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Screening of *Cucumis melo* L. Cultivars from Iran for Resistance against Soil-Borne Fungal Pathogens

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Abstract

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. 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. A second screening was performed for resistance to these pathogens under greenhouse conditions. In the second screening, '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.

Keywords: 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) [1]. 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 to be known.

Monosporascus root rot is an important disease affecting melons worldwide [2], 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 [1]. 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 [3]. 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 [4-6], most available chemicals are expensive. Screening experiments have identified several sources of intermediate resistance to *M. cannonballus* [7,8]. Crosby [8] screened germplasm accessions of the melon (*Cucumis melo* L. var. *agrestis*), alongwith 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 the stem canker. All these diseases lead either to premature plant death and/or decreased yield [9,10]. *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 [11,12]. To avoid the disease, farmers often abandon infested areas and migrate to non-infested fields. This practice causes large economic losses, due to both 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. [13] 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

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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 [14], 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. 10-day old culture discs of *M. phaseolina*, were used to inoculate the melon. Five-mm-diameter discs (6×10^4 CFUs of *M. phaseolina* per disc) 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 [15], 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

M. cannonballus was grown on a double sterilized mixture of washed sea sand and ground oat hulls (1:10) in 1 L flasks [16]. The flasks were kept at room temperature under 12 h of fluorescent light/day for 5 weeks, and yielded 60 CFUs of *M. cannonballus* per gram of the sand medium [16,17]. 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 upto 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 [14]. 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 [13]. The colonized substrate was placed in paper bags and dried for 48 h at 30°C with constant luminosity. Later, the substrate was grounded in a blender for five minutes and weighed to prepare aliquots for incorporation into the soil. Sterilized sandy-clay soil [14] 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 10 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 [18] 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 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 the least significant difference (LSD) test 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

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

[z]Means within a column followed by the same letter are not significantly different at P=0.01 according to the least significant difference test. HS, Highly susceptible; SU, susceptible; MR, moderately resistant

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

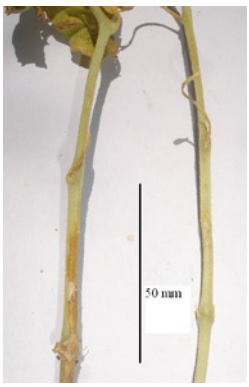

Figure 1: *M. phaseolina* inoculated shoots of 'Jajrood' (left) and 'Sfidak khatdar' (right), demonstrating high rot of shoot tissues of plant of 'Jajrood' and less rot of shoot tissues of plant of 'Sfidak khatdar'.

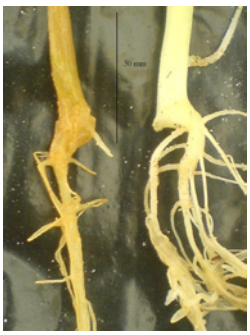

Figure 2: *M. cannonballus* inoculated roots of 'Khaghani' (left) and 'Nabijani' (right), demonstrating more necrosis of fine roots on the plant with introduction of 'Khaghani' and less necrosis of fine roots on the plant with introduction of 'Nabijani'.

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

'Jajrood' (most susceptible cultivar). Percentage of infected shoot tissues was higher for 'Jajrood' (60%) than for 'Sfidak 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' (%).

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 the Sistan region. 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

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

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

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 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 [19].

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* [6-8,14,20,21] and *R. solani* [13], 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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