

Evolution of Some Melon Genotypes for *Fusarium oxysporum* f. sp. *melonis* Race 1 and 1.2 Resistance

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ABSTRACT

Fusarium oxysporum is a major wilt pathogen of many economically important crop plants. Four melon genotypes (Isabelle, Ogon9, NBD-929 and NBD-943) were screened for resistance to *Fusarium oxysporum* f.sp. *melonis* race 1 and 1. 2 using artificial inoculation procedure. The experiment was conducted in the 2019 cropping season in the research greenhouse of the Islamic Azad University, Khorasgan Branch. The analysis of variance showed significant differences within genotypes, *Fusarium* spp pathotype and days after inoculation, and their interactions. Isabelle, Ogon9 and NBD-929 had a good performance when inoculated with race 1 and therefore classified as resistance. The line NBD-943 showed more sever susceptibility to race1 comparing to other genotypes. All of evaluated genotypes were susceptible to race 1.2. Finally, the line NBD-929 was considered as a novel and suitable candidate for future resistance to *Fusarium* race1 in breeding projects of melon.

Keywords: *Cucumis melo* L. *Fusarium* spp. Disease resistance

INTRODUCTION

Melons (*Cucumis melo* L.) are commercially important crops worldwide, which are grown extensively in arid and semi-arid climates. Iran, with a production of 1.73 million tons, is fourth largest melons producer in the world (FAO, 2018).

Melons production faces various abiotic and biotic stresses. This adverse factors can have devastating economic consequences on a large scale in the country. In the melon production season, powdery mildew, downy mildew, *Phytophthora*, *Anthracnose* and *Fusarium* wilt

(FW) are main diseases found in Iran. It caused by *Fusarium oxysporum* f.sp. *melonis* (*F.o.m.*), is a limiting factor for melon production worldwide (Martyn and Gordon, 1996; Mas et al., 1981).

F.o.m. isolates classified into four physiological races (0, 1, 2, and 1.2) Based on the host resistance genes associated with variants of this pathogen (Risser et al., 1976). Two dominant resistance genes, *Fom-1* and *Fom-2*, control resistance to races 0 and 2 and 0 and 1, respectively. Many sources of resistance to *F.o.m.* races 0, 1, and 2 have been reported (Alvarez et al., 2005; Pitrat et al., 1996). However, partial resistance to race 1.2 controlled by polygenic recessive genes was only detected in a few Far Eastern accessions such as Ogon 9 and Kogane Nashi Makuwa (Perchepied and Pitrat, 2004; Risser and Rode, 1973). Quality and availability of resistance source, effective methods of inoculation and evaluation of germplasm resistance to diseases are important factors of successful breeding (Burger et al., 2003). The degree of *Fusarium* wilt varies greatly depending on virulence level of the pathogen, soil type and temperature. However, *Fusarium* wilts may vary from field to field within an area (Sanogo, 2003). Screening of germplasm is also a very important step in identifying a resistant variety. This step may lead to the discovery of new resistance gene(s) which may overcome the new developing pathotypes (Solmaz et al., 2016). The necrotic lesions on old leaves, vascular discoloration, and wilting of plants are the symptoms of *Fusarium* wilt, these symptoms then spread throughout the plant and eventually cause death (Polat et al., 2014). The main objective of this study were to evaluate the response of four genotypes of melon to the wilt disease caused by *Fusarium oxysporum* f.sp. *melonis* and also detect the resistance genotypes for breeding programs.

MATERIAL AND METHODS

Four melon genotypes (Isabelle, Ogon 9 and the breeding lines NBD-929 and NBD-943) were used in this experiment for evaluation of *Fom* 1 and 1.2 resistance. Seeds of Isabelle and Ogon 9 were provided by Michel Pitrat, French National Institute for Agricultural Research (INRA). Seeds of the lines 929 and 943 were developed from breeding projects in NBD Seeds Company. The experiment was conducted in the 2019 cropping season in the research greenhouse of the Islamic Azad University, Khorasgan Branch. Fom *Fusarium oxysporum* f.sp. *melonis* (*F.o.m.*) race 1 and 1.2 was used for artificial inoculation of genotypes according to Madadkhah et al. (2012). The fungal strain was cultured on potato–dextrose–agar (PDA) and was incubated at 22 ± 1 C with a photoperiod of 14 h for 2 weeks for mass production. Spores were washed out from petri-dishes with distilled water and the concentration of spores was adjusted to 1×10^6 spores/ml. To Melon seeds from the tested accessions were sown into sterilized sand and grown in a growth chamber (24 C: 16-h day, 16 C: 8-h night) When the first true leaf began to emerge, seedlings were uprooted, and their roots were washed under running tap water. The plants were introduced into plastic pots containing 200 mL of a Hoagland nutrient solution (Gamborg and Wetter, 1975) and a conidial suspension ($3 \cdot 10^6$ conidia/mL).

Ten seedlings per genotype were inoculated. The symptom evaluation was done 14 d after inoculation according to the scale described subsequently. Disease severity was assessed using the Following scale: 0 = no symptoms; 1 = beginning of wilting or yellowing on leaves; 2 = leaves heavily affected by wilting or yellowing; 3 = all leaves completely wilted, stem standing; and 4 = dead plant. Plants were classified as either healthy, with no symptoms, or diseased, i.e., displaying leaf yellowing with some necrotic spots or wilting symptoms, stunting, or eventual death. The proportion of each class out of the total seedling number was calculated. Plants were rated for symptoms 10, 12, 15, 17 and 24d after inoculation using the 0 to 4 scale described previously.

Analysis of variance was performed for each pathotype to evaluate the significance of the differences in the level of resistance among the genotypes. Means were separated using the Tukey's b test ($\alpha = 0.05$). Data analysis was performed with the Statistical Analysis System SAS 9.3 (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

The analysis of variance (Table 1) showed significant differences within cultivars, *Fusarium* spp pathotype, and this was also observed for the interactions. The cultivar effect differed significantly from the two-way interactions (Cultivar \times *Fusarium* spp pathotype), indicating a relative stability of the resistance expression under different conditions.

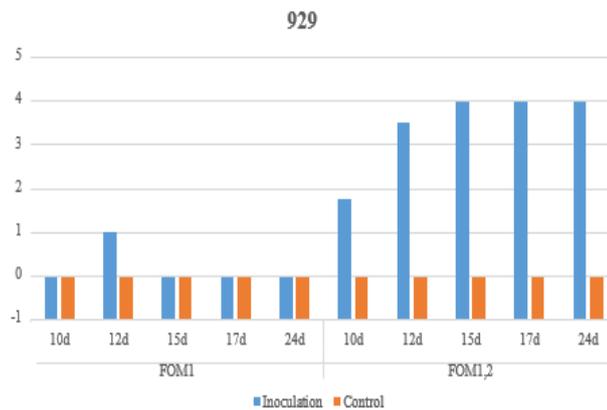
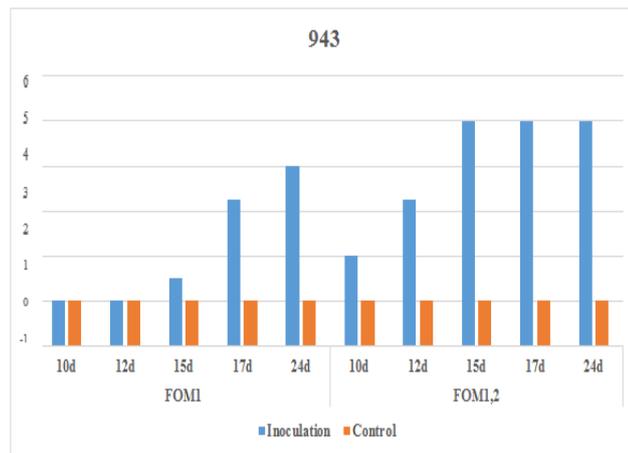
Table 1. Analysis of variance for resistance to *Fusarium* in four melon genotypes

Source of variation	d.f	Mean of square	F value
Cultivar	3	2.07	29.10**
<i>Fusarium</i> spp. pathotype	2	46.36	651.24**
Cultivar \times <i>Fusarium</i> spp. pathotype	6	1.77	24.90**
Error	120	0.07	

Results of the inoculations are shown in Table 2. For this the experiment, ratings for incidence of *Fusarium* wilt were made 10, 12, 15, 17 and 24 days after inoculation. Of the 4 cultivars, 3 were classified as resistant, 1 as mixtures of asymptomatic and symptomatic plants for race1. Cultivar 943 ranked semi-tolerance among genotypes tested, with scores, 0, 0, 0.5, 2.25 and 3 in 10, 12, 15, 17 and 24 days. Our breeding line 929, like the two cultivars Isabelle and Ogon 9, showed complete tolerance to race 1, and all of seedlings remaining healthy under all of the test duration. For race 1, 2 all of the evaluated cultivars showed susceptibility response.

Table 2. reaction of melon genotypes to *Fusarium* pathotype at different duration

Cultivar	<i>Fusarium spp</i> pathotype	Days after inoculation					Results
		10	12	15	17	24	
929	Control	0	0	0	0	0	No infected
	Fom 1	0	1	0	0	0	Tolerant
	Fom 1.2	1.75	3.5	4	4	4	Susceptible
943	Control	0	0	0	0	0	No infected
	Fom 1	0	0	0.5	2.25	3	Semi-tolerant
	Fom 1.2	1	2.25	4	4	4	Susceptible
Ogon 9	Control	0	0	0	0	0	No infected
	Fom 1	0	0	0	0	0	Tolerant
	Fom 1.2	0	0.3	0.3	1.3	4	Semi-susceptible
Isabell	Control	0	0	0	0	0	No infected
	Fom 1	0	0	0	0	0	Tolerant
	Fom 1.2	0	0.25	1	3.5	4	Susceptible



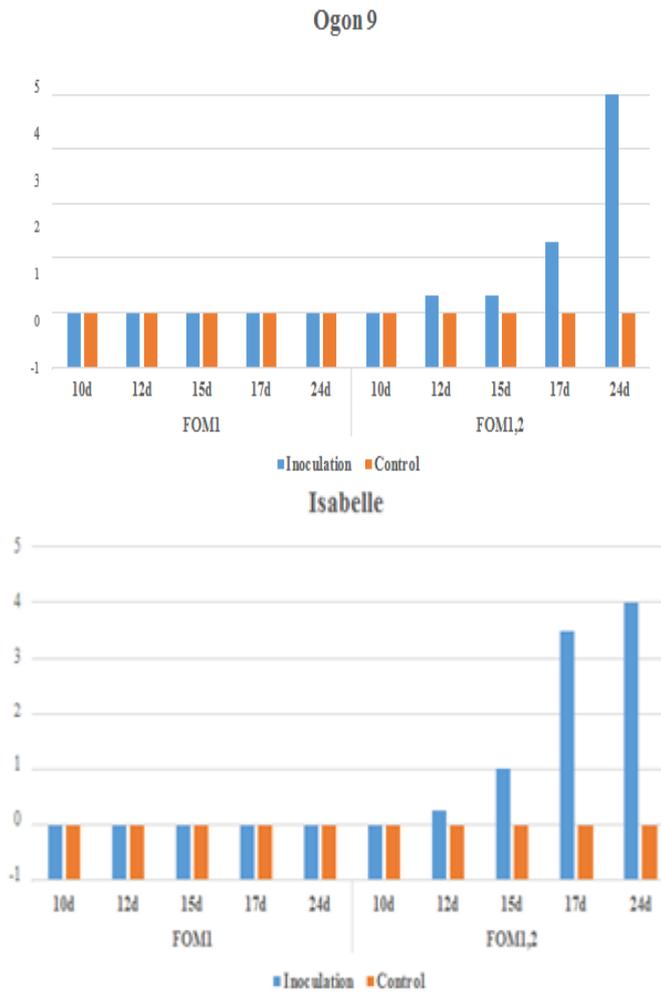


Figure 1. Reaction of four melon genotypes to *Fusarium* race1 and race 1.2

Figure 1 shows the trend of *Fusarium* resistance in four evaluated cultivars. For race 1, Isabel and Ogon 9 not shown any symptom of *Fusarium* wilt throughout the test period, indicating the high resistance of these cultivars to this pathotype. All seedling of breeding line 929 remained healthy during the entire experimental period and their health was monitored daily based on their symptoms. Therefore, this breeding line can be used for later hybridization program.

The other breeding line, 943, seedling showed no symptoms at 10 and 12 days after inoculation and at next days, seedlings exhibited more severe symptoms. At the 10 days after inoculation (DAI), the breeding lines 929 and 943 showed symptoms and cultivars Ogon9 and Isabelle also exhibited susceptibility at the 15 days after inoculation. Eventually, all evaluated seedlings died on the 24th day after inoculation.

Fusarium spp have a global distribution and are economically important and as infective agents of crops (Leslie and Summerell, 2006). Screening of genetic material for *Fusarium* resistance and comparing them to control cultivars is a prerequisite for resistance breeding programs. In this study our breeding lines had similar performance to resistance

cultivar (Isabelle and Ogon9) when inoculated with *Fusarium* race 1 and race1, 2. Early and accurate detection of phytopathogenic through phenotypic taxonomy methods is used routinely in plant pathology laboratories, improves the quantity and quality of crop through the implementation of various strategies to control plant diseases (Sanzani *et al.*, 2014). Although rot caused by *Fusarium* spp. has been controlled with synthetic fungicides, the use of biocontrol agents and plant extracts can be an efficient alternative for the control of various species of *Fusarium* (Zhao *et al.*, 2013; Gopi and Thangavelu, 2014; Vargas-Gonzalez *et al.*, 2016). Finally our breeding line, 929 showed resistance to *Fusarium* race 1 in this experiment, which is suggested to be further studied as a suitable candidate in breeding programs.

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