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RESEARCH PAPER

Determination of Resistance Levels of Some Onion Cultivars or Inbreed Lines with Fusarium Testing at Seedling Stage

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Abstract

This study was carried out to determine the resistance levels of some onion genotypes in Yalova onion gene pool by Fusarium testing during seedling stage. The isolate used in the study was *Fusarium oxysporum* f. sp. *cepae*, which causes damping off during the seedling stage and later basal rot in onion bulbs. The variance analysis for the onion seedling test and the mean differences against control were analyzed by using General Linear Model of the Tukey test. The germination rate of control seeds varied between 72% to 98%, while the germination rate of inoculated seeds varied between 39% to 93%. Texas Early Grano 502 showed the highest level of resistance with a survival rate of 83.8%, and resistance levels of 19Y07 and 19Y142 genotypes were higher than other genotypes. Resistance levels of 19Y51, 19Y15 and 19Y73 genotypes were lower than other genotypes. Akgün 12 showed moderate resistance with a survival rate of 59.6%. Determining the resistance levels of these onion genotypes during the seedling stage may be a preliminary step towards further studies.

1. Introduction

Onion (*Allium cepa* L.), belonging to the Alliaceae family is a significant vegetable crop worldwide. Onion is the herbaceous biennial crop, and it has a wide range of landraces and cultivars with edible bulbs (Nasr Esfahani, 2018; Singh et al., 2018). The onion plant can be infected by soil- and seed-borne fungal pathogens, resulting in significant yield and quality losses.

Fusarium oxysporum Schlechtend.: Fr. f. sp. *cepae* (Hans) Synder and Hans (FOC) is caused seedborne and soilborne diseases such as damping off and basal root (Köycü and Özer, 1997; Özer et al., 2003). This pathogen may be seen and noticed in different growth stages of onion and can cause serious loss in the field and during storage (Fantino and Schiavi, 1987; Özer et al., 2003). Symptoms of Fusarium basal rot (FBR) appear on leaves, roots, basal plates, on the bulb scales of small seedlings, on the mature and dormant plants (Cramer, 2000; Özer et al., 2003). Symptoms are pre- and post-emergence damping-off of seedling in the field, root rot in older plants, discoloration in onion stem plate, basal rot in bulbs during storage (Abawi and Lorbeer, 1972; Cramer, 2000).

Fusarium oxysporum f. sp. *cepae* (FOC) exist in many countries such as the United States, Brazil, South Africa, the Netherlands, India, England, Iran, Sweden, Japan, and Uruguay, where onions are grown around the world (Cramer, 2000; Galvan et al., 2008; Dissanayake et al., 2009; Lager, 2011; Ghanbarzadeh et al., 2014; Ünsal et al., 2019) and also FOC exist in Turkey in onion production areas (Türkkan and Karaca, 2006; Bayraktar and Dolar, 2011).

Fusarium oxysporum f. sp. *cepae* can lead to losses reaching up to 50% in the field and 75% in

the greenhouse (Brayford, 1996; Stadnik and Dhingra, 1997; Ünsal et al., 2019). For this reason, the development and application of the most effective control methods of this destructive pathogen are vital. This pathogen can be controlled with some control methods such as resistance of host plant, crop rotation, solarization, various biological applications, and fungicide applications (Cramer, 2000; Ünsal et al., 2019). However, the using of resistant varieties is economic, applicable on a large scale, and stated as the best option (Cramer, 2000; Nasr Esfahani et al., 2012; Özer et al., 2003; Özer et al., 2004).

Many management strategies have been developed in the world for the detection and control of FOC. But over the time, control methods have become limited and inadequate. Therefore, it has become very important to identify and develop varieties that are tolerant or resistant to this pathogen. Resistance studies against FOC in onion have been performed before (Özer et al., 2003; Özer et al., 2004; Saxena and Cramer, 2009; Nasr Esfahani et al., 2012; Taylor et al., 2013). However, in this study, no previous study was conducted to define the resistance levels of 13 onion genotypes that were taken from the Yalova gene pool with Akgün 12 and grown in the same environment.

The goal of this study is to determine onion genotypes tolerance levels to develop tolerant varieties for commercial production. Developing resistance cultivars will prevent the loss of crops in the field and storehouse and will contribute to the national economy. This study aims to determine the tolerance/resistance level of some onion genotypes at the seedling stage. In this study, resistance levels of some onion genotypes to this pathogen were determined.

2. Materials and Methods

The plant materials were formed from 13 onion genotypes and 2 onion varieties. The onion genotypes were labeled 19Y01, 19Y06, 19Y07, 19Y15, 19Y16, 19Y17, 19Y18, 19Y19, 19Y34, 19Y46, 19Y51, 19Y73 and 19Y142. Akgün 12 and Texas Early Grano 502 have used as onion varieties. The thirteen genotypes and Akgün 12 were provided from Yalova onion gene pool and Texas Early Grano (TEG) 502 was provided by Bayram Seed Company. Whereas Akgün 12 onion variety was used as tolerant, TEG 502 variety was used as susceptible against *F. oxysporum* f. sp. *cepae* as reported according to previous research (Ko et al., 2002; Özer et al., 2003). The susceptibility levels of other onion genotypes were not known. As fungal material, one pathogenic and virulence isolate to be used in this study was provided from Ankara University, Agriculture Faculty, Plant Protection Department.

Onion seedling test was carried out by the method of inoculation of onion seeds. Onion seedling test was mainly included sterilization of onion seeds, preparation of spore suspension, inoculation of onion seeds, sowing of onion seeds in the soil, and counting of seeds and seedlings. Initially, surface sterilization of onion seeds from each genotype was performed in the flow cabinet in the Mycology Laboratory, Plant Protection Department, Atatürk Horticultural Crops Central Research Institute. The onion seeds were kept in 1000 µl 1% sodium hypochlorite for 3 minutes in the Eppendorf tubes to disinfect the surface, then seeds were rinsed in the sterile distilled water 3 times and left to get dry on sterile filter papers. Then *Fusarium oxysporum* f. sp. *cepae* was cultured on PDA medium at 20°C for 10 days. Sterile water was added into the petri dish to allow the conidia to pass into the water onto the developing culture and filtered through the sterile cheesecloth by gentle mixing and the intensity of the spore was adjusted to a density of 1×10^6 ml^{-1} by the hemocytometer.

After preparation of spore suspension, each onion genotype was inoculated by standing in 1 ml of spore suspension for 1 hour and the seeds used for control were kept in 1 ml sterile purified water for 1 hour. After 1 hour, seeds were rinsed and put back into petri dishes.

Before sowing the seeds, the 1/3 garden soil + 1/3 farm manure + 1/3 stream sand mixture was filled in the fireproof bag and placed in the autoclave machine (60 min, 121°C) to be a sterile soil mixture. For each treatment, 100 seeds were planted for each genotype with independent 4 replications in each replication with 25 seeds.

The seeds planted in the seedling trays (10×12 cells per tray) were placed in the climate chamber and the climate chamber was adjusted to be 25 °C day / 18°C night and 16-hour light / 8-hour dark and 60% relative humidity. The counting of survival seedlings was performed twice a week after 10 days of planting and continued for 3 weeks (Özer et al., 2004; Taylor et al., 2013). The survival percentage of the genotypes was calculated according to control genotypes to ensure a variation of natural in the seed germination.

The data obtained before and after the seedling emergence were compared with the control. The variance analysis for the onion seedling test was analyzed by using the General Linear Model of the Tukey test. Variance analyses were performed with Minitab® 16.2.4 (e-academy version). Paired Sample T-Test for comparison of control and inoculated alive seedlings were analyzed by using Microsoft Excel. Counting of dead and alive seeds and seedlings were taken after two weeks and four weeks and the percentage of emergence and survival were calculated with the formula below (Saxena and Cramer, 2009).

$$\%Emergency = \frac{Emerged\ seedlings}{Total\ planted\ seed} \times 100$$

$$\%Survival = \frac{Survival\ seedlings}{Total\ emerged\ seedling} \times 100$$

3. Results and Discussion

3.1. Observation of disease symptoms

FOC caused germination, plant death and adverse effects on the length and weight of the root and stem in plant disease studies (Behrani et al., 2015). On that account, significant differences were noted between plant growths of inoculated and control plants (Figure 1). Ten days after inoculation, germination almost completed for inoculated and control seeds.

Twelve days after inoculation, the initial disease symptoms began to seem such as the appearance of white mycelium on the soil and the death of several small seedlings (Figure 2). Before germination, it either germinated later than controls or death occurred. After germination, inoculated seedlings developed later than control. Yellowing occurred on the onion leaves (Figure 3). Cramer (2000) noticed that white mycelium may be on the basal plate of external bulb scales. Like to this work, white mycelium was noticed on the soil of the onion seedling during seedling stage in this study (Figure 2). It was noteworthy that when the root part of the seedlings that looked healthy in the soil was removed from the soil, it was already brown and broken. If the study had continued, seedlings would continue to die (Figure 4).

3.2. Onion seedling test

A total of 13 onion genotypes and 2 onion varieties inoculated by the seed inoculation method were evaluated about resistance against FOC. There were highly significant differences among onion genotypes due to infection caused by FOC.

In this study, the percentage of germinated seeds, the percentage of alive seedlings and the percentage of dead seedlings in the inoculated onion seeds were calculated by comparing them with the control seedlings. At the end of the 4-week counts, the obtained data were evaluated, analyzed and the resistance levels against damping-off were determined among the onion genotypes (Table 1).

Dead seeds shown in the control seeds were non-germinated seeds. These dead seeds were percentage of seeds that were not germinated by their nature. In the case of inoculated seeds, it was showed the percentage of seedlings that died after germination. It was observed that germinated seeds and alive seedlings of onion genotypes had similar values, while their dead seedlings had significant differences. While the mean percentage of germinated seeds was found to be 70.26, the mean percentage of alive seedlings was found to be 57.00. The mean percentage of dead seedlings was found to be 21.40. In addition, when looking at dead seedlings, significant differences were observed

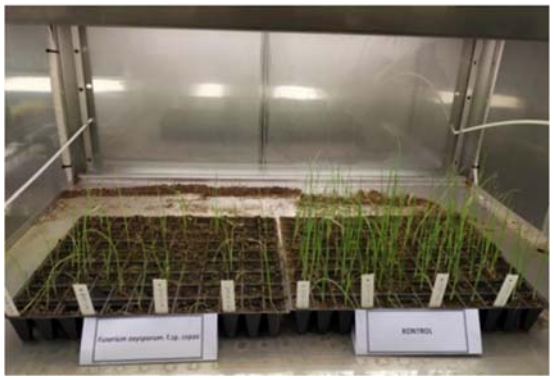

Figure 1. Final comparison between inoculated and control seedlings

Figure 2. View of dead seed and seedling and the formation of white mycelium (a, b)

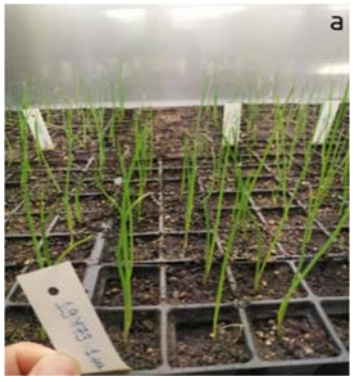

Figure 3. Comparison of control (a) and inoculated seedlings (b) of 19Y73

Figure 4. Comparing of the alive and dead seedling in the same inoculated genotype

between genotypes both according to control genotypes and among themselves (Table 1).

The mean of control dead seedlings was unexpectedly higher than inoculated dead seedlings in some genotypes (Table 1). These genotypes can be listed as follows: 19Y51, 19Y15, Akgün 12, 19Y46, 19Y34, 19Y01, and 19Y07. In the 19Y16 genotype, the mean of control and inoculated dead seedlings were equal. Other onion genotypes had higher average dead seedlings in inoculated genotypes as expected in the Table 1. Another remarkable point was that TEG 502 onion variety has more average germinated seeds than other genotypes in both control and inoculated seeds in the Table 1. Then the mean of control and inoculated alive seedlings at the end of the experiment were given comparatively in the Table 2.

Significant difference was not observed between control and inoculated live seedlings of some genotypes. Examples of these genotypes were 19Y06, 19Y19, 19Y142, Akgün 12 and TEG 502 ($P > 0.05$). Significant differences ($P < 0.05$) were seen in other onion genotypes (Table 2). When mean of the control and inoculated alive seedlings were compared, there were no statistically significant differences for some onion genotypes such as TEG *502 ($P = 0.253$), Akgün 12 ($P = 0.110$), 19Y142 ($P = 0.223$), 19Y19 ($P = 0.063$) and 19Y06 ($P = 0.427$).*

Two onion varieties and 13 onion genotypes were inoculated by a FOC isolate. Then, seedling emergence rate and survival rate of onion seedlings at 2nd and 4th week were calculated and compared with each other in the Table 3. This evaluation was done with using the formula given by Saxena and Cramer (2009).

The survival seedling rates among onion genotypes ranged from 89.7% to 100% in the 2nd week, while survival seedling rates ranged from 39.02% to 83.87% in the 4th week (Table 3). If the study had continued, it would have estimated that the survival seedling rates, would continue to decrease in the following weeks.

Each onion genotype did not differ significantly at 2nd and 4th weeks. For example, FOC isolate caused a little change of pre- and post-emergence damping-off, by a decrease from 100% survival rate after 2 weeks to 83.87% survival rate after 4 weeks in TEG 502. However, FOC isolate caused a significant amount effect of pre- and post-emergence damping off, with a decrease from 90.24% survival rate after 2 weeks to 39.02%

Table 1. Genotypes, experiment, mean percentages of the germinated seeds, alive and dead seedlings of control and inoculated seeds.

Genotypes	Experiment	N	Germinated seeds		Alive seedlings		Dead seedlings	
			Mean	G	Mean	G	Mean	G
TEG 502	Control	4	98.00	a*	98.00	a	2.00	c
TEG 502	FOC	4	93.00	ab	78.00	a	15.00	ac
19Y18	Control	4	88.00	ac	88.00	a	12.00	bc
19Y18	FOC	4	64.00	bh	38.00	cd	26.00	ab
19Y142	Control	4	85.00	ad	85.00	a	15.00	ac
19Y142	FOC	4	71.00	ag	50.00	bc	21.00	ac
19Y17	Control	4	84.00	ad	84.00	a	16.00	ac
19Y17	FOC	4	62.00	ch	37.00	cd	25.00	ac
19Y19	Control	4	83.00	ae	83.00	a	17.00	ac
19Y19	FOC	4	60.00	ch	31.00	cd	29.00	ab
19Y16	Control	4	80.00	ae	80.00	a	20.00	ac
19Y16	FOC	4	60.00	ch	40.00	cd	20.00	ac
19Y07	Control	4	79.00	ae	79.00	a	21.00	ac
19Y07	FOC	4	43.00	gh	32.00	cd	11.00	b
19Y06	Control	4	79.00	ae	79.00	a	21.00	ac
19Y06	FOC	4	72.00	ag	36.00	cd	36.00	a
19Y01	Control	4	77.00	ae	77.00	a	23.00	ac
19Y01	FOC	4	44.00	gh	28.00	cd	16.00	ac
19Y73	Control	4	77.00	ae	77.00	a	23.00	ac
19Y73	FOC	4	45.00	fh	21.00	d	24.00	ac
19Y34	Control	4	74.00	af	74.00	ab	26.00	ab
19Y34	FOC	4	54.00	eh	30.00	cd	24.00	ac
19Y46	Control	4	74.00	af	74.00	ab	26.00	ab
19Y46	FOC	4	47.00	fh	26.00	cd	21.00	ac
Akgün 12	Control	4	74.00	af	74.00	ab	26.00	ab
Akgün 12	FOC	4	57.00	dh	34.00	cd	23.00	ac
19Y15	Control	4	72.00	ag	72.00	ab	28.00	ab
19Y15	FOC	4	39.00	h	17.00	d	22.00	ac
19Y51	Control	4	72.00	ag	72.00	ab	28.00	ab
19Y51	FOC	4	41.00	h	16.00	d	25.00	ac
General Mean			70.26		57.00		21.40	
General SE Mean			5.45		4.89		4.26	

*Different letters following the mean in the same column signify that the mean is statistically significant difference. (ANOVA p = 0.05, Tukey test). N: Number of replication. G: Grouping.

Table 2. Genotypes, comparison of control and inoculated alive seedlings.

Genotypes	Control alive seedling		Inoculated alive seedling		p-value*
	Mean	Std Dev	Mean	Std Dev	
19Y01	0.77	0.09	0.44	0.12	0.005
19Y06	0.79	0.14	0.72	0.09	0.427
19Y07	0.79	0.02	0.43	0.23	0.021
19Y15	0.72	0.03	0.39	0.07	0.000
19Y16	0.80	0.09	0.60	0.05	0.012
19Y17	0.84	0.09	0.62	0.10	0.016
19Y18	0.88	0.09	0.64	0.09	0.008
19Y19	0.83	0.11	0.60	0.16	0.063
19Y34	0.74	0.11	0.54	0.07	0.043
19Y46	0.74	0.07	0.47	0.07	0.002
19Y51	0.72	0.09	0.41	0.13	0.009
19Y73	0.77	0.04	0.45	0.12	0.003
19Y142	0.85	0.10	0.71	0.10	0.223
TEG 502	0.98	0.04	0.93	0.04	0.253
Akgün 12	0.74	0.16	0.57	0.04	0.110

*(Paired Sample T-Test, p = 0.05).

Table 3. Genotypes, percentages of emergence and survival rate of onion seedlings.

Genotypes	Inoculated seeds		
	% Emergence*	% Survival (2nd week)	% Survival (4th week)
19Y51	41.00	90.24	39.02
19Y15	39.00	89.70	43.58
19Y73	45.00	93.33	46.66
19Y06	72.00	93.05	50.00
19Y19	60.00	90.00	51.66
19Y46	47.00	97.87	55.31
19Y34	54.00	96.29	55.55
19Y18	64.00	93.75	59.37
Akgün 12	57.00	91.22	59.64
19Y17	62.00	90.32	59.67
19Y01	44.00	95.45	63.63
19Y16	60.00	98.33	66.66
19Y142	71.00	98.59	70.42
19Y07	43.00	97.67	74.41
TEG 502	93.00	100.00	83.87
Mean	56.80	94.38	58.63

*Emergence rate and survival rate of onion seedlings at 2nd and 4th week was calculated using the formula given by Saxena and Cramer (2009).

survival rate after 4 weeks in 19Y51. As another example, although 19Y07 onion genotype has more loss than 19Y142 in terms of damping off before germination, 19Y07 onion genotype has less loss in terms of damping-off after germination than 19Y142 (Table 3). Moreover, among genotypes, while the survival rate of genotypes 19Y15 and 19Y51, where the first symptoms were seen, was the lowest compared to others, TEG 502 onion variety showing the last symptom had the highest survival rate (Table 3).

Özer et al. (2003) measured disease severity of onion varieties 7 days after inoculation and they observed that the disease severity of TEG 502 was higher than the disease severity of Akgün 12. Ko et al. (2002) remarked that TEG 502 was the most susceptible to FOC. For another study, Galvan et al. (2008) noted that the TEG 502 onion variety was less resistant in their study. Nasr-Esfahani et al. (2013) announced that TEG was one of the susceptible onion genotypes in field and greenhouse conditions. In contrast, according to the data obtained in this study, at the end of the 4th week, in the inoculated seeds, TEG 502 showed 83.87% survival rate and 93.0% emergence rate in the Table 3. Thus, unlike other studies, it was observed that the resistance level of TEG 502 was highest against damping-off before and after germination (Figure 5).

According to a study by Özer et al. (2004), while Akgün 12 variety was found to be resistant in all bulb stages in their all experiments, in this study, the survival rate of Akgün 12 was showed 59.64% (Table 3). In this case, it was observed that Akgün 12 showed a moderate level of resistance against damping-off during the seedling stage. Akgün 12

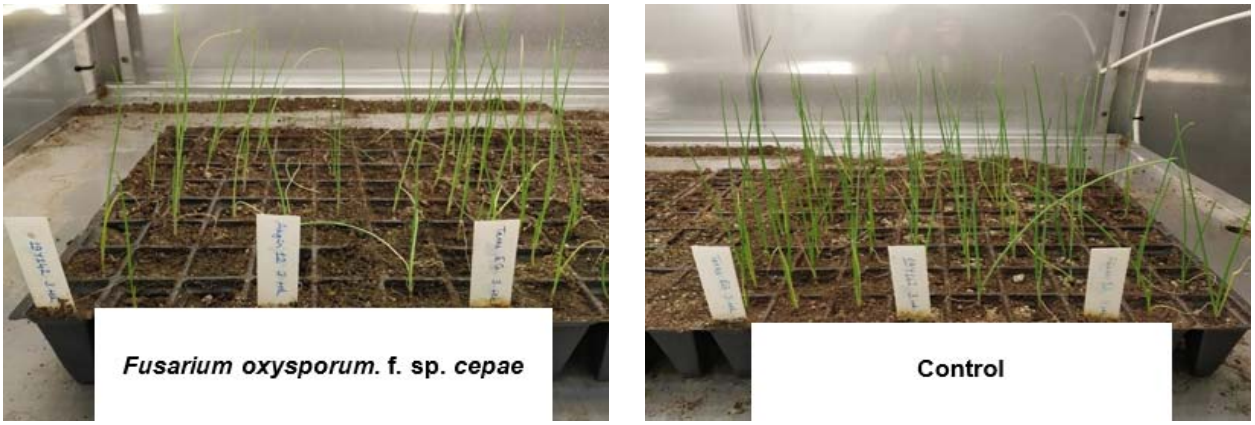

Figure 5. Comparison of inoculated and control seedlings of 19Y142, Akgün 12, and TEG 502.

may not appear more resistant compared to TEG 502. However, regarding the germination of control seeds, it should be considered that Akgün 12 has a 74.0% germination rate and TEG 502 has a 98.0% germination rate.

It has been thought that differences in resistance levels changed over time and the result obtained in the 4th week gave a more accurate result. According to observation, pre-damping off refers to the percentage of seeds that die before germination, while post-damping off refers to the percentage of seedlings that die after germination. The rate of dead seeds before germination were significantly higher than the rate of dead seedlings after germination.

4. Conclusion

These 13 onion genotypes have never been used in a disease study. The results of Akgün 12 used in this study were like to previous studies. Accordingly, one can have an idea about the resistance level of 13 other onion genotypes grown under the same conditions as Akgün 12. However, the result of the Texas Early Grano 502 variety used in the study was not compatible with previous studies (Özer, 1998; Ko et al., 2002; Özer et al., 2004; Galvan et al., 2008; Nasr-Esfahani et al., 2013).

When the obtained results were analyzed, according to the survival percentages at the end of the 4th week, it is possible to specify the resistance levels of onion genotypes as follows: Texas Early Grano 502, 19Y07, 19Y142, 19Y16, 19Y01, 19Y17, Akgün 12, 19Y18, 19Y34, 19Y46, 19Y19, 19Y06, 19Y73, 19Y15 and 19Y51. Since it was observed that the pre-damping-off severity was higher than the post-damping-off severity, the production of onion at the seed stage should be done more carefully and controlled. Since the aggressiveness of pathogens changes from region to region and onion varieties found as resistant will not always show the same performance in time.

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References

Abawi, G.S., & Lorbeer, J.W. (1972). Several aspects of the ecology and pathology of *Fusarium oxysporum* f. sp. *cepae*. *Phytopathology,* 62:870-876.

Bayraktar, H., & Dolar, F.S. (2011). Molecular identification and genetic diversity of Fusarium species associated with onion fields in Turkey. *Journal of Phytopathology,* 159:28-34.

Behrani, G.Q., Syed, R.N., Abro, M.A., Jiskani, M.M., & Khanzada, M.A. (2015). Pathogenicity and chemical control of basal rot of onion caused by *Fusarium oxysporum* f. sp. *cepae*. *Pakistan Journal of Agriculture, Agricultural Engineering and Veterinary Sciences,* 31:60-70.

Brayford, D. (1996). IMI Descriptions of Fungi and Bacteria, Set No. 127, Nos. 1261-1270.

Cramer, C.S. (2000). Breeding and genetics of Fusarium basal rot resistance in onion. *Euphytica,* 115:159-66.

Dissanayake, M.L.M.C., Kashıma, R., Tanaka, S., & Ito, S.I. (2009). Genetic diversity and pathogenicity of *Fusarium oxysporum* isolated from wilted Welsh onion in Japan. *Journal of General Plant Pathology*, 75:125-130.

Fantino, M.G., & Schiavi, M. (1987). Onion breeding for tolerance to *Fusarium oxysporum* f. sp. cepae in Italy. *Phytopathology Mediterranea,* 26:108-112.

Galván, G.A., Koning-Boucoiran, C.F.S., Koopman W.J.M., Burger-Meijer K., González P.H., Waalwijk C., Kik C., & Scholten O.E. (2008). Genetic variation among fusarium isolates from onion and resistance to fusarium basal rot in related allium species. *European Journal of Plant Pathology,* 121:499-512.

Ghanbarzadeh, B., Goltapeh, E.M., & Safaie, N. (2014). Identification of Fusarium species causing basal rot of onion in East Azarbaijan province, Iran and evaluation of their virulence on onion bulbs and seedlings. *Archives of Phytopathology and Plant Protection*, 47:1050-1062.

Ko, S.S., Chang, W.N., Wang J.F., Cherng, S.J., & Shanmugasundaram, S. (2002). Storage variability

among short-day onion cultivars under high temperature and high relative humidity, and its relationship with disease incidence and bulb characteristics. *Journal of American Society for Horticultural Science*, 127:847-854.

Köycü, N.D., & Özer, N. (1997). Determination of seedborne fungi in onion and their transmission to onion sets. *Phytoparasitica,* 25:25-31.

Lager, S. (2011). Survey of Fusarium species on yellow onion (*Allium cepa*) on Öland. MSc Thesis, Swedish University, Sweden.

Nasr Esfahani, M., Hossaini, M., & Ashrafi, N. (2012). Screening of Iranian onion seed sets genotypes for resistance to *Fusarium oxysporum* f. sp. *cepae*. *International Journal of Farming and Allied Sciences,* 1:9-15.

Nasr Esfahani, M., Hosseini, M., Nasehi, A., & Golkhandan, E. (2013). Screening of onion seed sets for resistance against new Iranian isolates of *Fusarium oxysporum* f. sp. *cepae*. *Archives of Phytopathology and Plant Protection,* 46:1864-1873.

Nasr Esfahani, M. (2018). Genetic and virulence variation in *Fusarium oxysporum* f. sp. *cepae* causing root and basal rot of common onion in Iran. *Journal of Phytopathology,* 166:572-580.

Özer, N. (1998). Reaction of some onion cultivars to *Aspergillus niger* V. Tiegh. and *Fusarium oxysporum* Schlecht. *Journal of Turkish Phytopathologu*, 27:17-26.

Özer, N., Köycü, D., Chilosi, G., Pizzuolo, P.H., Coşkuntuna, A., & Magro, P. (2003). Pectolytic isoenzymes by *Fusarium oxysporum* f. sp. *cepae* and antifungal compounds in onion cultivars as a response to pathogen infection. *Canadian Journal of Plant Pathology,* 25:249-57.

Özer, N., Köycü, N.D., Chilosi, G., & Magro, P. (2004). Resistance to fusarium basal rot of onion in greenhouse and field and associated expression of antifungal compounds. *Phytoparasitica,* 32:388-394.

Saxena, A., & Cramer, C.S. (2009). Screening of onion seedlings for resistance against New Mexico isolates of *Fusarium oxysporum* f. sp. *cepae*. *Journal of Plant Pathology,* 91:199-202.

Singh, D., Dhillon, T.S., Singh, R., & Kumar, R. (2018). Present status and future opportunities in onion research: A review. *International Journal of Chemical Studies,* 6:656-665.

Stadnik, M.J., & Dhingra, O.D. (1997). Root infection by *Fusarium oxysporum* f. sp. *cepae* at different growth stages and its relation to the development of onion basal rot. *Phytopathologia Mediterranea,* 36:8-11.

Taylor, A., Vagany, V., Barbara, D.J., Thomas, B., Pink, D., Jones, J.E., & Clarkson, J.P. (2013). Identification of differential resistance to six *Fusarium oxysporum* f. sp. *cepae* isolates in commercial onion cultivars through the development of a rapid seedling assay. *Plant Pathology,* 62:103-11.

Türkkan, M., & Karaca, G. (2006). Determination of fungal root rot disease agents associated with onion fields in Amasya province. *Journal of Agricultural Sciences*, 12:357-363.

Ünsal, İ., Kaş, S., & Türkkan, M. (2019). Effect of some calcium salts on the growth and development of *Fusarium oxysporum* f. sp*. cepae,* the causal agent of Fusarium basal rot of onion. *Akademik Ziraat Dergisi,* 8:35-42.

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RESEARCH PAPER

HORTICULTURAL STUDIES

Flowering and Non-Flowering Spur Leaf Characteristics of 'Amasya' Apple and Its Comparison with Other Cultivars

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Abstract

Understanding the attributes of spur leaves in apple trees is essential to gain more insight into the complex process of fruit development and quality. However, limited literature is available about the spur leaf characteristics of the 'Amasya' apple cultivar. In this work, the Soil Plant Analysis Development (SPAD) and leaf area were investigated in flowering and non-flowering spur leaves of 'Amasya' and six common apple cultivars. Significant differences among cultivars were observed for the SPAD and spur leaf area. The median SPAD readings in 'Amasya' were 32.63 and 26.23 for the flowering and non-flowering spurs, respectively, which were the relatively low values among studied cultivars. The maximum SPAD value was measured in flowering spurs of 'Cripps Pink' (45.03). SPAD values were found to be lower in non-flowering spurs compared with flowering ones for all the studied cultivars, which confirms that decline in chlorophyll content coincided with a gradual decline in productivity. A notably significantly lower spur leaf area was found in 'Amasya', whereas the highest spur leaf area was in 'Cripps Pink'. However, no statistical difference was observed between flowering and non-flowering spur leaf areas within the same cultivars. The present results provided a general framework of SPAD and spur leaf areas in 'Amasya' and other apple cultivars when spurs had flowers or not. Knowledge of these characteristics provides a basis for building a model related to yield elaboration of apple trees.

1. Introduction

Unlike shoot leaves, spur leaves are produced firstly on an apple tree in spring and enlarged from the spurs, non-extension and shorter shoots (Proctor and Palmer, 1991; Elsysy and Hirst, 2017). These primary spur leaves can usually make up 30 to 60 percent of the total leaf area on an apple tree, depending on the cultivar, whereas they are the main part of the tree canopy until fruit set (Van den Ende, 2018). Therefore, they are strongly associated with the fruit set, fruit growth, quality, and long-term productivity (Ferree and Palmer, 1982; Proctor and Palmer, 1991; Ferree et al., 2001). In addition, studying spur leaf attributes of the cultivars is needed to understand these complex processes of agronomic importance.

'Amasya' apple is characterized by its unique eating quality and aroma (Atay et al., 2016). It also faces several production constraints such as nonfunctional tree architecture, low production, alternate bearing, earlier bloom, and poor coloration (Atay et al., 2018). For this reason, a better definition of its spur leaf habits would be of great relevance for overcoming these constraints, which complicate the management of the cultivar.

SPAD, also called chlorophyll meter or leaf greenness index, has been proposed to analyze leaf chlorophyll content or leaf nitrogen concentration through a non-destructive spectral

practice (Uddling et al., 2007; Munoz-Huerta et al., 2013). It is widely accepted that tight coordination between the SPAD readings and plant nitrogen status in apple trees as well as citrus, grapevine, wheat, rice, maize, oak, sycamore and maple (Castelli et al., 1996; Argenta et al., 2004; Jifon et al., 2005; Percival et al., 2008; Cerovic et al., 2015; Treder et al., 2016). The SPAD value assigned on the principles of leaf transmittance or reflectance of a red and an infrared light provides immediate and valuable approaches to assess the relative amount of leaf nitrogen (Markwell et al., 1995). While differences in the leaf color in early spring may have been observed among cultivars and flowering and non-flowering spurs of the same cultivar, previous research on 'Amasya' is limited.

Within the tree canopy, the leaf area of fruit trees is a vital component favoring orchard productivity. Various methods have been used to assess the leaf area in the orchard (Breda, 2003; Demirsoy, 2009; Mora et al., 2016; Bairam et al., 2017; Atay et al., 2019). Generally, these methods take into account shoot leaves, or total leaf area represented the ratio of leaf to a specific area of soil. Indeed, spur leaves and shoot leaves growth vary spatially in an apple tree (Van den Ende, 2018). However, for reflecting the potential of the spurs, the information on the spur leaf area is crucial.

In the present study, the SPAD and spur leaf area were investigated in flowering and non-flowering spur leaves of 'Amasya' and six common apple cultivars 'Granny Smith', 'Braeburn', 'Jerseymac', 'Cripps Pink', 'Mondial Gala', and 'Golden Reinders'. The objectives were to (i) reveal a detailed description of spur leaf characteristics in 'Amasya' cultivar, (ii) compare commercially important apple cultivars which exhibit varied branching and bearing behaviors by evaluating the SPAD and leaf area of spur leaves, (iii) to disclose and quantify the SPAD of flowering and non-flowering spur leaves in apple trees.

2. Materials and Methods

The study was carried out at Fruit Research Institute, Egirdir-Isparta in Turkey, in 2018 on a five-year-old apple trees cv. 'Amasya', 'Braeburn', 'Granny Smith', 'Cripps Pink', 'Jerseymac', 'Golden Reinders', and 'Mondial Gala', grafted onto M.9 rootstock. Trees were planted at 4.0 m row spacing and 1.0 m tree spacing and trained as a trellis spindle system with minimal pruning. Orchard management practices, including irrigation, nutrition, pest, disease, and weed control, were performed according to local commercial orchards.

The experimental design was randomized blocks with three replications (two trees each). The SPAD and spur leaf area values were obtained in flowering (Figure 1a) and non-flowering spurs (Figure 1b), sampled randomly, in the last week of April, nearly 5-7 days after full blooming, when the spur leaves were steady. The data were collected from sixty tagged spurs (30 for flowering and 30 for non-flowering) for each cultivar. The SPAD values were obtained using a portable chlorophyll meter (SPAD-502Plus, Konica Minolta, Japan) between 12:00 and 14:30 each measurement day. Three successive readings were taken from the three uppermost fully expanded leaves of spurs tagged. The mean of the three measurements was taken as the mean SPAD value of a spur.

Spur leaf area of the same tagged spurs for each cultivar was visually ranked on a scale of 1 to 5 (1=0-10 cm^2, progressively to 5=>41 cm^2 spur leaf area (Figure 2). Using this scale, established in previous experiments, is time-saving for predicting the vigorous and weak spurs in mature apple trees.

All statistical analyses and graphs were performed using R statistical software version 4.1.2 (R Core Team, 2021). When the F-test was significant in the one-way analysis of variance (ANOVA), means were separated using the Least Significant Difference (LSD) test ('agricolae'

Figure 1. (a) Non-flowering and (b) flowering spurs with spur leaves in apple.

Figure 2. Changes in spur leaf areas in apples and the scale values of total spur leaf area according to a 5–point scale (1:0-10 cm^2, 2:11-20 cm^2, 3:21-30 cm^2, 4:31-40 cm^2 and 5:more than 41 cm^2).

package). A correlation analysis was performed with the 'PerformanceAnalytics' package to investigate the relationship level between SPAD and spur leaf area values. Box plot and violin graphs were created with the 'ggplot2' package.

3. Results and Discussion

When apple trees were at full bloom individual flowering and non-flowering spurs of each cultivar were tagged and separated to examine spur leaf attributes. SPAD values were varied from 45.73 to 25.91 and differed among the cultivars and spur type (P=0.001). The median SPAD values were higher in all cultivars' flowering spurs than non-flowering spurs. The median SPAD values in 'Amasya' were 31.55 and 26.05 for the flowering and non-flowering spurs, respectively, which were relatively low when considering the cultivars studied. 'Golden Reinders' showed pretty similar SPAD values to 'Amasya' (median values 32.00 and 27.20 for flowering and non-flowering spurs, respectively). The flowering spurs of 'Cripps Pink' displayed the highest SPAD value (median=44.85), followed by the flowering spurs of 'Braeburn' (median=41.80). In non-flowering spurs, the lowest mean for SPAD values were observed in 'Amasya' (median=26.05) and 'Golden Reinders' cultivars (median=27.20), while the highest values were recorded from 'Granny Smith' (34.65) and 'Jerseymac' (35.55). SPAD values of 'Mondial Gala' did not differ significantly by spur type (Figure 3).

The greenness index determined by SPAD is an indirect measurement of the chlorophyll content of leaves (Markwell et al., 1995; Uddling et al., 2007). SPAD differences between cultivars in our study indicated the changes in their chlorophyll content. As reported in previous studies, the SPAD readings is a robust and satisfactory method to estimate the leaf chlorophyll concentrations or leaf nitrogen content, which is a significant component of chlorophyll molecule structure (Rostami et al., 2008; Brunetto et al., 2012; Romero et al., 2013; Benati et al., 2021). Moreover, the relationship between leaf chlorophyll content and SPAD values may be affected by the changes in cultivars, environmental factors, and management strategies (Argenta et al., 2004; Munoz-Huerta et al., 2013; Xiong et al., 2015). The high variability of the SPAD among the cultivars observed in this study can be attributed to their wide range of flowering and fruiting patterns and growth habits. Biennial cultivars such as 'Amasya' and 'Golden Reinders' displayed relatively low mean SPAD values. However, SPAD reached relatively high values for the cultivars with annual bearing capacity such as 'Cripps Pink' and 'Braeburn'.

Moreover, most previous studies using SPAD indicators focused on the nitrogen nutritional status to adjust fertilizer management practices during critical plant growth periods (Wang et al., 2006; Xiong et al., 2015; Cerovic et al., 2015; Benati et al., 2021). Our results found a better relationship between the SPAD and spur type (flowering vs non-flowering) for each cultivar. The potential of using the SPAD for yield indicator purposes has been evaluated for grain cereals, such as maize (Bishnu, 2020; Szulc et al., 2021), rice (Zhang et al., 2019; Hou et al., 2021), but it is underutilized in fruit trees. The current study is the first to disclose and quantify the relationship between flowering, and non-flowering spur leaves for SPAD measurements in apples and particularly in the Turkish cultivar 'Amasya'. For all cultivar when spurs had flowers, the SPAD values were much higher in the current

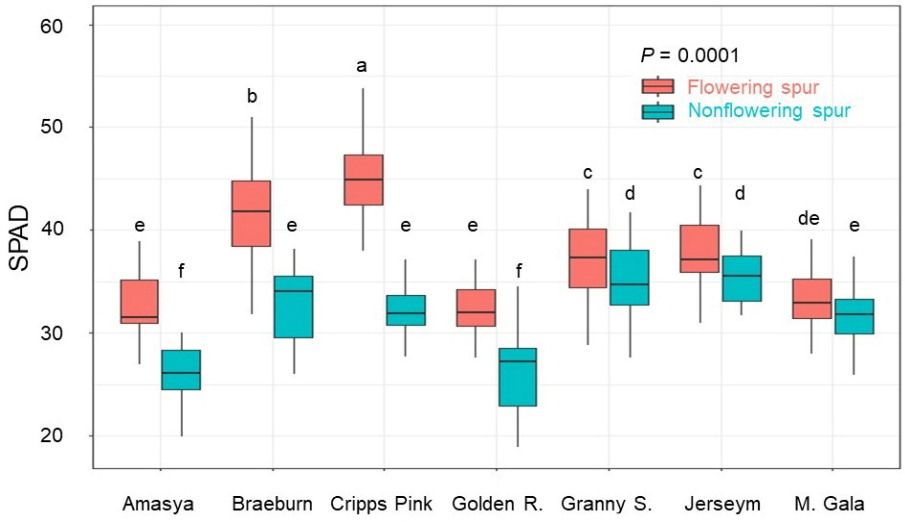

Figure 3. Box plot representation of leaf Soil Plant Analysis Development (SPAD) readings in apple cultivars for each spur leaf category (flowering and non-flowering). The bold horizontal line indicates the median value of SPAD values. The whiskers below and above the boxes denote the minimum and maximum SPAD values, respectively. Different letters indicate statistically different values at $P < 0.0001$ considering both flowering and non-flowering spurs of all the cultivars together.

Figure 4. Distribution of spur leaf area in apple cultivars for the two bearing statuses (flowering and non-flowering) considering together the seven cultivars. The expansion and shrinkage of the letter-value plots denote their corresponding quantiles. Different letters indicate statistically different values at $P < 0.0001$, considering all the cultivars' flowering and non-flowering spurs.

study, which confirms that decline in chlorophyll content or SPAD values coincided with a gradual decline in productivity. There were significant differences in spur leaf area between cultivars ($P<0.001$). The spur bearing behavior had no direct effect on the leaf area of the cultivar. A slight difference was observed among flowering and non-flowering spur leaf areas in the cultivars 'Cripps Pink' and 'Granny Smith'. Spur leaf area was relatively high in 'Cripps Pink' with the mean values 4.93 and 4.70 for flowering and non-flowering spur, respectively. 'Granny Smith' also showed relatively high spur leaf area values (mean values 4.63 and 4.47 for flowering and non-flowering spur, respectively). The flowering and non-flowering spurs of other cultivars showed similar distributions with no differences statistically for spur leaf area. 'Golden Reinders' (mean values 4.57 and 4.43 for flowering and non-flowering spur, respectively) showed a similar spur leaf area pattern to 'Mondial Gala' (mean values 4.60 and 4.53 for flowering and non-flowering spur, respectively) for flowering and non-flowering spur. However, there was a greater distribution in 'Mondial Gala' for flowering and non-flowering spur. The latter the main spur leaf area were determined at 3.73, 3.43, 3.07 and 2.83 for flowering and non-flowering spurs of the cultivars ‘Jerseymac’ and ‘Braeburn’, respectively. A significantly lower spur leaf area was found in 'Amasya' with mean values 2.40 and 2.20 for flowering and non-flowering spur, respectively (Figure 4).

In previous studies, shoot leaves or total leaf areas of different apple cultivars have been described (Wünsche and Palmer, 1997; Lauri and Kelner, 2001; Knerl et al., 2018). However, to our knowledge, there are limited reports on the spur leaf area of the cultivars we studied in the current study. Indeed, the spur leaf area is one of the critical factors for improved productivity of apple cultivars (Ferree and Schmid, 2004). The presence of a sufficiently large leaf area per spur in apple is a significant factor favoring fruit set and quality (Ferree et al., 2001). Different spur leaf areas were observed in our study depending on the cultivar. The lowest spur leaf area was determined in 'Amasya', whereas the highest spur leaf area was in 'Cripps Pink'. Like all yield components, spur leaf area can typically vary depending on the genotype (Ferree and Palmer, 1982; Elsysy and Hirst, 2017), probably due to differences in their photosynthetic performances (Proctor and Palmer, 1991). Generally, it was determined that the spur leaf area was more prominent in the cultivars with superior yield and fruit quality, except for 'Braeburn'.

Interestingly, no statistical difference was observed between flowering and non-flowering spur leaf areas within the same cultivars, unlike the SPAD value. A relatively weak growth for flowering buds than for vegetative buds is expected chiefly in apple trees since the presence of the flowers/fruits can reduce the amount of carbon required for growth, as previously shown in other studies (Costes, 2003; Willaume et al., 2004; Lauri et al., 2008). However, these studies generally considered shoot leaves, and this prediction is not entirely relevant for spur leaves. The current study examined the spur leaves, which are often more vigorous to initiate flowers. Indeed, weak spurs are not expected to be florally induced, which could explain the similarity in spur leaf area between flowering and non-flowering spur.

The role of SPAD in the spur leaf area was highlighted in the correlation plot illustrated in Figure 5, which clearly showed that the SPAD value was positively correlated with spur leaf areas. This positive impact can be attributed to the relationship between photosynthesis and SPAD values, as previously observed in other studies (Thompson et al., 1996; Miah et al., 1997; Pallas et al., 2018). Increased photosynthetic activity may likely develop nutrient status and leaf growth of the spurs. These results appear to confirm the observations of Vrignon-Brenas et al. (2019). They suggested that decreasing SPAD value accompanies the lower leaf area.

4. Conclusion

To conclude, this research shows the corresponding changes in spur leaf characteristics of 'Amasya' and six common apple cultivars. We determined a significant variability in the SPAD and spur leaf area amongst cultivars/ flowering and non-flowering spurs. 'Amasya' had a relatively low SPAD value and spur leaf area compared to other cultivars. It is vital to have good growth of spur leaves to maximize the productivity of apple trees. Therefore, the knowledge of spur leaf properties of the cultivars studied here could be helpful to organize the management practices or breeding programs. Furthermore, SPAD readings were higher for flowering spurs than non-flowering spurs for all cultivars. The SPAD may play an essential role in estimating apple yielding potential. This study would be the basis for future research over many environmental conditions and long years.

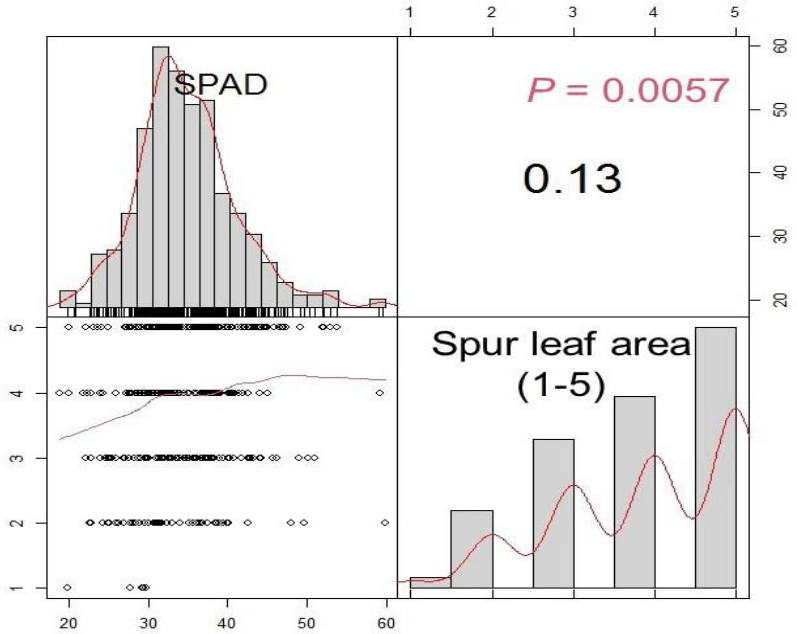

Figure 5. Correlation analysis between Soil Plant Analysis Development (SPAD) value and spur leaf area in flowering and non-flowering spurs of studied apple cultivars.

References

Argenta, G., Ferreira da Silva, P., & Sangoi, L. (2004). Leaf relative chlorophyll content as an indicator to predict nitrogen fertilization in maize. *Ciência Rural,* 34:1379-1387.

Atay, A.N., Özongun, S., Seymen, T., Bayav, A., & Atay, E. (2016). Variability and heritability of floral development in apple full-sib offsprings. *Genetika*, 48:383-394.

Atay, A.N., Atay, E., Lauri, P.E., Kunter, B., & Kantoglu, K.Y. (2018). Phenotyping gamma-ray-induced mutant population of 'Amasya' apple for architectural traits, precocity, floral phenology and fruit characteristics. *Scientia Horticulturae*, 233:195-203.

Atay, E., Crete, X., Loubet, D., & Lauri, P.E. (2019). A moderate regulated deficit irrigation does not negatively affect flowering, fruit-set and return-bloom in a late apple cultivar. *Acta Horticulturae*, 1253:63-70.

Bairam, E., Delaire, M., Le Morvan, C., & Buck-Sorlin, G. (2017). Models for predicting the architecture of different shoot types in apple. *Frontiers in Plant Science,* 8:65.

Benati, J.A., Nava, G., & Mayer, N.A. (2021). SPAD index for diagnosis of nitrogen status in 'Esmeralda' peach. *Revista Brasileira de Fruticultura Jaboticabal,* 43:(e-093).

Bishnu, P.K. (2020). SPAD value varies with age and leaf of maize plant and its relationship with grain yield. *BMC Research Notes*, 13:475.

Breda, N.J.J. (2003). Ground-based measurements of leaf area index: a review of methods, instruments and current controversies *Journal of Experimental Botany,* 54: 2403-2417.

Brunetto, G., Trentin, G., Ceretta C., Girotto E., Lorensini F., Miotto A., Moser, G.R.Z., & Melo, G. W. de. (2012). Use of the SPAD-502 in estimating nitrogen content in leaves and yield in grapevines in soils with different texture. *American Journal of Plant Sciences,* 3:1546-1561.

Castelli, F., Contillo, R., & Miceli F (1996). Non-destructive determination of leaf chlorophyll content in four crop species. *Journal of Agronomy and Crop Science,* 177:275–283.

Cerovic, Z.G., Ghozlen, N.B., Milhade, C., Obert, M., Debuisson, S., & Le Moigne M. (2015). Nondestructive diagnostic test for nitrogen nutrition of grapevine (*Vitis vinifera* L.) based on Dualex leafclip measurements in the field. *Journal of Agricultural and Food Chemistry*, 63:3669–3680.

Costes, E. (2003). Winter bud content according to position in 3-year-old branching systems of 'Granny Smith' apple. *Annual Botany,* 92:581–588.

Demirsoy, H. (2009). Leaf area estimation in some species of fruit tree by using models as a non-destructive method. *Fruits*, 64:45-51.

Elsysy, M.A., & Peter M.H. (2017). The role of spur leaves, bourse leaves, and fruit on local flower formation in apple: an approach to understanding biennial bearing. *HortScience,* 52: 1229-1232.

Ferree, D.C., & Palmer, J.W. (1982). Effect of spur defoliation and ringing during bloom on fruiting, fruit mineral level, and net photosynthesis of Golden Delicious apple. *Journal of the American Society for Horticultural Science*, 107:1182-6.

Ferree, D., Bishop, B., Schupp, J., Tustin, D., & Cashmore, W. (2001). Influence of flower type, position in the cluster and spur characteristics on fruit set and growth of apple cultivars. *Journal of Horticultural Science and Biotechnology*, 76:1-8.

Ferree, D.C., & Schmid, J.C. (2004). Spur leaf and flower characteristics of apple cultivars in the 1995 NE-183 trial. *Journal of the American Pomological Society,* 58:90-97.

Hou, W., Shen, J., Xu, W., Khan, M.R., Wang, Y., Zhou, X., Gao, Q., Murtaza, B., & Zhang, Z. (2021). Recommended nitrogen rates and the verification of effects based on leaf SPAD readings of rice. *PeerJ,* 9:e12107.

Jifon, J.L., Syvertsen, J.P., & Whaley, E. (2005). Growth environment and leaf anatomy affect nondestructive estimates of chlorophyll and nitrogen in *Citrus* sp. leaves. *Journal of the American Society for Horticultural Science*, 130:152–158.

Knerl, A., Anthony, B., Serra, S., & Musacchi, S. (2018). Optimization of leaf area estimation in a high-density apple orchard using hemispherical photography. *HortScience,* 53:799-804.

Lauri, P.E., & Kelner, J.J. (2001). Shoot type demography and dry matter partitioning: a morphometric approach in apple (*Malus* × *domestica*). *Canadian Journal of Botany*, 79:1270–1273.

Lauri, P.E., Bourdel, G., Trottier, C., & Cochard, H. (2008). Apple shoot architecture: evidence for strong variability of bud size and composition and hydraulics within a branching zone. *New Phytology,* 178:798–807.

Mora, M., Avila, F., Carrasco-Benavides, M., Maldonado, G., Olguín-Cáceres, J., & Fuentes, S. (2016). Automated computation of leaf area index from fruit trees using improved image processing algorithms applied to canopy cover digital photograpies, *Computers and Electronics in Agriculture,* 123:195-202.

Markwell, J., Osterman, J.C., & Mitchell, J.L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. *Photosynth Research*, 46:467–472.

Miah, M.N.H, Yoshida, T., & Yamamoto, Y. (1997). Effect of nitrogen application during ripening period on photosynthesis and dry matter production and its impact on yield and yield components of semidwarf iniica rice varieties under water culture conditions. *Soil Science and Plant Nutrition*, 43:205-217.

Munoz-Huerta, R.F., Guevara-Gonzalez, R.G., Contreras-Medina, L.M., Torres-Pacheco, I., Prado-Olivarez, J., & Ocampo-Velazquez, R.V. (2013). A review of methods for sensing the nitrogen status in plants: Advantages, disadvantages and recent advances. *Sensors*, 13:10823.

Pallas, B., Bluy, S., Ngao, J., Martinez, S., Clément-Vidal, A., Kelner, J.J., & Costes, E. (2018). Growth and carbon balance are differently regulated by tree and shoot fruiting contexts: an integrative study on apple genotypes with contrasted bearing patterns, *Tree Physiology*, 389:1395–1408.

Percival, G.C., Keary, I.P., & Noviss, K. (2008). The potential of a chlorophyll content SPAD meter to quantify nutrient stress in foliar tissue of sycamore (*Acer pseudoplatanus*), English Oak (*Quercus robur*). *Annals of Forest Science*, 77:11-30.

Proctor, J.T.A., & Palmer, J.W. (1991). The role of spur and bourse leaves of three apple cultivars on fruit set, growth, and calcium content. *Journal of Horticultural Science*, 66:275-282.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical

Computing, Vienna, Austria. URL https://www.R-project.org/.Foundation.

Romero, I., Garcia-Escudero, E., & Martin, I. (2013). Leaf blade versus petiole analysis for nutritional diagnosis of *Vitis vinifera* L. cv. Tempranillo. *American Journal of Enology and Viticulture*, 64:50–64.

Rostami, M., Koocheki, A., Mahallati, M.N., & Kafi, M. (2008). Evaluation of chlorophyll meter (SPAD) data for prediction of nitrogen status in corn (*Zea mays* L.). *American-Eurasian Journal of Agricultural & Environmental Sciences,* 3:79-85.

Szulc, P., Bocianowski, J., Nowosad, K., Zielewicz, W., & Kobus-Cisowska, J. (2021). SPAD leaf greenness index: green mass yield indicator of maize (*Zea mays* L.), *genetic and agriculture practice relationship. Plants*, 10:830.

Thompson, J.A., Schweitzer, L.E., & Nelson, R.L. (1996). Association of specific leaf weight, an estimate of chlorophyll, and chlorophyll concentration with apparent photosynthesis in soybean. *Photosynth Research*, 49:1-10.

Treder, W., Klamkowski, K., Kowalczyk, W., Sas, D., & Wójcık, K. (2016). Possibilities of using image analysis to estimate the nitrogen nutrition status of apple trees. *Zemdirbyste-Agriculture*, 103:319-326.

Uddling, J., Gelang-Alfredsson, J., Piikki, K., Pleijel, H. (2007). Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynth Research,* 91:37-46.

Van den Ende, B. (2018). Why apples need both spur and shoot leaves to grow. https://www.treefruit.com.au. Date accessed: December 01, 2021.

Vrignon-Brenas, S., Aurélie, M., Romain, L., Shiva, G., Alana, F., Myriam, D., Gaëlle, R., & Anne, P. (2019). Gradual responses of grapevine yield components and carbon status to nitrogen supply. *OENO One*, 53:2431.

Wang, S., Zhu, Y., Jiang, H., & Cao, W. (2006). Positional differences in nitrogen and sugar concentrations of upper leaves relate to plant N status in rice under different N rates. *Field Crop Research*, 96:224–234.

Willaume, M., Lauri, P.E., & Sinoquet, H. (2004). Light interception in apple trees influenced by canopy architecture manipulation. *Trees,* 18:705–713.

Wünsche, J.N., & Palmer, J.W. (1997). Comparison of non-destructive methods of estimating leaf area in apple tree canopies. *Acta Horticulturea,* 451:701-708.

Xiong, D., Chen, J., Yu, T., Gao, W., Ling, X., Li, Y., Peng, S., & Huang, J. (2015). SPAD-based leaf nitrogen estimation is impacted by environmental factors and crop leaf characteristics. *Scientific Reports,* 5:13389.

Zhang, K., Liu, X., Tahir Ata-Ul-Karim, S., Lu, J., Krienke, B., Li, S., Cao, Q., Zhu, Y., Cao, W., & Tian, Y. (2019). Development of chlorophyll-meter-index-based dynamic models for evaluation of high-yield Japonica rice production in Yangtze River Reaches. *Agronomy,* 9:106.

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RESEARCH PAPER

Determination of The Response of Wild and Cultivated Tomato Genotypes to Some Disease and Pests by Molecular Markers

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Tomato

Abstract

Fusarium oxysporum f. sp. *lycopersici, Verticillium* spp., and nematodes, as well as virus diseases that negatively affect production with limited chemical control cause significant losses in greenhouse tomato cultivation. The practical and effective side of controlling diseases is genetic control by breeding. Using a genomic approach for plant breeding is a more sustainable and effective way to control disease and pests. The development of the resistant line is improved by conventional breeding methods that can be conducted over a long period. However, molecular markers make these processes considerably shorter with identifying resistant individuals. In this study, 14 wild and 188 cultivated tomato genotypes have been tested against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), *Meloidogyne* spp. (root-knot nematodes), Tomato spotted wilt virus (TSWV), Tomato mosaic virus (ToMV), Tomato yellow leaf curl virus (TYLCV) and *Verticillum dahliae* Kleb. (Ve) diseases and pests with using MAS (Marker- Assisted Selection) technique. According to these results, it has been determined that the selected markers can be used effectively in breeding studies to determine the diseases mentioned above.

1. Introduction

The cultivated tomato (*Solanum lycopersicum* L.) is a self-pollinated plant with 2n=24 chromosomes and its origin is Middle and South America. It is thought that it has been brought to the European continent in the 16th century from Peru, Ecuador and Bolivia. It is a very popular type of vegetable that entered Turkey in the 1900s (Kaya et al., 2018). It has a genetic potential since it has been cultivated in the world from ancient times to the present day. It is very important to determine these genetic differences and using inbreeding studies based on biotic and abiotic stress that may occur today and in the future. There are serious crop losses due to these diseases and pests during cultivation. Therefore, disease and pest resistance genotypes are very important in tomato breeding studies. On the other hand, intensive use of pesticides for plant pests and diseases both negatively affect the environment and human health. For this reason, the search for less chemical control or alternative methods continues in Turkey and the world. The most important way is to develop resistant varieties against diseases and pests. The common fungal diseases encountered in greenhouse tomato cultivation in Turkey are *Fusarium oxysporum* f. sp. *lycopersici* and *Verticillium* spp. These soil-borne diseases cause significant economic damage. In addition, it is reported that yield losses caused by virus diseases negatively affect and have no chance of chemical control (Hanson et al., 2016). For this reason, studies on developing resistant cultivars are very important in the control of viruses. For resistance studies, it is necessary to identify the virus effectively, to know its molecular structure and disease mechanism. Various methods have been

used in the identification of viral diseases. Multiple identification methods such as biological indexing, cellular structures, vector transport status, serological tests, particle morphology, and molecular methods can be used to characterize any virus in plant diseases. World tomato cultivation is restricted because of many virus diseases which are not controlled by chemicals. The most common of these viruses are; Tomato Mosaic Virus (ToMV), Tomato yellow leaf curl virus (TYLCV), Cucumber mosaic virus (CMV), Tomato spotted wilt virus (TSVW), Potato Y Virus (PVY), Tomato chlorosis virus (ToCV), Tomato brown rugose fruit virus (ToBRFV), which has been increasing rapidly of last years, can cause significant damage in Turkey and the world (Guldur et al.,1994; Yurtmen et al.,1999; Nie and Singh, 2002; Mason et al., 2003; Çevik and Erkış, 2007; Fidan, 2020). Molecular markers have been used extensively in many plants since 1980 in the development of breeding lines that are resistant or tolerant to these viruses. In tomatoes, the use of molecular marker techniques in disease resistance has been developed in the last 10 years and marker-assisted selection (MAS) is a quite common method that has been used in plant breeding programs by researchers (Grube et al., 2000). Owing to these markers, screening of the genotypes to biotic factors and use for breeding programs is more rapid and reliable. Inbreeding studies, MAS selection can be used against the *Fusarium*, *Verticillium,* bacterial spot disease, ToMV, TYLCV and Root-Knot Nematode routinely.

The advantages of MAS studies can be summarized as follows:

- It is an easier method than phenotypic screening.
- It saves time by providing selection during the germination phase.
- Its reliability is high as it is not affected by environmental factors.
- Allows the selection of a single plant by selecting homozygous and heterozygous plant.
- Contributes to the creation of breeding records by determining the genetic status
- It provides a more precise and accurate selection of genotypes with specific characteristics.
- In recent years, markers such as Randomly Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), Sequence Characterized Amplified Region (SCAR), Cleaved Amplified Polymorphic Sequence (CAPS), Single Nucleotide Polymorphism (SNP) and Insertion-Deletion (InDel) have been used in tomato. It is one of the common techniques used to identify genotypes (Gebhardt, 2007).

In this study, tomato lines, other characteristics (yield, fruit quality, plant height, fruit weight, plant vigor etc.), determined according to IPGRI (International Plant Genetic Research Institute) rules, were used. The purpose of the study was to test these lines against some diseases and pests such as *F. oxysporum* f. sp. *radicis-lycopersici*, *V. dahliae*, Tomato spotted wilt virus, Tomato mosaic virus, Tomato yellow leaf curl virus, *Meloidogyne* spp., by using molecular marker methods and to provide material for future tomato breeding programs.

2. Materials and Methods

A total of 202 tomato genotypes including released or commercial cultivars up to F5-F8 level for breeding program and accessions were used in this research. Tomato accessions (LA series) were provided by the Tomato Genetics Resource Center, University of California, Davis, CA 95616 (TGRC, 2014).

All tomato genotypes were used for testing against *F. oxysporum* f.sp. *radicis-lycopersici*, *Meloidogyne* spp., Tomato spotted wilt virus, Tomato mosaic virus, Tomato yellow leaf curl virus and *V. dahliae*. LA 2830 (*S. habrochaites*), LA 0722 (*S. pimpinellifolium*), LA 4442 (*S. penelli*), LA 4353 (*S. lycopersicum* var. *cerasiforme*), LA 4252 (*S. pimpinellifolium*), LA 3325 (*S. juglandifolium*), LA 2332 (*S. habrochaites*). For DNA extraction, 20 seeds of each line were sown in seeding dishes filled with substrates that were artificially mixed with a proportion of peat: perlite in a 1:1 ratio. When the plants had 3-4 true leaves, they were used for DNA analysis.

DNA isolation was performed according to the CTAB method developed by Doyle and Doyle (1987). In this method, 0.1 g of young leaf samples taken from each genotype were thoroughly crushed in a mortar with liquid nitrogen (-195°C), then the leaf frozen transferred into 2.2 ml microfuge tubes and added 0.9 ml extraction buffer [(2% w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris–HCl (pH 8.0), 1% PVP, 0.1% sodium bisulfite, and 0.2% (v/v) 2-mercaptoethanol] tubes to which buffer solution was added were kept in a hot water bath at 65°C for 1 hour with stirring every 10 minutes. The samples removed from the water bath were kept at room temperature for 5-10 minutes, and then 0.9 ml of chloroform: isoamyl alcohol (24:1) was added into the tubes. Tubes containing chloroform-isoamyl alcohol were mixed continuously and very slowly for 15 minutes and then centrifuged at 14000 rpm for 15 minutes. The supernatants of the centrifuged samples were taken (approximately 0.75 ml) and transferred to a new tube, and cold (incubated at-20°C) isopropanol (0.5 ml) was added to it, and the tubes were shaken slowly and the DNA was allowed to precipitate. The supernatant was then removed from the tube. Then, 1 ml of 76% ethanol containing 10 mM ammonium acetate was added to the tube and the tubes were shaken for 15 minutes. After the washed DNA was centrifuged at 2000 rpm for 30 seconds, the supernatant was poured and the DNA was dried at room temperature. The dried DNA was thawed after one day by adding ultrapure water.

Then, the concentration of the obtained DNA was adjusted to 20 ng μl^{-1} for use in PCR analysis.

The DNA markers used in the research are given in Table 1. PCR conditions vary for each DNA marker, but in each reaction solution 1X PCR buffer (50 mM KCl, 10 mM Tris-HCL, pH 9.0, 0.1% Triton X-100), 0.2 mM dNTPs, 2 mM $MgCl_2$, 0.5 U Taq Polymerase, 5 pmol forward and reverse primer and 20-100 ng DNA sample were added. In the reactions, the first denaturation was started at 94°C for 5 minutes and the cycle was performed 37 times, including denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds (it may vary according to each DNA marker) and 45 seconds at 72°C, and this cycle was performed for 5 minutes at 72°C. The PCR products obtained as a result of the study were conditioned on 2-3% agarose gel and the results were evaluated. When the 2-3% agarose gel used was insufficient, high-resolution agarose gel was used in the study. The gel was visualized in a UV device after staining with 0.5 mg ml^{-1} ethidium bromide solution.

3. Results and Discussion

In this study, 14 wild tomatoes genotype and 188 *S. lycopersicum* genotypes were screened with 6 SCAR and 1 CAPS marker providing resistance against nematode (Mi), *V. dahliae*, *F. oxysporum* f. sp. *radicis-lycopersici*, Tomato spotted wilt virus, Tomato yellow leaf curl virus and Tomato mosaic virus.

Pmi markers developed in association with the Mi-1.2 gene were used for resistance to nematodes. Homozygous resistance band of 550 bp and susceptible genotype was determined in 350 pb with Pmi3 marker (Table 2). According to these results, 32 homozygous resistant, 1 heterozygous resistant and 139 susceptible genotypes were determined in the cultivated tomato (*S. lycopresicum*) genotype (Table 3). While no resistance was found in S. pennelii (LA4442), S. peruvianum, *S. lycopersicum* var. *cerasiforme* (LA4353), *S. juglandifolium* (LA3325) and *S. pimpinellifolium* (LA4252, LA0722), 1 in *S. habrochaites* (LA0407) line with resistance to nematodes was determined. Devran and Elekçioğlu (2004), tested the Pmi and Mi23 SCAR markers and found similarly homozygous-susceptible at 350 bp homozygous-resistance bands at 550 bp in tomato lines. Generally, studies show that *S. peruvianum* species have nematode resistance (El Mehrach et al., 2005; Seah et al., 2007; Kaur et al., 2014). However, according to the results of our research, nematode resistance was not found in the *S. peruvianum* line. However, genotypes with nematode resistance were determined in *S. lycopersicum* and *S. habrochaites* species. In this case, it is thought that the origins of the cultivated tomato (*S. lycopersicum*) may have crossed with different resistance wild types lines.

Ve2 marker, developed in association with the Ve gene, was used for resistance to Verticillium wilt and 242 bp of resistance and 131 bp of susceptible bands were obtained. According to these results, 47 homozygous-resistant, 66 heterozygous-resistant and 45 susceptible genotypes were determined in the *S. lycopresicum* genotype (Table 3). *S. pennelii* (LA4442) and *S. peruvianum* genotype had one

Table 1. Primer sequences used in the study.

Gene	Marker	Primer sequence		Marker type	References
Tm-2a	Tm2	F: CAC CTT TCC CTC TCC AA R: CAC CTT TCC CCT AAA GC	Co-dominant	SCAR	Dax et al. (1998)
Ve	Ve2	F:GGA TCT TAG CTC ACT TTA TGT TTT GAA C R: GGT GCT GGT TTC AAC TCT GAA GT	Co-dominant	SCAR	Kawchuk et al. (2001)
Mi	PMi3	F:GGT ATG AGC ATG CTT AAT CAG AGC TCT C R:CCT ACA AGA AAT TAT TGT GCG TGT GAA TG	Co-dominant	SCAR	El Mehrach et al. (2005)
Sw-5	Sw5-2	F:AAT TAG GTT CTT GAA GCC CAT CT R:TTC CGC ATC AGC CAA TAG TGT	Co-dominant	SCAR	Dianese et al. (2010)
Ty3	P6-25	F:GTA GTG GAA ATG ATG CTG CTC R:CTC TGC CTA TTG TCC CAT ATA TAA CC	Co-dominant	SCAR	Ji et al. (2007a)
Ty1	JB1	F: AAC CAT TAT CCG GTT CAC TC R: TTT CCA TTC CTT GTT TCT CTG	Co-dominant	CAPS	De Castro et. al. (2007)
Frl	$SCAR_{Frl}$	F:TTG GCC ATT GAA TGA AGA AC R: CAT CTG TTT TTA GTC TAT TC	Co-dominant	SCAR	Mutlu et al. (2015)

Table 2. DNA fragment sizes (bp) identify resistant and susceptible individuals linked to resistance genes.

Resistance	Tm-2a	Ve	Mi	Sw	Ty3	Ty1	Frl
			Base pairs (bp)				
H	703	242	550	574	630	500	1000
R	703-538	242-131	550-350	574-470	630-350	500-400	1000-950
S	538	131	350	510-470	350	400	950

H: Homozygous resistant, R: Heterozygous resistant, S: Susceptible

Table 3. Tomato genotypes used in the study and their resistance situation (number).

	Tm-2a	*Ve*	*Mi*	*Sw*	*Ty3*	*Ty1*	*Frl*
S. lycopersicum							
H	16	47	32	6	22	17	14
R	11	66	1	7	4	1	80
S	85	45	139	121	128	153	63
S.pimpinellifolium							
H	0	2	0	0	0	0	0
R	1	2	0	0	0	0	2
S	2	0	4	2	4	4	1
S.penelli							
H	0	0	0	0	0	0	0
R	0	1	0	0	0	0	0
S	2	0	2	2	2	2	2
S.habrochaites							
H	1	3	1	0	0	0	1
R	1	1	0	0	0	0	1
S	2	0	4	5	5	5	3
S.peruvianum							
H	0	0	0	0	0	0	0
R	0	1	0	0	0	0	1
S	1	0	1	1	1	1	0
S.lycopersicum var. *cerasiforme*							
H	0	1	0	0	0	0	0
R	0	0	0	1	0	0	1
S	0	0	0	0	0	0	0
S. juglandifolium							
H	0	1	0	0	0	0	0
R	0	0	0	0	0	0	0
S	1	0	1	1	1	1	1

H: Homozygous resistant, R: Heterozygous resistant, S: Susceptible

heterozygous resistance, one homozygous resistance in *S. lycopersicum* var. *cerasiforme* (LA4353) and *S. juglandifolium* (LA3325) genotypes, two homozygous resistance in *S.pimpinellifolium* (LA0722 and LA4252) species two heterozygous resistance, three homozygous and one heterozygous resistance against Verticillium wilt were determined in *S. habrochaites* (LA 2830 and LA2332) line (Table 3). In different studies, it has been reported that Ve1 and Ve2 markers developed against Verticillium wilt were resistant in the range of 242 bp and susceptible in the range of 131 bp and could be easily used in MAS studies (Acciaari et al., 2007; Morid et al., 2017).

SCARFrl marker was used for resistance to *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL). Accordingly, resistance bands were obtained in 1000 bp and susceptible bands in 950 bp. 14 homozygous resistant, 80 heterozygous resistant and 63 susceptible genotypes were determined in *S. lycopersicum* (Table 3). Two susceptible genotypes in *S. pennelii* (LA4442) species, one heterozygous resistant in S. peruvianum and *S. lycopersicum* var. *cerasiforme* (LA4353) species, one susceptible in *S. juglandifolium* (LA3325) and two heterozygous resistants, one susceptible in *S. pimpinellifolium* (LA0722) species, *S. habrochaites* (LA 2830, LA2332) one homozygous, two heterozygous resistant and three susceptible genotypes were determined (Table 3). It has been determined that there are many genotypes of FORL resistance used in our research. For this reason, is thought to be the genetic resistance of FORL controlled by a single dominant gene (Roberts et al., 2000). In addition, in a study, *S. peruvianum* is shown as a source of FORL resistance (Laterrot and Moretti, 1991). It has been reported that the SCARFrl marker (1000 bp resistant and 950 bp susceptible) that we used in our research was easily used in other studies and very successful results were obtained (Mutlu et al., 2015). These studies support our study (Table 3).

It was tested with the Sw-5-2 SCAR marker, which was developed in association with the Sw-5 gene, which provides resistance to Tomato spotted

wilt virus. Resistant bands were obtained with the relevant marker at 574 bp and susceptible bands at 470 bp. In *S. lycopresicum* genotypes, 6 homozygous, 7 heterozygous resistant and 121 susceptible genotypes were determined (Table 3). One susceptible in *S. peruvianum* and *S. juglandifolium* (LA3325) species, two susceptible in *S. pimpinellifolium* (LA0722) and *S. pennelii* (LA4442) species, 5 susceptible genotypes in S. habrochaites (LA2332, LA2830) and *S. lycopersicum* var. *cerasiforme* (LA4353) 1 heterozygous resistance was determined (Table 3). Nascimento et al. (2009), in their study with tomato genotypes, screened populations created by hybridization of elite lines known to be resistant to tomato spotted wilt virus with the SCAR marker developed for resistance and reported that the marker was useful in distinguishing heterozygous and homozygous lines. Again, in a similar study conducted by Fidan and Sarı (2019) on tomatoes, the band range of the Sw-5 gene are similar to our study.

In the test for resistance to tomato yellow leaf curl virus, 2 markers (JB1 for Ty-1 and P6-25 for Ty-3) developed with Ty1 and Ty3 alleles, which are 2 of the 4 genes that provide the marker resistance, were used. For Ty1, homozygous resistance was obtained with the JB-1 CAPS marker at 500 bp, while susceptible bands were obtained at 400 bp (De Castro et al., 2007). For Ty3, homozygous resistance was obtained at 630 bp with the P6-25 SCAR marker, while susceptible bands were obtained at 350 bp (Ji et al., 2007a). According to results, in *S. lycopresicum* genotypes, 17 homozygous, 1 heterozygous resistant and 153 susceptible genotypes were determined for the Ty1 gene (Table 3). One each in *S. peruvianum* and *S. juglandifolium* (LA3325), 4 susceptible in *S. pimpinellifolium* (LA0722, LA4252), 2 susceptible in *S. pennelii* (LA4442) species, 5 susceptible in *S. habrochaites* (LA2332, LA2830) species and no result was obtained for *S. lycopersicum* var. *cerasiforme* species (Table 3).

In *S. lycopresicum* genotypes, 22 homozygous, 4 heterozygous resistant and 128 susceptible genotypes were determined for the Ty3 gene (Table 3). One each in *S. peruvianum* and *S. juglandifolium* (LA3325), 4 susceptible in *S. pimpinellifolium* (LA0722, LA4252), 2 susceptible in *S. pennelii* (LA4442), 5 susceptible in *S. habrochaites* (LA2332, LA2830) and *S. lycopersicum* var. *cerasiforme* species, no results were obtained.

It has been demonstrated in all of the studies carried out to date that Ty1 and Ty3 with the widest spectrum among the 6 genes (Ty1, Ty2, Ty3, Ty4, Ty5, Ty6) detected in the development of lines resistant to TYLCV are effective sources of resistance. Ty-1 and Ty-3 have been adopted by different researchers as the markers that show resistance to TYLCV virus in tomatoes and are published in MAS (Zamir et al., 1994; Agrama and Scott, 2006; Ji and Scott, 2007a; Ji et al., 2007b; Lee et al., 2015). A study determined that *S. peruvianum* (LA1589) species showed resistance to TYLCV (Anbinder et al., 2009). However, it was determined that the *S. peruvianum* species used in our study did not show any resistance. While resistance was found in only *S. lycopresicum* species, it was determined that other species were susceptible. While TYLCV was mostly seen in genotypes of commercial origin, it was determined to be susceptible in locally collected species. In this case, it is thought that this resistance is due to the transfer to previously commercial varieties. As a matter of fact, in a study conducted by Kaya et al. (2009), in their screening with the Ty-1 CAPS marker developed for tomato yellow leaf curl virus in the tomato population in the F3 stage, 15 of 131 plants were determined to be heterozygous susceptible and no homozygous resistance could be obtained in any of the plants in a study in which wild species were predominantly used, it was determined that the lines of *S. chilense*, LA1932, LA2779 and LA1938 were resistant to TYLCV (Agrama and Scott, 2006). Again, in a similar study, it was reported that TYLCV resistance could be found in *S. pimpinellifolium*, *S. peruvianum*, *S. chilense*, and *S. habrochaites* species (Ji et al., 2007b). However, it was determined that the wild lines used in our study did not have any resistance against TYLCV virus disease. It is thought that this situation is caused by the different lines used within the species.

It was tested with the Tm2 SCAR marker, which was developed with the Tm-2a gene, which provides resistance to Tomato mosaic virus (Zengin and İlbi, 2016). With the relevant marker, 703 bp of the resistance band and 538 bp of the susceptible band were obtained. In *S. lycopresicum* genotypes, 16 homozygous, 11 heterozygous resistant and 85 susceptible genotypes were determined (Table 3). *S. peruvianum* and *S. juglandifolium* (LA3325) 1 heterozygous resistant, *S. pimpinellifolium* 1 heterozygous resistant and 2 heterozygous resistant (LA0722), *S. pennelii* (LA4442) 2 heterozygous resistant, *S. habrochaites* 1 homozygous resistant (LA2830), 1 heterozygous, 2 susceptible (LA2332) genotypes were determined and no results were obtained for *S. lycopersicum* var. *cerasiforme* (LA4353) (Table 3).

The most common genes conferring tolerance to tomato mosaic virus are known as Tm-1, Tm-2 and Tm-22 (Pelham, 1966; Hall, 1980; Levesque et al., 1990; Lanfermeijer et al., 2003; Foolad, 2007). The Tm-1 gene is also found on chromosome 5 in the *S. habrochaites* (Levesque et al., 1990; Ohmori et al., 1996; Foolad and Sharma, 2004; Foolad, 2007). Tm-2 and Tm-22 were identified on chromosome 9 in *S. peruvianum* species (Hall, 1980; Tanksley et al, 1992; Ohmori et al., 1995; Pelham, 1966; Lanfermeijer et al., 2003). However, it is known as the Tm-2 gene, which is the most widely used in the molecular marker-based selection method (Foolad and Sharma, 2004).

4. Conclusion

Among the most common fungal diseases encountered in greenhouse tomato cultivation, soil-borne diseases and pests such as *F. oxysporum* f. sp. *lycopersici, Verticillium* spp., nematodes, and virus diseases are very difficult to control. Therefore, developing cultivars that can tolerate these diseases and pests during cultivation is one of the most important strategies. It may take a long time to develop a variety with conventional breeding methods, but these periods can be shortened by molecular markers that have developed in recent years. In this study, breeding lines of which all characteristics were determined before, have been tested against *F. oxysporum* f. sp. *radicis-lycopersici*, *Meloidogyne* spp., Tomato spotted wilt virus, Tomato mosaic virus, Tomato yellow leaf curl virus and *V. dahliae* diseases. It has been determined that these markers can be used in future breeding studies in light of this information and the disease and pest resistance of some wild lines.

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References

Acciarri, N., Rotino, G.L., Tamietti G., Valentino, D., Voltattorni, S., & Sabatini E. (2007). Molecular markers for Ve1 and Ve2: *Verticillium* resistance genes from Italian tomato germplasm. *Plant Breeding,* 126:617-621.

Agrama, H.A., & Scott, J.W. (2006). Quantitative trait loci for Tomato Yellow Leaf Curl Virus and Tomato Mottle Virus resistance in tomato. *Journal of the American Society for Horticultural Science,* 131:267-272.

Anbinder, I., Reuveni, M., Azari, R., Paran, I., Nahon, S., Shlomo, H., & Levin, I. (2009). Molecular dissection of Tomato Leaf Curl Virus resistance in tomato line TY172 derived from *Solanum peruvianum*. *Theoretical and Applied Genetics*, 119:519-530.

Çevik, B., & Erkış, G. (2008). First report of Tomato chlorosis virus in Turkey. *Plant Pathology*, 57(4).

Dax, E., Livneh, O., Aliskevicius, E., Edelbaum, O., Kedar, N., Gavish, N., & Sela, I. (1998). A SCAR marker linked to the ToMV resistance gene, *Tm2 2*, in tomato. *Euphytica,* 101:73-77.

De Castro, A.P., Blanca, J.M., Díez, M.J., & Viñals, F.N. (2007). Identification of a CAPS marker tightly linked to the Tomato Yellow Leaf Curl Disease resistance gene *Ty-1* in tomato. *European Journal of Plant Pathology*, 117:347-356.

Devran, Z., & Elekçioğlu, İ.H. (2004). The screening of F_2 plants for the Root-Knot Nematode resistance gene, *Mi* by PCR in tomato. *Turkish Journal of Agriculture and Forestry*, *28*:253-257.

Dianese, E.C., Fonseca, M.E. Goldbach, R., Kormelink, R., Inoue-Nagata, A.K. Resende, R.O., & Boiteux, L.S. (2010). Development of a locus-specific, co-dominant SCAR marker for assisted-selection of the *Sw-5* (Tospovirus resistance) gene cluster in a wide range of tomato accessions. *Molecular Breeding*, 25:113-142.

Doyle, J.J., & Doyle, J. (1987). A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochemical bulletin*, 19:11-15.

El Mehrach, K., Gharsallah Chouchane, S., Mejia, L., Williamson, V. M., Vidavski, F., Hatimi, A., & Maxwell, D.P. (2005). PCR-based methods for tagging the *Mi-1* locus for resistance to Root-Knot Nematode in Begomovirus-resistant tomato germplasm. *Acta Horticulturae*, 695:263-270.

Fidan, H., & Sarı, N. (2019). Phenotypic characterization of the isolate that breaks resistance to Tomato Spotted Wilt Virus in tomato. *Mediterranean Agricultural Sciences*, *32*:307-314.

Fidan, H. (2020). Tomato Brown Rugose Fruit Virus (ToBRFV): current situation and future prospects. *Mediterranean Agricultural Sciences*, 33:43-49.

Foolad, M.R., & Sharma, A. (2004). Molecular markers as selection tools in tomato breeding. *Acta Horticulturae,* 695:225-240.

Foolad, M.R. (2007). Genome mapping and molecular breeding of tomato. *International Journal of Plant Genomics*. 2007:1-52.

Gebhardt, C. (2007). Molecular markers, maps and population genetics. *In Potato Biology and Biotechnology*, Elsevier Science B.V. p. 77-89.

Grube, R.C., Radwanski, E.R., & Jahn, M. (2000). Comparative genetics of disease resistance within the solanaceae. *Genetics*, 155:873–887.

Guldur, M.E., Ozaslan, M., Baloglu, S., & Yilmaz, M. A. (1994). Pepper mild mottle virus in pepper in Turkey. In *Proceedings of the 9th Congress of the Mediterranean Phytopathological Union, Kusadası,* pp. 465-467.

Hall, T.J. (1980). Resistance at the Tm-2 locus in the tomato to tomato mosaic virus. *Euphytica*, *29:*189-197.

Hanson, P., Lu, S.F., Wang, J.F., Chen, W., Kenyon, L., Tan, C.W., & Yang, R.Y. (2016). Conventional and molecular marker-assisted selection and pyramiding of genes for multiple disease resistance in tomato. *Scientia Horticulturae*, 201:346-354.

Ji, Y., Schuster, D.J., & Scott, J.W. (2007a). *Ty-3*, a Begomovirus resistance locus near the Tomato yellow leaf curl virus resistance locus *Ty-1* on chromosome 6 of tomato. *Molecular Breeding*, 20:271-284.

Ji, Y., Scott, J.W., Hanson, P., Graham, E. & Maxwell, D.P. (2007b). Sources of resistance, inheritance, and location of genetic loci conferring resistance to members of the tomato-infecting Begomoviruses. In Tomato Yellow Leaf Curl Virus disease. *Springer*, Dordrecht. p. 343-362.

Kaur, S., Dhaliwal, M.S., Cheema, D.S., Jindal, S.K., & Gaikwad, A.K. (2014). Screening of tomato (*Solanum lycopersicum L.*) germplasm for Root-Knot Nematode resistance using conventional and molecular marker techniques. *Indian Journal of Nematology*, *44*:56-61.

Kawchuk, L.M, Hachey, J., Lynch, D.R., Kulcsar, F., van Rooijen, G., Waterer, D.R., Robertson, A., Kokko, E., Byers, R., Howard, R.J., Fischer, R., & Prüfer, D. (2001). Tomato *Ve* disease resistance genes encode cell surface-like receptors. *PNAS,* 98:6511–6515.

Kaya, H.B., & Tanyolaç, B. (2009). Screening of F3 segregation population lines revealed by *Ty-1* markers linked to resistance locus of Tomato Yellow Leaf Curl Disease (TYLCD) in tomato (*Lycopersicum esculentum*). *International Journal of Natural and Engineering Sciences*, 33:149-153.

Kaya, Y., Al-Remi, F., Arvas, Y.E., & Durmus, M. (2018). Tomato plant and ıts ın vitro micropropagation. *Journal of Engineering Technology and Applied*

and Applied Sciences, 3:57-73.

Lanfermeijer, F.C., Dijkhuis, J., Sturre, M.J., de Haan, P., & Hille, J. (2003). Cloning and characterization of the durable Tomato Mosaic Virus resistance gene *Tm-2 2* from *Lycopersicon esculentum*. *Plant Molecular Biology*, 52:1039-1051.

Laterrot, H., & Moretti, A. (1991). Allelism of various FORL resistance sources. *Report of the Tomato Genetics Cooperative,* 41:28-30.

Lee, H.J., Kim, B., Bae, C., Kang, W.H., Kang, B.C., Yeam, I., & Oh, C.S. (2015). Development of a single-nucleotide polymorphism marker for the *Sw-5b* gene conferring disease resistance to Tomato Spotted Wilt Virus in tomato. *Horticultural Science & Technology,* 33:730-736.

Levesque, H., Vedel, F., Mathieu, C., & De Courcel, A.G.L. (1990). Identification of a short rDNA spacer sequence highly specific of a tomato line containing *Tm-1* gene introgressed from *Lycopersicon hirsutum*. *Theoretical and Applied Genetics*, 80:602-608.

Mason, G., Roggero, P., & Tavella, L. (2003). Detection of Tomato Spotted Wilt Virus in its vector *Frankliniella occidentalis* by reverse transcription polymerase chain reaction. *Journal of Virological Methods*, 109:69-73.

Morid, B., & Haj Mansour, S. (2017). Assessment of tomato genotypes resistance to *Verticillium* and *Fusarium wilt* diseases using molecular markers. *Journal of Microbial World*, 10:80-93.

Mutlu, N., Demirelli, A., Ilbi, H., & Ikten, C. (2015). Development of codominant SCAR markers linked to resistant gene against the *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Theoretical and Applied Genetics*, 128:1791-1798.

Nascimento, I.R.D., Maluf, W.R., Figueira, A.R., Menezes, C.B., Resende, J.T.V.D., Faria, M.V., & Nogueira, D.W. (2009). Marker assisted identification of Tospovirus resistant tomato genotypes in segregating progenies. *Scientia Agricola*, 66:298-303.

Nie, X., & Singh, R.P. (2002). A new approach for the simultaneous differentiation of biological and geographical strains of Potato Virus Y by uniplex and multiplex RT-PCR. *Journal of Virological Methods*, 104:41-54.

Ohmori, T., Murata, M., & Motoyoshi, F. (1995). Identification of RAPD markers linked to the *Tm-2* locus in tomato. *Theoretical and Applied Genetics*, 90:307-311.

Ohmori, T., Murata, M., & Motoyoshi, F. (1996). Molecular Characterization of RAPD and SCAR Markers Linked to the *Tm-1* Locus in Tomato, *Theoretical and Applied Genetics*, 92:151-156.

Pelham, J. (1966). Resistance in tomato to Tobacco Mosaic Virus. *Euphytica*, 15:258-267.

Roberts, P.D., McGovern, R.J., & Datnoff, L.E. (2000). *Fusarium crown* and root rot of tomato in Florida. *Plant Pathology Fact Sheet*, 184:1-4.

Seah, S., Williamson, V.M., Garcia, B.E., Mejia, L., Salus, M.S., Martin, C.T. & Maxwell, D.P. (2007). Evaluation of a co-dominant SCAR marker for detection of the *Mi-1* locus for resistance to Root-Knot Nematode in tomato germplasm. *Tomato Genetic Cooperative Report*, *57*:37-40.

Tanksley, S.D., Ganal, M.W., Prince, J.P., De Vicente, M.C., Bonierbale, M.W., Broun, P., & Martin, G. (1992). High density molecular linkage maps of the tomato and potato genomes. *Genetics,* 132:1141-1160.

TGRC (2014). Tomato Genetics Resource Center, University of California, Davis, CA 95616. https://tgrc.ucdavis.edu/. Accessed date: 10 September, 2021.

Yurtmen, M., Guldur, M.E. & Yilmaz, M.A. (1999). Tomato Spotted Wilt Virus on peppers in Icel Province of Turkey. *Petria,* 9:243-344.

Zamir, D., Ekstein-Michelson, I., Zakay, Y., Navot, N., Zeidan, M., Sarfatti, M., & Czosnek, H. (1994). Mapping and introgression of a Tomato Yellow Leaf Curl Virus tolerance gene, *Ty-1*. *Theoretical and Applied Genetics*, 88:141-146.

Zengin, S. & İlbi, H. (2016). Development of Tomato Lines Resistant to Viral (Tomato Yellow Leaf Curl Virus, Tomato Spotted Wilt Virus), Fungal (Root and Root Rot Rot), Diseases and Nematodes (*Meloidogyne Incognita*) by Molecular Marker Assisted Selection. PhD Thesis, Ege University, İzmir (in Turkish).

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RESEARCH PAPER

Identification of Races 1, 2, 4 and 8 of *Fusarium oxysporum* f. sp. *dianthi* in Turkey by Using Molecular Markers

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Abstract

Fusarium oxysporum f.sp. *dianthi* (Prill and Delacr.) (*Fod*) is a causal agent of wilt disease of carnation (*Dianthus caryophyflus* L.) with its different physiological races. Although eleven physiological races of *Fod* have been reported in the world, a polymerase chain reaction (PCR)-based diagnostic tool was developed for identification of *Fod* races 1, 2, 4, and 8, which are the most commonly found in many countries. On the other hand, there is no information about which races are found in Turkey. A total of 91 isolates were collected from plants with Fusarium-like symptoms in the most grown carnation areas in Antalya, İzmir, Isparta and Yalova provinces and all isolates were identified using molecular methods. As a conclusion, the results showed that *Fod* isolates collected from the most carnation grown provinces of Turkey consist mostly of race 1, 2 and 8. This is the first study reporting *Fod* races causing wilt disease on carnation plants in Turkey.

1. Introduction

Carnation (*Dianthus caryophyllus* L.) is one of the highly valued plant species among the cut flowers in Turkey and worldwide. The biggest carnation exporters in the world are Colombia (52.78%), the Netherlands (23.04%), and Turkey (5.75%), respectively (Kazaz et al., 2020). Export values of all three countries between 2015-2019 have increased by years. Colombia alone carries out more than half of the world's carnation exports. According to the data of 2018, carnation is the most produced cut flower in Turkey with an area of 494 ha. According to the data of the same year, 607 070 350 branches of carnations were produced in Turkey. The provinces where the most carnation is produced in Turkey are Antalya (54.11%), İzmir (31.83%), and Isparta (13.07%) (Kazaz et al., 2020).

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi* (Prill and Delacr.) with its different physiological races, is responsible for the most devastating disease on carnation cultivation worldwide (Vergara et al., 2007; Poli et al., 2013; Castano et al., 2014; Deng, 2018). Moreover, it is widely seen and constitutes economic damage in Turkey (Tezcan et al., 2004; Coskuntuna and Yıldız, 2006; Arıcı and Kazaz, 2013; Arıcı et al., 2018; Kazaz et al., 2020; Atakan and Özgönen Özkaya, 2020). Breeders in the world have been developing carnation cultivars resistance to *Fod* because it is the most destructive pathogen on carnation (Scovel et al., 2001). On the other hand, the genetic basis for race resistance and pathogen virulence have been studied (Chiocchetti et al., 1999; Sarrocco et al., 2007; Werner and Irzykowska, 2007; Poli et al., 2013; Castano et al., 2014). Until today, eleven races of *Fod* have been identified in different countries (Chiocchetti et al., 1999). However, race 3 of *Fod* was recently reclassified as *F. redolens* f. sp. *dianthi* race 3 (Baayen et al., 1997; Bogale et al., 2007). Besides, race 2 is commonly found in all

carnation cultivation areas in the world (Baayen et al., 1997; Bogale et al., 2007; Castano et al., 2014). Furthermore, *Fod* races 1 and 8 were found on carnation cultivation areas in Italy, France, and Spain (Garibaldi et al., 1986; Baayen et al., 1997), while race 4 in the United States, Israel, Italy, Colombia and Spain (Ben-Yephet et al., 1992; Baayen et al., 1997; Sarrocco et al., 2007), races 5, 6, and 7 in Great Britain, France, and the Netherlands (Gabrialdi, 1983; Garibaldi et al., 1986), race 9 in Australia (Kalc Wright et al., 1996), race 10 and 11 in the Netherlands (Baayen et al., 1997).

Physical and chemical managements are not always useful for control of *Fod* due to environmental factors, cost, and limited efficacy. Using resistant cultivars and pathogen-free propagative material offers the most effective approach to Fusarium wilt control (Scovel et al., 2001; Prados-Ligero et al., 2007; Deng, 2018). Race-specific resistance carnation varieties usually show low levels of disease under a range of environmental conditions (Ben-Yephet and Shtienberg, 1997). The determination of races provides relevant information of practical significance (Gabrialdi and Gullino, 1987; Scovel et al., 2001; Prados-Ligero et al., 2007; Baysal et al., 2009; Castano et al., 2014; Polat et al., 2014), which is helpful to breeding programs aimed at selecting resistance cultivars that can reduce the devastating effects of Fusarium wilt (Garibaldi and Gullino, 1987). However, the races of *Fod* are morphologically indistinguishable and pathogenicity tests have been required for race determination (Manulis et al., 1994). Moreover, there are many obstacles such as time-consuming and high dependency on environmental factors etc. (Migheli et al., 1998; Poli et al., 2013). Molecular markers are specific fragments of DNA that can be identified within the whole genome. Nowadays, molecular markers for the determination of *Fod* race 2, race 4, races 1-8 together (Chiochetti et al., 1999), race 1, and race 8 (Migheli et al., 1998) were reported.

The aim of present study was to survey the racial diversity and prevalence of a *Fod* population in the most carnation cultivation provinces in Turkey using specific molecular markers.

2. Material and Methods

2.1. Sampling process

Symptomatic carnation plants showing leaf yellowing and wilting symptoms collected from 91 randomly selected greenhouses in Antalya, İzmir, Isparta and Yalova provinces in Turkey between 2019 and 2020, were sampled (Table 1 and Figure 1). The provinces where the most carnation is produced in Turkey are Antalya (54.11%), İzmir (30.83%), Isparta (13.07%), and Yalova (1.08%) (Kazaz et al., 2020).

2.2. Pathogen isolation

Plants were cut at the stem base and surface sterilization of small pieces of dark discolour vascular tissues were done using 2% (v/v) sodium hypochlorite for 2-3 min. After rinsing two times in sterile distilled water (SDW), tissues were dried on sterile filter paper and placed in Komada's Fusarium semi selective medium containing potato dextrose agar (PDA) and incubated at 25±1°C in 12 h light/dark photoperiod. When hyphae were observed emerging from the tissues, isolates were purified by sub-culturing hyphal tips on fresh PDA. Fungi stored on silica gel were grown on sterile petri dishes on PDA and incubated at 25°C for 7 to 8 days.

2.3. Genomic DNA isolation

Total genomic DNA was extracted from sporangia of *F. dianthi* using a DNA isolation kit (Promega, Wizard Genomic DNA Purification Kit, Madison, US) according to the manufacturer's instructions. Obtained extract was resuspended with DNase-free RNase (Roche Diagnostics, Germany) and checked with a spectrophotometer (Thermo Scientific™ NanoDrop, V, Finland) and quantified in high-resolution agarose gels (1%) using standard lambda DNA for comparison.

2.4. PCR analysis

PCR amplifications specific for *Fod* races 1, 2, 4 and 8 were carried out with the specific primer sets. These primer sets are Ft3 (5′- GGC GAT CTT GAT TGT ATT GTG GTG-3′) / R2.1 (5′-CTT GTT TCT CGA TTT CTG TCT CAC G-3′) to race2, Ft3 / R8.1 (5′-CGA TGA AGT CGG TTT GCG ATT-3′) to jointly race 1 and 8, IMP2 (5′-AAT CCT ATA GAG AAT CTG TGG-3′) / R4.2 (5′-GGT GAT TGG AGG AGG AAT ACC-3′) to race 4 (Chiocchetti et al., 1999) and OPE-08 RAPD (5′-TCA CCA CGG T-3′) to race 1 and 8 (Migheli et al., 1998).

PCR amplification to identify race 2, race 1-8 together, and race 4 were conducted as described by Chiocchetti et al. (1999) with some modifications. PCR amplification was carried out in reaction volumes of 25 µl containing 12.5 µl of 2X master mix (AmpMaster Taq master mix), 1 µl 0.5 mM of each primer, 10 µl double distilled water and 3 µl 20 ng of genomic DNA. A DNA Thermal Cycler (Applied Biosystems SimpliAmp Termal Cycler) was used at the cycling parameters included 5 min of denaturing at 94°C, 30 cycles of 3 steps [30 s of denaturing at 94°C, 30 s of annealing at 55°C and 1 min of elongation at 72°C], and 1 cycle of 10 min at 72°C for the extension. On the other hand, PCR amplification to separately identify races 1 and 8 were conducted as described by Migheli et al. (1998) with some modifications. PCR amplification was conducted in 20 µl of reaction volume containing 10 µl of 2X master mix (AmpMaster Taq

Table 1. PCR amplification of genomic DNA performed with race-specific primers: (+) presence of amplicon, (-) absence of amplicon

Province	Isolate code	Ft3-R8.1 (Race 1 and 8)	Ft3-R2.1 (Race 2)	IMP2-R4.2 (Race 4)	OPE-08 (Race 1)	OPE-08 (Race 8)
Antalya	Tm 1-1	-	-	-	-	-
Antalya	Tm 1-2	+	-	-	-	+
Antalya	Tm 2-1	+	-	-	-	+
Antalya	Tm 2-2	+	-	-	-	+
Antalya	AG 1	+	+	-	+	-
Antalya	AG 2-1a	-	-	+	-	-
Antalya	AG 2-1b	+	-	-	-	+
Antalya	S 1-1	-	+	-	-	-
Antalya	S 1-2	-	+	-	-	-
Antalya	S 6-1	-	-	-	-	-
Antalya	S 6-2	+	-	-	+	-
Antalya	S 6-3	-	+	-	-	-
Antalya	S 7-1	+	-	-	+	-
Antalya	S 7-2	-	+	-	-	-
Antalya	S 8-1	-	+	-	-	-
Antalya	S 8-2	-	-	-	-	-
Antalya	Ant	-	-	-	-	-
Antalya	I-4	+	+	-	-	+
Antalya	I-5	+	+	-	+	-
Antalya	I-6	+	+	-	-	+
Antalya	KA 1	+	-	-	+	-
Antalya	KA 2	+	-	-	+	-
Antalya	KA 3	+	-	-	+	-
Antalya	KA 4	+	-	-	+	-
Antalya	AK 1	+	-	-	+	-
Antalya	AK 2	+	-	-	-	+
Antalya	AK 3	+	-	-	+	-
Antalya	AK 4	+	-	-	+	-
Antalya	AK 5	+	-	-	-	+
Antalya	AK 6	+	-	-	+	-
Antalya	HD 1-1	-	-	-	-	-
Antalya	HD 1-2	-	-	-	-	-
Antalya	BT 1-1	+	-	-	-	+
Antalya	BT 2-1	-	-	-	-	-
Antalya	KT 1-1	-	-	-	-	-
Antalya	KT 1-2a	-	-	-	-	-
Antalya	KT 1-2b	-	-	-	-	-
Isparta	YB 1-2	-	-	-	-	-
Isparta	YL 1-2	-	-	-	-	-
Isparta	TT 1	-	-	-	-	-
Isparta	TT 2-1	+	-	-	-	+
Isparta	TT 3-1	+	-	-	-	+
Isparta	TT 3-2	+	-	-	-	+
Isparta	Tm 2-1	-	-	-	-	-
Isparta	Tm 2-2	-	-	-	-	-
Isparta	UT 1-1	-	-	-	-	-
Isparta	UT 1-2	-	-	-	-	-
Isparta	FT 1	-	-	-	-	-
Isparta	FT 3-1	-	-	-	-	-
İzmir	MA 1	+	-	-	+	-
İzmir	MA 2	+	+	-	+	-
İzmir	MS 1	+	+	-	-	+
İzmir	MA 3	+	+	-	-	+
İzmir	MA 4	+	+	-	-	+
İzmir	MA 5	+	-	-	-	+
İzmir	MA 6	+	+	-	-	+
İzmir	MA 7	+	-	-	+	-
İzmir	MA 8	+	+	-	-	+
İzmir	MA 9	+	+	-	-	+
İzmir	MA 10	+	-	-	-	+
İzmir	MA 11	+	+	-	+	-
İzmir	MA 12	+	+	-	+	-
İzmir	MA 13	+	+	-	+	-
İzmir	MA 14	+	+	-	-	+
İzmir	MA 15	+	+	-	-	+

Table 1. PCR amplification of genomic DNA performed with race-specific primers: (+) presence of amplicon, (-) absence of amplicon (continued)

Province	Isolate code	Ft3-R8.1 (Race 1 and 8)	Ft3-R2.1 (Race 2)	IMP2-R4.2 (Race 4)	OPE-08 (Race 1)	OPE-08 (Race 8)
İzmir	MA 16	+	-	-	-	+
İzmir	İ-1	+	+	-	-	+
İzmir	İ-2	+	+	-	+	-
İzmir	İ-3	+	+	-	+	-
İzmir	İ-4	+	+	-	-	+
İzmir	İ-5	+	-	-	-	+
İzmir	İ-6	+	-	-	+	-
İzmir	U-1	+	-	-	-	+
İzmir	U-2	+	-	-	-	+
İzmir	U-3	+	-	-	+	-
İzmir	U-4	+	-	-	+	-
İzmir	U-5	+	+	-	+	-
İzmir	U-6	+	+	-	+	-
İzmir	U-7	+	+	-	-	+
İzmir	U-8	+	-	-	-	+
İzmir	U-9	+	+	-	-	+
Yalova	K-1	+	-	-	+	-
Yalova	K-2	+	+	-	-	+
Yalova	M-1	+	+	-	-	+
Yalova	M-2	+	+	-	+	-
Yalova	YK 1-2	-	-	-	-	-
Yalova	YK 2-1	+	-	-	+	-
Yalova	YK 2-2	+	-	-	+	-
Yalova	YK 3-1	+	-	-	+	-
Yalova	YK 5-1	+	-	-	+	-
Yalova	YK 5-2	+	-	-	+	-

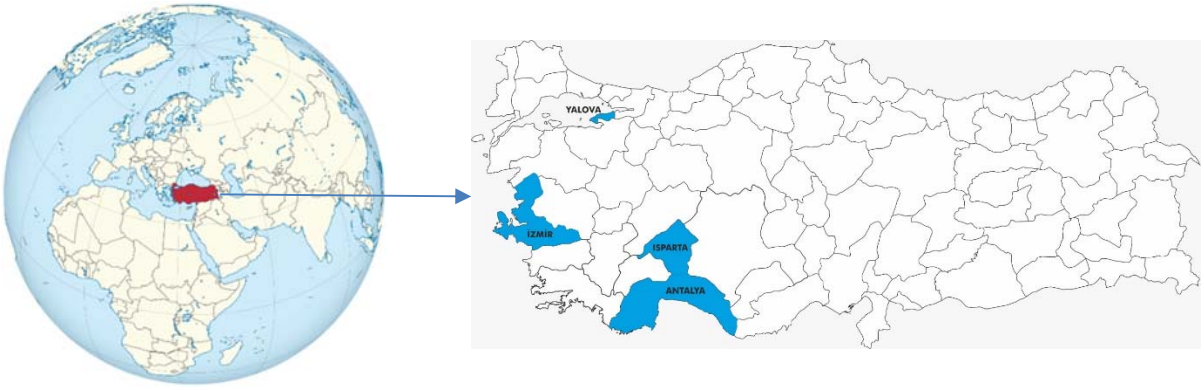

Figure 1. *Fusarium oxysporum* f.sp. *dianthi* sampled in the carnation cultivation provinces of Turkey.
(Antalya: 262.5 ha production area, 11.7 ha sampled area, and 37 isolate; İzmir: 159.6 ha production area, 9.6 ha sampled area, and 32 isolate; Isparta: 51.4 ha production area, 3.5 ha sampled area, and 12 isolate; and Yalove: 3.5 ha production area, 1.0 ha sampled area, and 10 isolate).

master mix), 1 µl 0.5 mM primer, 5 µl double distilled water and 4 µl 20 ng of genomic DNA. PCR conditions were conducted at 94°C for 2.5 min followed by 45 cycles of 94°C for 30 s, 1 min at 36°C, 72°C for 2 min, and followed by final extension of 5 min at 72°C. PCR products were separated on 2.5% high-resolution agarose gel in 1X TAE buffer at 100 V for 3.0 h. A 100 bp DNA ladder (Vivantis) was used as molecular standard. The fragment patterns were photographed under UV light (ENDURO GDS Gel Documentation System) in dye (EZ-ONE N472-KIT, Ambresco) for further analysis. All PCR experiments were repeated three times to confirm the reproducibility of the banding patterns.

3. Results and Discussion

In this study, 82 greenhouses in 4 provinces (Table 1) were sampled. A total of 91 isolates displaying Fusarium morphology were isolated (Figure 1). All collected isolates did not differ in terms of their macroscopic and microscopic characteristics. Amplification with the primer pair Ft3/R2.1, Ft3/R8.1, IMP2/R4.2 and primer OPE-08 RAPD resulted in amplicons of about 564, 295, 1315 and 2000 bp, corresponding to race 2, jointly race 1 and 8, race 4, and separately race 1 and 8, respectively. During the research process, the following *Fod* fungal disease races were analyzed: 1 isolate race 4, 32 isolates race 2, 65 isolates race

1 and 8 (32 isolates of them race 1 and 33 isolates of them race 8) (Table 1). Analysis with specific primers showed a prevalence of 71.43%, 1.09%, 35.16%, 35.16% and 36.26% for jointly race 1 or race 8, race 4, race 2, race 1 and race 8, respectively. Moreover, 1 isolate presence race 4, 5 isolates displayed only Fod race 2, 16 isolate race 8, 22 isolates had only race 1, while *Fod* race 2 and race 1 or race 8 were located together in 27 isolates. However, nineteen isolates were not determined by molecular markers.

Fusarium wilt (*Fod*) is responsible for the most serious and severe disease affecting carnation and economically important species complex within the genus Fusarium in the world (Vergara et al., 2007; Werner and Irzykowska, 2007; Poli et al., 2013; Castano et al., 2014). Similarly, *F. dianthi* was determined as the Fusarium species affecting carnation in Turkey (Tezcan et al., 2004; Arıcı et al., 2018; Arıcı and Kazaz, 2013; Atakan and Özgönen Özkaya, 2020). This study has made an important contribution to the determine of the *Fod* races except that it is first report of detection of *F. dianthi* races on carnation in Turkey.

Determination of the races provides relevant information of practical significance for developing resistance breeding programs and management for disease control (Gabrialdi and Gullino, 1987; Scovel et al., 2001; Prados-Ligero et al., 2007). However, the races of *F. dianthi* has morphologically indistinguishable and race determination requires pathogenicity tests (Manulis et al., 1994). Moreover, it has many obstacles such as time consuming and affected by high dependency on environmental factors etc. (Migheli et al., 1998; Poli et al., 2013). Molecular markers are specific fragments of DNA that can be identified within the whole genome. Nowadays, molecular markers for the determination of *Fod* races 2, 4, and, jointly, 1 and 8 (Chiocchetti et al., 1999), 1 and 8 (Migheli et al., 1998) were reported.

Of ninety one isolates, 32 were identified as belonging to race 1, 32 were identified as belonging to race 2, 33 isolates were identified as race 8 and one isolates were identified as race 4 indicating that *Fod* race 1, 2 and 8 was spatially more widespread than *Fod* race 4 in Turkey. However, race 2 is the most widespread in all areas of carnation cultivation in the world (Ben-Yephet et al., 1992; Chiocchetti et al., 1999; Prados-Ligero et al., 2007; Zahiri et al., 2013; Castano et al., 2014, Poli et al., 2014; Deng, 2018). Race 4 is found in carnation cultivars in the United States, Colombia, Spain, Italy, and Israel (Chiocchetti et al., 1999; Bogale et al., 2007). On the other hand, races 1 and 8 are reported in France, Italy, Iran, Colombia and Spain (Katan et al., 1989; Manulis et al., 1993; Baayen et al., 1997; Bogale et al., 2007; Zahiri et al., 2013; Poli et al., 2013). However, survey studies carried out in greenhouse cut flower carnation areas in Izmir province of Turkey, it was determined that 67 of 84 *Fod* isolates were *Fod* race 2 and 16 were *Fod* race 1/8 (Cer, 2021). The biggest reason for the races to be seen in many countries is migration.

4. Conclusion

In this paper, we show that specific molecular markers can efficiently be used for determination of the race 1, 2, 4 and 8 between and among isolates of *Fod*. Overall, the results showed that *Fod* isolates in the most carnation cultivation province of Turkey consist mostly of race 1, 2 and 8. However, some isolates did not generate any amplification by using molecular markers the reason is that we could not determine molecular as may belong to other races. Yet, highly specific molecular markers are required to better identification among other races isolates of *F. dianthi.* Therefore, molecular markers for identifying these isolates could be developed in future studies.

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References

Arıcı, Ş.E., & Kazaz, S. (2013). Occurrence of carnation root and crown rot under greenhouse condition in Isparta. *Reserach Journal of Agricultural Sciences,* 6:159-162 (in Turkish).

Arıcı, Ş.E., Tuncel, Z.N., & Erdoğan, O. (2018). The effect of some biological preparations against Fusarium wilt of carnation (*Fusarium oxysporum* f.sp. *dianthi*). 6th ASM International Congress of Agriculture and Environment Congress, 11-13 October, 2018, Antalya, Turkey.

Atakan, A., & Özgönen Özkaya, H. (2020). Determination of Fusarium species in carnation greenhouses in Antalya, Turkey. *Communications Faculty of Sciences University of Ankara Series C Biology*, 29:131-147.

Baayen, R.P., van Dreven, F., Krijger, M.C., & Waalwijk, C. (1997). Genetic diversity in *Fusarium oxysporum* f. sp. *dianthi* and *Fusarium redolens* f. sp. *dianthi*. *European Journal of Plant Pathology,* 103:395-408.

Baysal, Ö., Siragusa, M., Ikten, H., Polat, I., Gumrukcu, E., Yigit, F., Carimi, F., & Texeira da Silva, J.A. (2009). *Fusarium oxysporum* f. sp *lycopersici* races and their genetic discrimination by molecular markers in West Mediterranean region of Turkey. *Physiological and Molecular Plant Pathology*, 74:68-75.

Ben-Yephet, Y., Reuven, M., Lampel, M., Nitzani, Y., & Mor, Y. (1992). *Fusarium oxysporum* f. sp. *dianthi* races in carnation. *Phytoparasitica*, 20:225.

Ben-Yephet, Y., & Shtienberg, D. (1997). Effects of the host, the pathogen, the environment and their interactions, on Fusarium wilt in carnation. *Phytoparasitica,* 25:207-216.

Bogale, M., Wingfield, B.D., Wingfield, M.J., & Steenkamp, E.T. (2007). Species-specific primers for *Fusarium redolens* and a PCR-RFLP technique to distinguish among three clades of *Fusarium oxysporum. FEMS Microbiology Letters,* 271:27-32.

Castano, R., Sherm, B., & Avilés, M. (2014). Genetic diversity of *Fusarium oxysporum* f. sp. *dianthi* in Southern Spain. *Journal of Mycology*, 2014:1-14.

Cer, C. (2021). Occurrence and control measures of Fusarium wilt (*Fusarium oxysporum* f. sp. *dianthi*) in carnation growing areas in İzmir province. PhD Thesis, Adnan Menderes University, Aydın.

Chiocchetti, A., Bernardo, I., Daboussi, M.J., Garibaldi, A., & Gullino, L. (1999). Detection of *Fusarium oxysporum* f. sp. *dianthi* in carnation tissue by PCR amplification of transposon insertions. *Phytopathology,* 89:1169-1175.

Coskuntuna, A., & Yıldız, F. (2006). The biological control of Fusarium wilt on carnation with fluorescent pseudomonads. *Journal of Turkish Phytopathology,* 35:43-56.

Deng, Z. (2018). Breeding for disease resistance in florists' crops. In: McGovern R, Elmer W (Eds) Handbook of Florists' Crops Diseases. Handbook of Plant Disease Management. Springer, Cham.

Garibaldi, A., Lento, G., & Rossi, G. (1986). Il *Fusarium oxysporum* f. sp. *dianthi* in Liguria. Indagine sulla diffusione dei diversi patotipi nelle colture di garofano. *Panorama Floricolo*, 11:1-4.

Garibaldi, A., & Gullino, M.L. (1987). Fusarium wilt of carnation: present situation, problems and perspectives. *Acta Horticulturae,* 216:45-54.

Kalc Wright, G.F., Guest, D.I., Wimalajeewa, D.L.S., & van Heeswijk, R. (1996). Characterisation of *Fusarium oxysporum* isolated from carnation in Australia based on pathogenicity, vegetative compatibility and random amplified polymorphic DNA (RAPD) assay. *European Journal of Plant Pathology,* 102:451-45.

Katan, T., Katan, J., & Hadar, E. (1989). Vegetative compatibility of *Fusarium oxysporum* f.sp. *dianthi* from carnation in Israel. *Plant Pathology*, 38:376-381.

Kazaz, S., Kılıç, T., Doğan, E., Mendi, Y., & Karagüzel, Ö. (2020). Süs bitkileri üretiminde mevcut durum ve gelecek. Türkiye Ziraat Mühendisliği Teknik Kongresi Bildiriler Kitabı-1, s: 673-698, (in Turkish).

Manulis, S., Kogan, N., Reuven, M., & Ben-Yephet, Y. (1994). Use of the RAPD techniques for identification *Fusarium oxysporum* f. sp. *dianthi* from carnation. *Phytopathology*, 84:98-101.

Migheli, Q., Briatore, E., & Garibaldi, A. (1998). Use of random amplified polymorphic DNA (RAPD) to identify races 1, 2, 4 and 8 of *Fusarium oxysporum* f. sp. *dianthi* in Italy. *European Journal of Plant Pathology*, 104:49-57.

Polat, İ., Baysal, Ö., Mercati, F., Kitner, M., Cohen, Y., Lebeda, A., & Carimi, F. (2014). Characterization of *Pseudoperonospora cubensis* isolates from Europe and Asia using ISSR and SRAP molecular markers. *European Journal of Plant Pathology,* 139:641-653.

Poli, A., Bertetti, D., Rapetti, S., Gullino, M.L., & Garibaldi, A. (2013). Characterization and identification of Colombian isolates of *Fusarium oxysporum* f. sp. *dianthi*. *Journal of Plant Pathology,* 95:255-263.

Prados-Ligero, A.M., Basallote-Ureba, M.J., López-Herrera, C.J., & Melero-Vara, J.M. (2007). Evaluation of susceptibility of carnation cultivars to Fusarium wilt and determination of *Fusarium oxysporum* fsp. *dianthi* races in Southwest Spain. *Hortscience,* 42: 596-599.

Sarrocco, S., Falaschi, N., Vergara, M., Nicoletti, F., & Vannacci, G. (2007). Use of *Fusarium oxysporum* f. sp. *dianthi* transformed with marker genes to follow colonization of carnation roots. *Journal of Plant Pathology,* 89:47-54.

Scovel, G., Ovadis, M., Vainstein ,A., Reuven, M., & Ben-Yephet, Y. (2001). Marker assisted selection for resistance to *Fusarium oxysporum* in the greenhouse carnation. *Acta Horticulture*, 552:151-156.

Tezcan, H., Karabulut, Ö.A., & İlhan, K. (2004). Investigations on the determination of fungal pathogens causing root and crown rot diseases of cut flowers in Yalova. *Journal of Agricultural Faculty of Bursa Uludag University*, 18:1-10 (in Turkish).

Vergara, M., Sarracco, S., Falaschi, N., Nicoletti, F., & Vannacci, G. (2007). *Fusarium oxysporum* f. sp. *dianthi* transformed with marker genes as a tool for studying resistance in dianthus. *Acta Phytopathologica et Entomologica Hungarica*, 42:297-303.

Werner, M., & Irzykowska, L. (2007). The pathogenicity and DNA polymorphism of *Fusarium oxysporum* originating from *Dianthus caryophyllus*, *Gypsophila* spp. and soil. *Phytopathologia Polonica,* 46:25-36.

Zahiri, P., Zamanizadeh, H.R., & Morid, B. (2013). Detection of *Fusarium oxysporum* f. sp. *dianthi* races in Iran. *Journal of Plant Pathology*, 95:69-77.

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RESEARCH PAPER

Evaluation of the Existence of a New Race of *Bremia lactucae* on Lettuce

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Abstract

Lettuce is one of the most consumed leafy vegetables in Turkey. The production of lettuce has been to get difficult due to infestation of cultivation areas with *Bremia lactucae*. This pathogen is genetically very variable. Pathogenic variation of *B. lactucae* has not been studied yet in Turkey. The objective of this study was to monitore the races of *B. lactucae* in the two geographic regions of Turkey. During the lettuce growing season in May and October 2018, totally 72 diseased leaf samples containing *B. lactucae* sporangia were collected from the fields of Bartın and Ankara provinces. From these samples 6 isolates were obtained. After a multiplication procedure, in which the susceptible lettuce cultivar 'Green Towers' was used, the *B. lactucae* sporangia were inoculated in differentiating lettuce cultivars. The response of the lettuce seedlings to pathogen was evaluated on the 7, 11 and 14 days after inoculation. The qualitative method was used for the assessment of infected seedlings. Sextet codes of Ankara and Bartın isolates were found as 44-00-01 and 13-03-04 respectively. The sextet codes of Turkish isolates did not match any of the present 37 races of *B. lactucae*. This result suggests that our isolates may be new races or pathotypes.

1. Introduction

Lettuce (*Lactuca sativa*), is the most widely used vegetable in Turkey for its availability all year-round. In Turkey, different types of lettuce are being cultivated on the area of 218 208 ha with an annual production of 520 151 tons (TUIK, 2020)

Many factors restrict the production of lettuce. Among these factors, downy mildew caused by *Bremia lactucae*, ranks first (Crute, 1992; Lebeda and Schwinn, 1994; Lebeda and Petrzelová, 2004). *B. lactucae* is can infect any lettuce growth stage from seedling to mature plant and produce symptoms of pale-yellow angular leaf spots on the upper leaf surface and whitish fluffy sporulation on the lower side of the leaves (Crute and Harrison, 1988; Lebeda and Petrzelova, 2010). Many factors affect the onset of the disease. Among these, humid and cool environments are of paramount importance following solar radiation and wind which influence spore production and dispersal. The severity of damage caused by *Bremia* can vary depending on environmental conditions. High humidity and cool temperatures are optimal for infection and spread of the disease (Fletcher, 1976; Scherm and van Bruggen, 1994; Wu et al., 2002; Su et al., 2004). The pathogen may spread quickly over large areas during cool (5-17ºC) and wet (100%) conditions, and may cause considerable damage, and economic losses (Lebeda and Petrzelova, 2010).

There are various alternative for the farmers to control disease. Although growing resistant varieties together with fungicide application seems as the most effective way, it is not applicable everywhere having many pathogenic variation and fungicide resistance. *Bremia lactucae,* which has been reported to have two mating types (B1 and B2)

(Michelmore and Ingram, 1980) shows great genetic variability. New races overcoming the resistance of the varieties occur very often (De Vries, 1997; Lebeda et al., 2009). Because of this, breeding new resistant varieties is a necessity in the control of the disease. For successful resistance breeding, the regional variation of virulence in the *B. lactucae* population has to be known (Lebeda and Zinkernagel, 2003). From the years 1998 to 2021, 37 races of *B. lactucae* were determined in the European region (Ettekoven and van der Arend, 1999; van der Arend et al., 2006; IBEB, 2021). Recently, The International Bremia Evaluation Board, European Union (IBEB-EU) reported that the most widely spread races are denominated Bl: 34EU, Bl: 35EU and Bl: 36EU.These three races appear in 20% of the samples. More than 60% of the isolates, had new virulence patterns.

According to the IBEB (2021), the most recently found race Bl:37EU (sextet code: 46-15-14) is widely spread in France but is also present in Spain, Portugal and Italy. Turkey is not in the scope of IBEB-EU. Therefore, resistance claims for lettuce varieties may be valid in Europe but not in Turkey. It is important to strengthen the scientific basis of the deployment of resistance in the Turkish market, given the importance of lettuce production for the Turkish economy and consumers. Therefore, identification of the races of *B. lactucae* in lettuce producing areas of Central Anatolia and West Black Sea regions of Turkey was taken into consideration in this study.

2. Materials and Methods

2.1. Collection and storage of the plant sample

Isolates of *Bremia lactucae* were obtained from lettuce fields in Bartın and Ankara (Beypazarı) provinces, during the lettuce growing season in May and October 2018. Leaves with typical symptoms of downy mildew (infected varieties: Cartagenas and Yedikule) were collected from the fields, placed in the cold plastic boxes with moistened filter paper, and transported to the laboratory as soon as possible. Part of the samples were used freshly in the experiments and the rest of the samples were stored at -20ºC.

2.2. Isolation, inoculation and incubation

Firstly, infected leaves were examination under a stereo microscope and light microscope to observe the sporangia and sporangiophore of the fungus on the lower surface of the leaves containing mycelial growth (Figure 1).

The inoculum was prepared by washing off spores from infected lettuce leaves by shaking the sporulating lesions in centrifuge tubes with tap water in a Vortex mixer. The suspension was filtered through a single layer cheesecloth. The concentration of suspension was determined by counting the number of sporangia with haemocytometer and adjusted to 8×10^4 sporangia ml^{-1}.

This initial inoculum was used to infect the susceptible lettuce variety 'Green Towers' on which the isolate was propagated (Figure 2). To determine of races of *Bremia*, seeds of the differential cultivars of IBEB in EU-C set (IBEB, 2019) were placed on moist filter paper in transparent plastic boxes. Then the seedlings with fully expanded cotyledons were sprayed to runoff with the sporangia suspension with small hand sprayer. The seedlings of the differentials set were kept in darkness for 24 hours (h) and then with a 12 h photoperiod (light intensity of 10,000 lux) in the climate chamber at night and day temperature of 15-17ºC, respectively. The experiment was conducted with three repetitions, each repetition consisting of a box containing fifteen seedlings in cotyledon stage.

2.3. Disease assessment

Isolates of *B. lactucae* were tested on a series of 16 differential cultivars of IBEB in EU-C set as mentioned above. Each experiment was conducted at least three times. The response of the lettuce seedlings was evaluated on the 7, 11 and 14 days after inoculation. The qualitative method was used for the assessment of infected seedlings (Lebeda

Figure 1. Infected lettuce leaf (a) with *Bremia lactucae*, sporangia (b).

Figure 2. Inoculated fully expanded cotyledons of cv 'Green Towers' lettuce.

Figure 3. *Bremia lactucae* symptoms with necrosis and chlorosis (a), sporulation (b, c).

and Petrželová, 2010). Symptoms were observed with or without necrosis, chlorosis and sporulation (Figure 3).

3. Results and Discussion

In this study, quite a lot of lettuce leaf samples infected with *B. lactucae* containing sporangia were collected from the fields of Bartın and especially from the high lettuce cultivation areas in the Beypazarı district of Ankara province. Sampling was carried out during the lettuce growing season in May and October 2018. However, it was seen that most of the isolates obtained in the spore viability tests lost their viability, possibly due to fungicide applications a few days before sampling. It has been observed that viable spores are associated with successful reproduction and non-viable spores with unsuccessful reproduction.

After a propagation procedure, in which the susceptible lettuce cultivar 'Green Towers' was used, the *B. lactucae* sporangia were inoculated in differentiating lettuce cultivars. The response of the lettuce seedlings was evaluated on the 7, 11 and 14 days after inoculation and the qualitative method was used for the assessment of infected seedlings.

Sextet codes of Ankara isolates (TR-1201 and BC-1) which were successful in propagation and inoculation were found as 44-00-01 and that of Bartın isolates (TB-1, TB-2, TB-3 and TB-4) were 13-03-04 (Table 1).

Bremia lactucae was prevalent in all major cultivation areas in Turkey before lettuce growers have started using fungicides and resistant varieties. However, in recent years, the losses caused by Bremia are relatively small due to the introduction of licensed fungicide against this disease in our country and the conscious preference of resistant varieties by the producer. Nevertheless, if the environmental conditions are favourable for the pathogen *B. lactucae* is still a major threat.

The pathogen is genetically very variable. Multiple isolates that differ in their ability to overcome resistance genes may be present even within one lettuce production field. Many isolates are of minor importance because they do not persist. Isolates with the same virulence that occur at several geographic locations, persist over multiple years, and have stable virulence are considered for nomination as a race. Between 2005 and 2010 in Australia, three distinct patterns of virulence in *B. lactucae* were identified and they were be named AUS4, AUS5 and AUS6 (Nordskog et al., 2014). During 2008 and 2009 *B. lactucae* races occurring in lettuce producing areas of São Paulo state were identified. Two *B. lactucae* codes SPBI:05 and SPBI:06 were identified (Castoldi et al., 2012). Another study was monitoring the races of *B. lactucae* in the state of Minas Gerais, Brazil, in 2010. The data have identified the sextet codes in the state of Minas Gerais 63/63/51/00, 63/63/03/00, 63/63/19/00 and

Table 1. Reactions of six *Bremia lactucae* isolates to the IBEB C set of differentials.

Isolates	TR-1201	BC-1	TB-1	TB-2	TB-3	TB-4
Green Towers	+	+	+	+	+	+
Dandie	-	-	+	+	+	+
R4T57D	-	-	-	-	-	-
UC Dm14	+	+	+	+	+	+
NunDm15	+	+	+	+	+	+
CGDm116	-	-	-	-	-	-
Coloeado	+	+	-	-	-	-
FrRsal-1	-	-	+	+	+	+
Argeles	-	-	+	+	+	+
RYZ 2164	-	-	-	-	-	-
RYZ910457	-	-	-	-	-	-
Bedford	-	-	-	-	-	-
Balesta	-	-	-	-	-	-
Bartoli	+	+	-	-	-	-
Design	-	-	-	-	-	-
Kibrille	-	-	+	+	+	+
Sextet code	44-00-01	44-00-01	13-03-04	13-03-04	13-03-04	13-03-04

63/63/02/00 (Vargas, 2017). There are a lot of races or pathotypes in the population of *B. lactucae* in Europe and 37 races were found on lettuce from 1998 to 2021 (Ettekoven and van den Arend, 1999; van der Arend et al., 2006; IBEB, 2021). IBEB-EU evaluated 800 *B. lactucae* isolates for pathogenic variation, 300 collected in 2018 and 500 in 2017. Most isolates in 2018 belonged to local races. However, BI: 36EU already found in 2016 and 2017 was established in many places. In 2021 a new race of *B. lactucae*, BI: 37EU (sextet code: 46-15-14) identified and denominated in Europe (IBEB, 2021). So far, information about the pathogenic variability or races of *B. lactucae* in Turkey have not been recorded. But, 37 races of the pathogen (EU BI 1-37) have been identified in Europe and 9 (US BI 1-9) races in the United States (IBEB, 2021). The sextet codes of none of the Turkish isolates of *B. lactucae* matched the sextet codes of the races detected in Europe (EU BI 1-37) and the United States (US BI1-9). This situation reveals the existence of different pathotypes or land races in our country.

4. Conclusion

Bremia lactucae, the causal organism of downy mildew in lettuce, is a major threat to lettuce production. Farmers often need to use both fungicides and resistance genes to prevent heavy losses. Reliable information about resistances in relation to the local strains of *Bremia* is essential for a successful and durable disease control strategy.

In the present study, it is seen that there is a difference between isolates collected from lettuce cultivation areas in two different regions (Central Anatolia and West Black Sea). While the Ankara isolates (TR-1201, BC-1) formed a group, all of the Bartın isolates (TB1, TB2, TB3, TB4) formed a separate group. It is seen that the pathogenicity of the isolates is different in two different geographical regions within the same country. The fact that the sextet codes obtained from the reactions of the six isolates tested on IBEB differentials did not match any of the present 37 races suggests a high pathogenic variability of the pathogen.

The future significance of the two local races that we describe here is hard to predict. Only continuous monitoring can further strengthen the scientific basis of resistance breeding.

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References

Castoldi, R., Charlo, H.C.D.O., Dalpian, T., Melo, D.M., Botelho, A.P., & Braz, L.T. (2012). Identification of new *Bremia lactucae* races in lettuce in São Paulo state. *Horticultura Brasileira*, 30:209-213.

Crute, I.R. (1992). The role of resistance breeding in the integrated control of downy mildew (*Bremia lactucae*) in protected lettuce. *Euphytica*, 63:95–102.

Crute, I.R., & Harrison, J.M. (1988). Studies on the inheritance of resistance to metalaxyl in *Bremia lactucae* and on the stability and fitness of field isolates. *Plant Pathology*, 37:231-250.

De Vries, I.M. (1997). Origin and domestication of *Lactuca sativa* L. *Genetic Resources and Crop Evolution*, 44:165-174.

Ettekoven, K.V., & van den Arend, A.J.M. (1999). Identification and denomination of “new” races of *Bremia lactucae*. In: Eucarpia Leafy Vegetables’ 99. Palacký University, Olomouc (Czech Republic), 8-11 Jun 1999. pp:171–175.

Fletcher, J.T. (1976). *Bremia lactucae*, oospores, sporangial dissemination, and control. *Journal of Applied Biology*, 84:294-298.

IBEB, (2021). International Bremia Evaluation Board Europe. https://worldseed.org/our-work/disease-resistance/other-initiatives/ibeb/. Date accessed: February 23, 2022.

IBEB. (2019). International Bremia Evaluation Board Europe. https://worldseed.org/our-work/plant-

health/other-initiatives/ibeb/. Date accessed: February 23, 2022.

Lebeda, A., & Petrželová, I. (2010). Screening for resistance to lettuce downy mildew (*Bremia lactucae*). In: Spencer, M & Lebeda, A. (eds.), *Mass Screening Techniques for Selecting Crops Resistant to Diseases* (p.245-257). International Atomic Energy Agency, Vienna, Austria.

Lebeda, A., Doležalová, I., Křístková, E., Kitner, M., Petrželová, I., Mieslerová, B., & Novotná, A. (2009). Wild Lactuca germplasm for lettuce breeding. *Euphytica*, 170:15-34.

Lebeda, A., & Petrželová, I. (2004). Variation and distribution of virulence phenotypes of *Bremia lactucae* in natural populations of *Lactuca serriola*. *Plant Pathology,* 53:316-324.

Lebeda, A., & Zinkernagel, V. (2003). Evolution and distribution of virulence in the German population of *Bremia lactucae*. *Plant Pathology*, 52:41–51.

Lebeda, A., & Schwinn, F.J. (1994). The Downy mildews-an overview of recent research progress. *Journal of Plant Diseases and Protection*, 101:225-254.

Michelmore, R.W., & Ingram, D.S. (1980). Heterothallism in *Bremia lactucae* Regel. *Transactions of the British Mycological Society*, 74:47-56.

Nordskog, B., Elameen, A., Gadoury, D.M., & Hermansen A. (2014). Virulence characteristics of *Bremia lactucae* populations in Norway. *European Journal of Plant Pathology*, 139:679-686.

Scherm, H., & van Bruggen, A.H.C. (1994). Weather variables associated with infection of lettuce by downy mildew (*Bremia lactucae*) in coastal California. *Phytopathology*, 84: 860-865.

Su, H., van Bruggen, A.H.C., Subbarao, K.V., & Scherm, H. (2004). Sporulation of *Bremia lactucae* affected by temperature, relative humidity, and wind in controlled conditions. *Phytopathology*, 94:396-401.

Subbarao, K.V., & Scherm, H. (2002). Incorporation of temperature and solar radiation thresholds to modify a lettuce downy mildew warning system. *Phytopathology*, 92:631-636.

TUIK (2020). Crop Production Statistics-Agricultural Production Areas. http://tuik.gov.tr/PreTablo.do?alt. Date accessed: February 23, 2022.

Van der Arend, A.J.M., Gautier, J., Grimault, V., Kraan, P., Laan, R., Mazet, J., Michel, H., Schut, J.W., Smilde, D., & Witte, I. (2006). Identification and denomination of new races of *Bremia lactucae* in Europe by IBEB until 2006. plantum.nl/pdf/IBEB_identification_and_nomination_2006.pdf.

Vargas, P.F. (2017). Monitoramento de raças de *Bremia lactucae* em alface no Estado de Minas Gerais em 2010. *Ciência et Praxis*, *5*, 7-10.

Wu, B.M., van Bruggen, A.H.C., Subbarao, K.V., & Scherm, H. (2002). Incorporation of temperature and solar radiation thresh-olds to modify a lettuce downy mildew warning system. *Phytopathology*, 92:631–636.