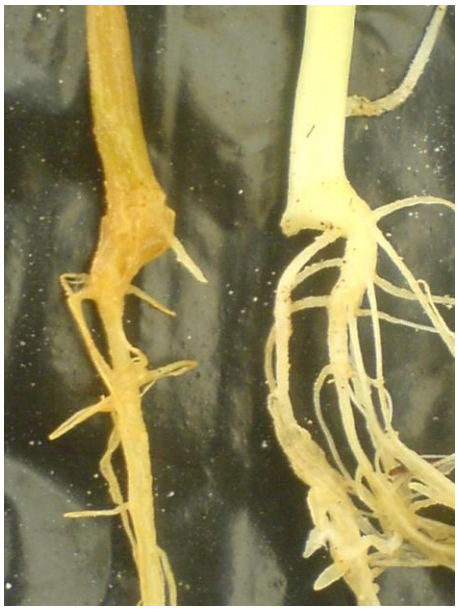

Figure 2. *M. cannonballus* inoculated roots of 'Khaghani' (left) and 'Nabijani' (right), demonstrating more necrosis of fine roots on the plant with introduction of 'Khaghani' and less necrosis of fine roots on the plant with introduction of 'Nabijani'.

khatdar' (15%). Cultivars 'Sfidak khatdar', 'Sfidak bekhat', 'Nabijani', 'Ghandak', 'Mollamosai', 'Chappat', 'Shadgan' and 'Hajmashallahi' were moderately resistant to *M. cannonballus*, while cultivars Moshi, Sooski, Termeh, Ahmady and Jajrood were susceptible, and cultivars 'Zard evanaki', 'Sadri', 'Janati' and 'Khaghani' were highly susceptible. Cultivar 'Nabijani' had the lowest level of disease severity. However, this cultivar was not significantly different from cultivars 'Sfidak khatdar', 'Sfidak bekhat', 'Ghandak' and 'Mollamosai' (Table 1). Figure 2 demonstrates the condition of fine roots, after inoculation of 'Nabijani' (most resistant cultivar) with *M. cannonballus*, as compared to 'Khaghani' (most susceptible cultivar). Percentage of fine roots between 0 and 0.5 mm was higher for 'Nabijani' (71%) than for 'Khaghani' (35%).

Cultivars 'Sfidak khatdar' and 'Ghandak' were highly resistant to *R. solani*, while cultivars 'Sfidak bekhat', 'Mollamosai', 'Hajmashallahi' and 'Ahmady' were moderately resistant; cultivars 'Nabijani', 'Zard evanaki', 'Shadgan', 'Sooski', 'Jajrood', 'Termeh, Janati', 'Cappat' and 'Zaboly' were susceptible and cultivars 'Moshi', 'Sadri' and 'Khaghani' were highly susceptible. 'Sfidak khatdar' and 'Ghandak' had the lowest levels of canker severity. However, these cultivars were not significantly different from cultivars 'Sfidak bekhat' and 'Mollamosai' (Table 1). Figure 3 demonstrates the condition of seedlings, after inoculation of 'Sfidak khatdar' (most resistant cultivar) with *R. solani*, as compared to 'Moshi' (most susceptible cultivar). Percentage of damping-off was higher for 'Moshi' (75%) than for 'Sfidak khatdar' (0%).

Assessment of the resistance of selected cultivars in the second trial

Immunity to all the soil-borne plant pathogenic fungi tested, namely *M. phaseolina*, *M. cannonballus* and *R. solani*, was not recorded for any of the cultivars studied (Table 2). Cultivars 'Sfidak khatdar' and 'Sfidak bekhat' were moderately resistant to *M. phaseolina*, while cultivar 'Ghandak' was susceptible, and cultivars 'Termeh' and 'Janati' were highly susceptible. Cultivars 'Sfidak khatdar' and 'Sfidak bekhat' had the lowest levels of disease severity and were significantly different from cultivar 'Ghandak' (Table 2). Cultivars 'Sfidak khatdar' and 'Sfidak bekhati' were moderately resistant to *M. cannonballus*, while cultivars 'Ghandak' and 'Termeh' were susceptible, and cultivar 'Janati' was highly susceptible. Cultivar 'Sfidak khatdar' and 'Sfidak bekhati' had the lowest level of disease severity, and were significantly different from cultivar 'Ghandak' (Table 2). Cultivars 'Sfidak khatdar', 'Sfidak bekhat' and 'Ghandak' were moderately resistant to *R. solani*, while cultivars 'Termeh' and 'Janati' were susceptible. Cultivars 'Sfidak khatdar' and 'Sfidak bekhati' had the lowest levels of canker severity and were significantly different from cultivar 'Ghandak' (Table 2).

DISCUSSION

M. phaseolina is sensitive to fungicides and the application of fungicide to seeds and soil can reduce fungal germination and infection. However, chemical control of this fungus is difficult and neither profitable nor advisable, because the pathogen is seed- and soil-borne. Moreover, fungicides are too costly for subsistence farmers in Sistan. Melon cultivars that are resistant to or tolerant to *M. phaseolina* would be the most efficient control measure, but these are not yet available. Solarization, the addition of organic matter to the soil, maintenance of high levels of soil moisture, fumigation and the use of biocontrol agents have shown potential in the control of soil-borne pathogens. However, there are no efficient control methods that can be used alone against charcoal rot. The disease pressure can only be reduced, if different preventive control measures are combined in an integrated management strategy.

The results of this experiment provided useful novel information about sources of resistance against *M. cannonballus*. This may be increasingly important in the Sistan region, where continuous melon culture has led to elevated levels of *M. cannonballus* in the soil. The capacity of the plant to restrict damage to the fragile fine roots was demonstrated by several entries. Figure 1 demonstrates this phenomenon in 'Nabijani' (most

Figure 3. *R. solani* inoculated seedlings of 'Sfidak khatdar' (left) and 'Moshi' (right), demonstrating lack of damping-off in 'Sfidak khatdar' seedlings and high incidence of damping-off in of 'Moshi' seedlings.

Table 2. Reaction of melon cultivars to *M. phaseolina*, *M. cannonballus* and *R. solani* in the second screening.

Cultivar	Charcoal rot		Monosporascus root rot		Rhizoctonia	
	Average	Reaction	Average	Reaction	Average	Reaction
Termeh	4.544[az]	HS	3.910[b]	SU	3.511[ba]	SU
Janati	4.391[b]	HS	4.699[a]	HS	3.361[b]	SU
Ghandak	3.328[c]	SU	3.312[c]	SU	1.873[c]	MR
Sfidak khatdar	1.799[d]	MR	2.623[d]	MR	1.415[d]	MR
Sfidak bekhat	1.811[d]	MR	2.617[d]	MR	1.429[d]	MR

[z]Means within a column followed by the same letter are not significantly different at $P = 0.01$ according to the Duncan's multiple range test. HS, Highly susceptible; SU, susceptible; MR, moderately resistant.

resistant cultivar) as compared to 'Khaghani' (most susceptible cultivar).

There was no difference in the speed of emergence caused by *R. solani* among cultivars. Thus, resistance reactions cannot be attributed to shorter exposure to the pathogen in the soil, which would interfere with cultivar response, since *R. solani* is known to act preferentially in young tissues (Baker and Martinson, 1970).

In conclusion, cultivars 'Sfidak khatdar' (moderately resistant to *M. phaseolina*, *M. cannonballus* and *R. solani*) and 'Sfidak bekhat' (moderately resistant to *M. phaseolina*, *M. cannonballus* and *R.* solani) collected from the Sistan region were resistant to all the soil-borne plant pathogenic fungi tested. Therefore, these cultivars are promising sources of resistance to *M. phaseolina*, *M. cannonballus* and *R. solani* and should be a preferential choice for melon grown in infested areas. Screening for, and the development of resistance to, these soil-borne plant pathogenic fungi would be of major benefit to melon growers throughout the Sistan melon-producing region. Successful melon production in areas affected by *M. phaseolina*, *M. cannonballus* and *R. solani* will include breeding for resistance against all these soil-borne plant pathogenic fungi, but the integration of complementary management strategies is required to maximize resistance durability. Among these strategies, field and crop rotation, as well as the destruction of crop remains, can be very effective.

Sources of resistance to some of these soil-borne plant pathogenic fungi, namely *M. cannonballus* (Crosby, 2000, 2001; Crosby et al., 2000; Wolff and Miller, 1998; Wolff, 1996; Mertely et al., 1993b) and *R. solani* (Michereff et al., 2008), have already been identified. However, no attempt has been made to develop a melon cultivar

resistant to multiple soil-borne plant pathogenic fungi. This study is the first report of an experiment that screened melon cultivars in Iran for resistance to *M. phaseolina*, *M. cannonballus* and *R. solani*, and the first report on the screening of melon cultivars for resistance to multiple soil-borne plant pathogenic fungi worldwide.

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