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Physiological Races of *Fusarium oxysporum* f.sp. *melonis* in Çeşme Melon Producing Areas of Urla Peninsula, Turkey

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ABSTRACT

Fusarium oxysporum f.sp. *melonis* (FOM), the causal agent of Fusarium wilt of melon, has various physiological races specialized to infect particular melon varieties. The aim of this project was determining the races of FOM causing wilting on 'Çeşme Melon', a widely-grown melon variety in Urla Peninsula. A total of 44 pathogenic FOM isolates collected from 'Çeşme Melon' fields in 2009 were subjected to race determination. Race of each isolate was identified by testing their virulence on a set of differential melon cultivars. Results indicated that all four races of FOM (Race 0, 1, 2 and 1.2) were found in the area. Race 1, represented by 24 isolates, was the most prevalent one. Race 0 and race 1.2, each was represented by 8 isolates and race 2 by 4 isolates. The findings will be beneficial for the researchers who are interested in managing Fusarium wilt of 'Çeşme Melon'.

Keywords: *Fusarium oxysporum* f.sp. *melonis,* Fusarium wilt, Melon, Çeşme Melon, Physiological races.

ÖZET

Urla Yarımadası Çeşme Kavunu Üretim Alanlarında *Fusarium oxysporum* f. sp. *melonis*'in Fizyolojik Irkları

Kavun Fusarium Solgunluğunun etmeni olan *Fusarium oxysporum* f.sp. *melonis (FOM),*'in belirli kavun çeşitlerine özelleşme gösteren çeşitli fizyolojik formları bulunmaktadır. Bu çalışma Urla Yarımadasında yaygın olarak yetiştiriciliği yapılan Çeşme Kavununda solgunluğa neden olan FOM ırklarının belirlenmesi amacıyla gerçekleştirilmiştir. 'Çeşme Kavunu' üretim alanlarında 2009 yılında toplanmış olan 44 patojenik FOM izolatı ırklarının belirlenmesi amacıyla testlenmiştir. Irk tanısı, izolatların bir dizi ayırıcı kavun çeşidine gösterdikleri virülenslik derecelerine bakılarak yapılmıştır. Çalışma sonuçları, FOM'un dört ırkının da (Irk 0, 1, 2 ve 1.2) bölgede varolduğunu ortaya koymuştur. Dört ırk arasında, 24 izolat ile temsil edilen ırk 1 yörede en yaygın ırk olarak bulunmuştur. Irk 0 ve ırk 1.2 den her biri 8'er izolat ile temsil edilirken ırk 2 ise 4 izolat ile temsil edilmiştir. Bu çalışmaki bulgular, Çeşme Kavunu Fusarium Solgunluğunun mücadelesine yönelik olarak çalışacak araştırıcılara yardımcı olacaktır.

Anahtar kelimeler: *Fusarium oxysporum* f.sp. *melonis,* Fusarium Solgunluğu, Kavun, Çeşme Kavunu, Fizyolojik ırklar

INTRODUCTION

Fusarium wilt caused by *Fusarium oxysporum* f.sp. *melonis* (Leach & Currence) Snyder & Hansen *(FOM)* is an economically important disease of melon worldwide (Jacobson and Gordon 1991; Chikh-Rouhou et al., 2013). The disease can be destructive in the favorable soil conditions and may cause crop losses of up to 100% (Luango et al., 2015). The pathogen first colonizes the root cortex then invades the vascular tissues, specifically the xylem, thus leading disruption of absorption and translocation of water within the plant (Michielse and Rep, 2009). The management of the disease is quite problematic since FOM is capable of surviving in the soil by colonizing crop residue and staying viable as chlamydospores for decades (Zuniga et al., 1997). The use of disease-resistant varieties is the most effective and economical method for controlling Fusarium wilt (Zink, 1992; Chikh-Rouhou et al., 2010; Sebastiani et al., 2017). To manage the disease successfully with the use of resistant varieties, proper variety selection regarding with the pathogen variants should be taken into consideration (Schreuder et al., 2000) .

F. oxysporum f.sp. *melonis* has various physiological races, which exhibit pathogenic specialization on particular melon varieties (Risser et al., 1976). This specialization is based on the host resistance genes associated with the four different races of *FOM*, designated as race 0, 1, 2, and 1.2. Resistance to race 1 and race 2 is controlled by single dominant genes *FOM*-2 and FOM-1, respectively (Risser et al, 1976). Both genes confer resistance also to race 0, but not race 1.2. Resistance to race 1.2 is complex and partial, probably involving multiple recessive genes (Perchepied and Pitrat, 2004).

The determination of the predominant *FOM* races in certain geographical melon-growing regions was the subject of great interest for the subsequent purposes of disease management and breeding (Zuniga et al., 1997; Schreuder et al., 2000; Kurt et al., 2002; Chikh-Rouhou et al., 2013). The race diversity of FOM in different melon growing sites of Turkey and reactions of numerous melon varieties and lines to FOM have been previously studied (Yıldız, 1977; Erzurum et al., 1999; Kurt et al., 2002; Şensoy et al., 2007) . 'Çeşme Melon', a local variety mainly grown in Urla Peninsula, is known to be susceptible to *FOM*. Since cultivation area is both limited and used intensively in Urla Peninsula, losses due to soil borne diseases in the production of 'Çeşme Melon' have been gradually risen. No updated scientific data on Fusarium wilt for the area are available, but yield losses as high as 80% were estimated by the growers (Personal communication). 'Çeşme Melon' is a high value crop in local markets so that losses have economic significance. Taking into account in subsequent management of Fusarium wilt, this study was performed to determine the *FOM* races in 'Çeşme Melon' producing areas in Urla Peninsula.

MATERIALS AND METHODS

Sampling and Isolation of FOM Isolates

Roots and crowns of the plants exhibiting Fusarium wilt symptoms (yellowing, vascular discoloration and wilting) were collected from 63 commercial 'Çeşme Melon' fields in three counties, including Çeşme, Urla and Karaburun, in Urla Peninsula in July-August 2009. Geographical coordinates of each sample were recorded by a hand held GPS device (Model Etrex Vista, Garmin International Inc.) and illustrated on a map in Figure 1. Tissue samples were placed in paper bags and transferred to laboratory for isolation. Each sample was washed under running tap water for approximately 30 seconds and air-dried at room temperature prior to isolation. Five or six tissue pieces cut from each sample were surface disinfected in a 2% solution of NaOCl for 3 min, then rinsed three times in sterilized water, and blotted dry of excess moisture on sterile filter paper. The sterilized pieces were placed in a 10-cm-diameter petri plate containing PDA amended with antibiotic (streptomycin sulfate 100 ml/L). After 7-10 days of incubation, colonies having Fusarium-like appearance were selected and transferred to potato dextrose agar (PDA) plates. Isolates were grown on carnation leaf agar (CLA) (Fisher et al., 1982) and the ones that produced three- to five-septate, sickle-shaped macroconidia with a foot-shaped basal cell, ellipsoid microconidia borne in false heads on short monophialides, and chlamydospores, were identified as *F. oxysporum* according to Leslie and Summerell (2006). Single microconidia cultures of all isolates of *F. oxysporum* were made and isolates were stored at 4°C in test tubes containing PDA.

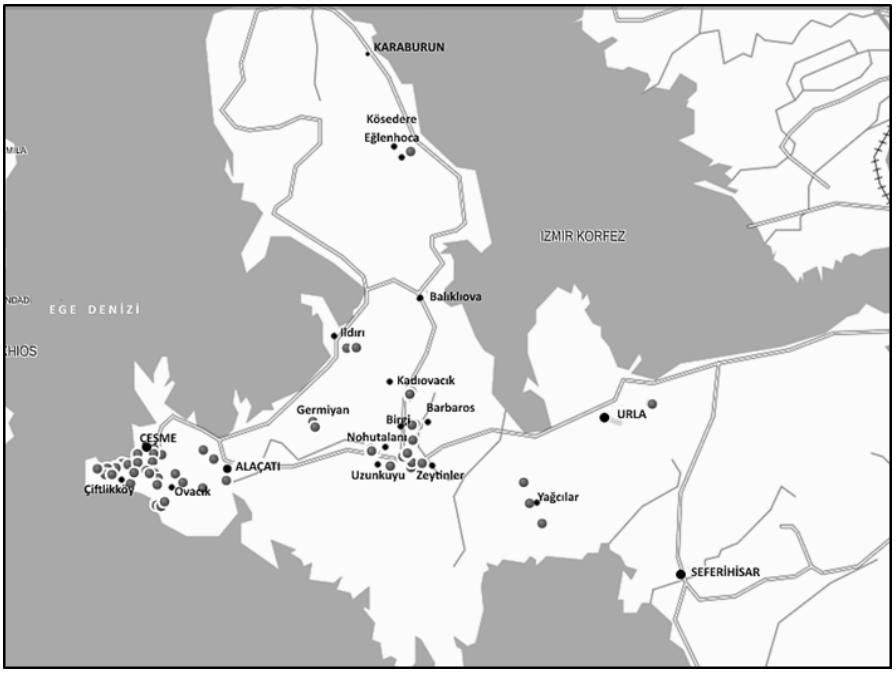

Fig 1. Map of the area where *Fusarium oxysporum* f.sp. *melonis* were sampled. Green dots are the sampling points.

Pathogenicity Tests

Pathogenicity of the isolates was performed by using root-dipped inoculation method (Burger et al., 2003). A spore (microconidia) suspension for each isolate was obtained from the culture grown in a potato dextrose broth (PDB) medium on a rotary shaker (125 rpm) under 12-h photoperiod at 25^{o} C for 8-10 days. Following the filtering of the suspension through two layers of cheesecloth, the concentration of the spore suspension was determined with a hemacytometer and adjusted to 10^{6} conidia/ml. Seedlings of cv. 'Ananas', known as susceptible to all races of FOM, were grown in a sterilized mixture of peat and sand (1:1 v/v). Two-week-old seedlings having fully expanded leaves were removed from the soil and their roots were first washed gently with water, then cut 1/3 of the root tips. Seedlings were inoculated by dipping the roots into the spore suspension for 5 min. Control plants were treated with only sterile PDB solution without inoculations. Seedlings were transplanted into pots (8 cm diameter) containing sterile soil and placed in a growth chamber under 14-h daily photoperiod at 25^{o} C. Nine seedlings were used per isolate and each three were grouped into one that served as a replication. Plants were evaluated for the presence of Fusarium wilt symptoms (yellowing, vascular discoloration, wilting and death) 14 days after inoculation. Isolates causing wilt incidence of ≥33% were identified as pathogenic and <33% were considered as non-pathogenic (Zhou et al., 2010).

Formae specialis determination

For formae specialis determination, randomly selected 44 pathogenic isolates of FOM were tested in cross pathogenicity on the susceptible varieties of cucumber (cv. 'Çengelköy'), watermelon (cv. 'Sugar Baby') and squash (cv. 'Sakız'). Inoculum preparation, planting, inoculation and disease rating were performed as described earlier in the pathogenicity test.

Race determination

Virulence tests for race determination were performed using seven differential melon cultivars based on the theory suggested by Risser et al. (1976). These cultivars were Charentais T', 'Vedrantais', 'Doublon' 'Isovac', 'CM 17187', 'Margot' and 'Isabelle', and presented in Table 1 (Risser et al., 1976; Perchepied and Pitrat, 2004). The

seeds of differential cultivars were provided by other researchers in Turkey and National Germplasm Resources Laboratory (ARS, USDA, Beltsville, Maryland, USA). Seeds of each cultivar were produced on the plants that were grown in isolated chambers and received a hand-polination treatment to maintain genetic purity. Forty pathogenic *FOM* isolates and a tester isolate for each race were tested on each of these cultivars. Inoculum preparation, planting, inoculation and incubation were performed using the same procedures described in the pathogenicity test. For each cultivar-isolate combination, 9 seedlings were inoculated. There were three pots for each cultivar-isolate combination and each pot had three seedlings serving as one replication. The incidence of seedlings exhibiting typical symptoms of Fusarium wilt (yellowing, stunting, wilting and death) was recorded 14 days after inoculation. Mean of wilt incidence for each cultivar-isolate combination was averaged across replications. Within each cultivar-isolate combination, any differantial cultivar giving mean wilt incidence of ≥33% was considered as susceptible and <33% was considered as resistant (Zhou et al., 2010).

Table 1. Differential melon cultivars used to identify races of *Fusarium oxysporum* f. sp. *melonis.*

Differential melon cvs. And their genes for resistance	Races of FOM			
	Race 0	Race 1	Race 2	Race 1.2
Charentais T	S	S	S	S
Vedrantais, Doublon FOM-1	R	S	R	S
Isovac, CM 17187 FOM-2	R	R	S	S
Margot	R	R	R	S
Isabelle (polygenic)	R	R	R	R

S=Susceptible; R=Resistant (Risser et al., 1976; Perchepied and Pitrat, 2004)

RESULTS AND DISCUSSION

Isolate characterization and race determination

On the basis of morphological characteristics of the isolates on CLA, 165 isolates were identified as *Fusarium oxysporum*. Pathogenicity tests revealed that 88 isolates were found to be pathogenic on melon cv. Ananas. The number of isolates found nonpathogenic in the pathogenicity test is quite high representing as nearly as half of the total isolates. It has been known that the nonpathogenic forms of *F. oxysporum* are very common in nature and they can effectively colonize the plant rhizosphere and roots without causing any symptoms (Gordon and Martyn, 1997). The growing of 'Çeşme Melon' in Urla Peninsula over the years probably has given a good opportunity for both pathogenic and nonpathogenic forms of *F. oxysporum* to multiply and build up their own populations.

Fortyfour isolates used in cross pathogenicity test did not cause any wilt symptoms on the other Cucurbit hosts, including cucumber, watermelon and squash. Since all tested isolates were found to be pathogenic only on melon, they were assigned as *Fusarium oxysporum* f.sp. *melonis*.

Race determination test showed that the four races of FOM, including race 0, race 1, race 2, and race 1.2, were found in 'Çeşme Melon' fields (Table 2). Eight isolates were virulent only on cv. Charentais T, and designated as as race 0. Twenty four isolates were virulent on cvs. Charentais T, Vedrantais and Doublon but avirulent on cvs. Isovac, CM 17187, Margot and Isabelle, and classified as race 1. Four isolates caused symptoms on cvs. Charentais T, Isovac and CM 17187 but did not on cvs Vedrantais, Margot, Doublon and Isabelle, and identified as race 2. Eight isolates were virulent on all differential cultivars, except Isabelle, a cultivar known as partially resistant to all races of FOM, and classified as race 1.2. Presence of the four races indicated that 'Çeşme Melon' appeared to be succeptible to all races of *FOM*. However, the reports from the previous studies on the susceptibility of 'Çeşme Melon' to FOM were conflicting. In an earlier study, 'Çeşme Melon' was characterized as highly resistant to race 0, but highly susceptible to race 1, 2 and 1.2 (Yıldız, 1977). More recently, high level of resistance to race 1 was reported in two 'Çeşme Melon' genotypes, however their reactions to the other races varied significantly (Kurt et al,. 2002). In another recent research, a significant susceptibility was found in a Çeşme Melon genotype to race 1 (Şensoy et al., 2007). Regarding all these

reports, it can be suggested that there might be a high genetic variation among Çeşme Melon genotypes. Since there is no available commercial seed of 'Çeşme Melon', the growers generally use their own seeds, selecting each year the best plants most suitable for their own criteria. Seeds generated by the growers might have a great potential of genetic diversity because of the cross pollination under field conditions.

Table 2. Race determination of *Fusarium osysporum* f.sp. *melonis* isolates collected from 'Çeşme Melon' fields based on their virulence on differential melon cultivars.

Isolate Code	Location (County/Village)	Differentail varieties							Race
		Charentais T	Vedrantais	Doublon	Isovac	CM 17187	Margot	Isabelle	
K-108-A	Çeşme/Çiftlikköy	S[z] (67)[y]	S (89)	S (78)	R (0)	R (0)	R (0)	R (0)	1
K-116	Çeşme/Çiftlikköy	S (67)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	0
K-131	Çeşme/Çiftlikköy	S (67)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	0
K135-B	Çeşme/Çiftlikköy	S(89)	R (11)	R (22)	R (0)	R (0)	R (0)	R (0)	0
K-136	Çeşme/Çiftlikköy	S (67)	S(89)	S (89)	R (0)	R (0)	R (0)	R (0)	1
K-159-A	Çeşme/Ovacık	S (78)	S (89)	S (100)	R (0)	R (0)	R (0)	R (22)	1
K-195-A	Çeşme/Ovacık	S (100)	S(78)	S (100)	S (55)	S (67)	S (89)	R (0)	1,2
K-219	Urla/Kadıovacık	S (89)	S (89)	S (89)	S (78)	S (89)	S (78)	R (0)	1,2
K-221-A	Urla/Kadıovacık	S (67)	S (100)	S (89)	R (0)	R (0)	R (22)	R (0)	1
K-222	Urla/Kadıovacık	S (67)	S (78)	S (67)	R (0)	R (0)	R (11)	R (0)	1
K-228	Urla/Kadıovacık	S (89)	R (0)	R (0)	S (78)	S (78)	R (0)	R (0)	2
K-231-A	Urla/Kadıovacık	S (78)	S (100)	S (89)	S (55)	S (67)	S (89)	R (0)	1,2
K-233	Urla/Barbaros	S (89)	S (100)	S (78)	S (67)	S (67)	S (89)	R(0)	1,2
K-234	Urla/Barbaros	S (78)	S (67)	S (67)	S (11)	S (0)	S (0)	R (0)	1
K-234-B	Urla Barbaros	S (67)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	0
K-235	Urla/Barbaros	S (67)	R (0)	R (0)	S (78)	S (89)	R(0)	R (0)	2
K-239	Urla/Barbaros	S (78)	S (78)	S (89)	R (0)	R (0)	R (22)	R (0)	1
K-240	Urla/Barbaros	S (78)	S (100)	S (100)	S (67)	S (89)	S(89)	R (22)	1,2
K241-I	Urla/Barbaros	S (78)	S (89)	S (100)	R (0)	R (11)	R (22)	R (22)	1
K-241	Urla/Barbaros	S (78)	S (100)	S (100)	R (0)	R (0)	R (0)	R (0)	1
K-243	Urla/Barbaros	S (78)	S (100)	S (78)	R (0)	R (0)	R (0)	R (0)	1
K-245-A	Urla/Barbaros	S (78)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	0
K-258-A	Urla/Zeytinler	S (67)	R (22)	R (0)	R (0)	R (0)	R (0)	R (0)	0
K-260	Urla/Zeytinler	S (67)	S (100)	S (89)	R (0)	R (0)	R (0)	R (0)	1
K-260-B	Urla/Zeytinler	S (100)	S (100)	S (100)	R (0)	R (0)	R (0)	R (0)	1
K-261	Urla/Zeytinler	S (78)	S (100)	S (89)	R (0)	R (0)	R (0)	R (0)	1
K-261-A	Urla/Zeytinler	S (89)	R (0)	R (0)	S (78)	S (100)	R (0)	R (0)	2
K-261-C	Urla/Zeytinler	S (100)	R (0)	R (0)	S (89)	S (89)	R (0)	R (0)	2
K-262-A	Urla/Uzunkuyu	S (55)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	0
K-270-B	Urla/Nohutalan	S (89)	S (100)	S (78)	S (55)	S (67)	S (78)	R (22)	1,2
K-271	Urla/Nohutalan	S (67)	S (78)	S (78)	R (0)	R (0)	R (0)	R (0)	1
K-272-C	Urla/Nohutalan	S (78)	S (89)	S (78)	R (0)	R (0)	R (0)	R (0)	1
K-273	Urla/Nohutalan	S (78)	S (100)	S (100)	R (0)	R (0)	R (0)	R (0)	1
K-280	Urla/Nohutalan	S (78)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	0
K286	Çeşme/Germiyan	S (89)	S (100)	S (78)	R (0)	R (0)	R (0)	R (0)	1
K-288	Çeşme/Germiyan	S (78)	S (100)	S (100)	S (67)	S (55)	S (78)	R (0)	1,2
K-292	Çeşme/Germiyan	S (78)	S (78)	S (78)	R (0)	R (0)	R (0)	R (0)	1
K-327	Urla/Birgi	S (67)	S (100)	S (100)	R (0)	R (0)	R (0)	R (0)	1
K-334-A	Urla/Birgi	S (78)	S (100)	S (100)	S (67)	S (78)	R (78)	R (0)	1,2
K-335	Urla/Birgi	S (89)	S (100)	S (89)	R (0)	R (0)	R (0)	R (0)	1
K-337-B	Urla/Birgi	S (78)	S (100)	S(100)	R (0)	R (0)	R (0)	R (22)	1
K-338	Urla/Birgi	S (89)	S (89)	S (78)	R (0)	R (0)	R (0)	R (0)	1
K-340	Urla/Birgi	S (55)	S (100)	S (89)	R (0)	R (0)	R (0)	R (0)	1
K-341	Urla/Birgi	S (78)	S (100)	S (89)	R (0)	R (0)	R (0)	R (0)	1
Race 0 Tester	-	S (67)	R (0)	R (0)	R (0)	R (0)	R (0)	R (0)	
Race 1 tester	-	S (78)	S (100)	S (89)	R (0)	R (0)	R (0)	R (0)	
Race 2 tester	-	S (100)	R (22)	R (22)	S (100)	S (100)	R (0)	R (0)	
Race 1,2 tester W	-	S (100)	S (100)	S (100)	S (100)	S (100)	S (100)	R (11)	
Race 1,2 tester Y		S (78)	S (89)	S (78)	S (89)	S (89)	S (78)	R (0)	
Control	-	0	0	0	0	0	0	0	

[y]Numbers in brackets in each column are the percentages of seedlings showing Fusarium wilt symptoms.
[z]S=Susceptible; R=Resistant. Disease incidence of ≥33% was considered susceptible and <33% was considered resistant (Zhou et al., 2010).

In our study, among *FOM* races, race 1 was the most widely distributed one, which was found in eight village districts and comprised of 55% of the total isolates (Figure 2). Race 0 and race 1.2 were found in equal frequency, and each represented 18% of the isolates. Race 2 was the lesser one (9%), which was found only in three villages, which are located in very close proximity to each other. Seventyseven percent of the isolates (34 isolates) were from the six villages of Urla, including Kadıovacık, Barbaros, Birgi, Zeytinler, Uzunkuyu and Nohutalan. Lack of or less irrigation opportunities in these villages make 'Çeşme Melon' a highly preferable crop since it can be grown without irrigation. Intensive cultivation of 'Çeşme Melon' in the area might have caused *FOM* to increase its inoculum density in the soil over the years.

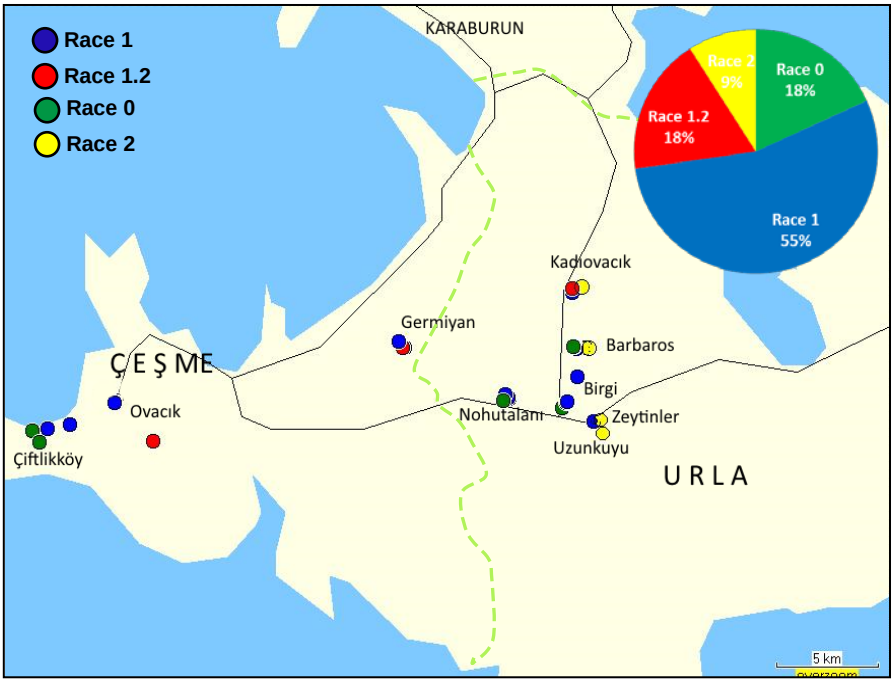

Figure 2. Distribution of the races of *Fusarium oxysporum* f.sp. *melonis* in Urla Peninsula.

The four races of FOM have been previously reported from different regions of Turkey but their frequencies varied depending on the region. Race 0, 1 and 1.2 appeared to be widely distributed in Turkey whereas race 2 is rare or does not existing in most locations (Yıldız, 1977; Kurt et al., 2002). Low frequency of race 2 was also detected in our study. Turkey is a rich source of gene for melon with numerous local varieties some of which are intensively grown in certain areas. Studies on the resistance of melon genotypes of Turkey indicated that most local genotypes were lack of resistance to FOM races; however, some of them have various degrees of resistance to particular ones (Kurt et al. 2002; Demir et al., 2006; Şensoy et al., 2007). This might be one of the reasons that certain races are widely distributed whereas the others are absent or rare in any given region of Turkey.

In conclusion, Fusarium wilt is one of the major factors limiting 'Çeşme Melon' production in Urla Peninsula. Fusarium wilt of melon is best controlled by the use of resistant cultivars. Unfortunately, all FOM races exist in the area and 'Çeşme Melon' appears to be susceptible to all of them. Improving of 'Çeşme Melon' for resistance to FOM is necessary for sustainable cultivation of this cultivar. In future studies, developing resistance in 'Çeşme Melon' both by breeding and grafting on resistant rootstocks should be considered to establish novel management strategies against Fusarium wilt.

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