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Investigation of resistance to Verticillium wilt disease (*Verticillium dahliae* Kleb.) in eggplant genotypes

Patlıcan genotiplerinde Verticillium solgunluk hastalığına (*Verticillium dahliae* Kleb.) dayanıklılığın araştırılması

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A B S T R A C T

This study was conducted in 2015, it was aimed to determine the resistance status of 42 eggplant genotypes for breeding studies against Verticillium wilt disease caused by *Verticillium dahliae* Kleb. disease, which limits eggplant production in Turkey. Disease resistance status of eggplant genotypes were determined by classical testing. The study revealed that it was determined the severity of the disease against *Verticillium dahliae* varied between 8.25-76.53% among the genotypes of different eggplant species. As a result of classical testing E4, E5, E7, E8, E10, E24, E33, E42 eggplant genotypes of different species; *Solanum torvum, Solanum incanum, Solanum linnaeanum, Solanum aethiopicum, Solanum sisymbriifolium, Solanum americanum* have been found resistant at 7.98-9.87% disease severity. It was also determined that 22 eggplant genotypes were moderate-level resistant and 13 eggplant genotypes were in susceptible groups. Eggplant genotypes, where the resistance status of Verticillium wilt determined in the study will contribute to the development of new hybrid eggplant varieties in future.

INTRODUCTION

Eggplant (*Solanum melongena* L.) is a hot climate plant originating in India. In recent years, it is known that eggplant has important vitamin and mineral content in terms of human health, like other vegetables, low calorie and low glycemic index values, and increased production of eggplant, such as salads, jams and pickles encouraged the increase of it (Çolak et al. 2018). Therefore, the eggplant has great economic value in many countries including Turkey. China is the larger eggplant producer in the first place in the world, followed by India, The United States of America and Turkey.

According to the Turkey Statistical Institute 2019 data, vegetable production in Turkey reached approximately 31.1 million tons by increasing 3.5%. In the production of vegetables in Turkey, eggplant is in fourth place after tomato, pepper and cucumber production (TÜİK 2019). Eggplant can be grown both in open field and in greenhouse; however, since the temperature demand is 25-30 °C during the day and minimum 15 °C at night, its cultivation is limited in certain regions (Aybak 2005). In 2019, eggplant production in Turkey was 836.284 tons, and the highest production was achieved in Antalya province as 190.125 tons, followed by Mersin (170.376 tons), Balıkesir (51.550 tons), Muğla (40.009 tons), Bursa (33.956 tons) and Adana (32.235 tons) (TÜİK 2019).

Verticillium wilt caused by the soil-borne wilting fungal pathogen, *Verticillium dahliae* Kleb. (VT), is one of the most important plant diseases that limits production in the eggplant cultivation areas of our country (Altınok et al. 2012, Derviş et al. 2009, Uslu-Kıran et al. 2007). It has been reported that there may be over 50% product losses in production areas where the disease is observed (Bletsos et al. 2003, Neshev et al. 1997, Tani et al. 2018). *V. dahliae* was first reported in 1970 as a wilt pathogen of eggplant in Turkey (Kamal and Saydam 1970). The disease occurs more than 200 plant species (Inderbitzin and Subbarao 2014). *Verticillium dahliae* Kleb. is a fungal pathogen of soil borne which remains in the form of microsclerotia in the soil for more than 10 years, causing wilt and blocking the transmission bundles of infected plants (Agrios 2005). The lesions are seen as V-shaped leaves and are seen in a part of the plant or leaf. While browning observed in xylem in infectious plants can reach phloem tissue in Fusarium wilt, it is limited to xylem tissue in Verticillium wilt. Both of soil-borne disease pathogens can cause more severe diseases in the infected areas with nematodes that change the physiology of the plant and open the door to the plant (Miller et al. 1996, Milton et al. 1971). A study determined that the rate of the areas of diseased plants caused by *V. dahliae* in eggplant was 43.2% where 18 isolates were from seven provinces of Western Anatolia (Aydın, Balıkesir, Bursa, Çanakkale, İzmir, Kütahya and Manisa), 28 isolates were from seven provinces of South Anatolia (Adana, Antalya Osmaniye, Hatay, Karaman, Konya and Mersin), 21 isolates were reported from five provinces of Southeast Anatolian provinces by Derviş et al. (2009). Further studies in Adana, Antalya and İçel regions revealed *Fusarium* sp. but nearly all samples diseased *Verticillium* sp. in Hatay region (Yücel 1994). Several survey studies conducted in Antalya, Mersin and Samsun provinces in greenhouse eggplant production areas exhibited prevalence rates and disease severity of Fusarium and Verticillium wilt pathogens. These studies provide that average prevalence rates of Fusarium and Verticillium wilt diseases 20% in Antalya, and approximately 35% in Mersin, and less than 5% Verticillium wilt determined in Samsun provinces, respectively. On the other hand, their macroscopic, microscopic analysis and pathogenicity tests of collected 176 isolates determined as 112 of fungal isolates were *Fusarium oxysporum* f. sp. *melongenae* and 64 of fungal isolates were *Verticillium dahliae* Kleb. (Altınok et al. 2012).

V. dahliae is common in the eggplant growing areas and is difficult to determine its symptoms in the host plants, the pathogen can be confused with other wilt pathogens easily. Cultural measures and chemical control are not very effective on the control against *V. dahliae*, and these control methods are not permanent and economical. Hence, appropriate control of *V. dahliae* needs alternative approaches including organic and integrated agriculture systems with reducing their negative effects of chemical control on other untargeted living organims for countries (Çolak et al. 2018). Today, breeding studies are primarily aimed at developing durable resistant varieties. Because of negative effects of chemical control a reliable resistance sources have been required immediately (Scott 2005). Unfortunately, there is no Verticillium wilt resistant cultivar present in eggplant. In this context, in hybridization programs, genotypes of eggplants that are known to be resistant to wilting disease within their species are needed. In this study, it was aimed to determine the resistance levels of 42 eggplant genotypes for use in breeding studies against Verticillium wilt disease caused by *V. dahliae* disease in eggplant.

MATERIALS AND METHODS

In experiments, 42 eggplant genotypes were used as material in this study from 2015 eggplant breeding programs carried out in Alata Horticultural Research Institute, Mersin, Turkey. In classical testing studies, high resistance AGR703 (*Solanum melongena* x *Solanum aethiopicum*), Köksal F1 (*Solanum melongena* X *Solanum incanum*) commercial eggplant rootstocks, and Kemer, Aydın Siyahı eggplant cultivars were used as susceptible controls (Derviş et al. 2009, Talhouni et al. 2019).

Classical testing for Verticillium dahliae resistance levels

Classical testing experiments were performed according to seedling root dipping method for determining the resistance of *V. dahliae* Kleb. in 42 eggplant genotypes. For this purpose, the high virulence VT isolate obtained from greenhouse eggplant areas was developed for 10 days in PDA (Potato Dextrose Agar). Fungal mycelial developed at the end of the incubation period of VT fungus isolate were passed through double-layer cheesecloth and the spores suspension was adjusted to $1x10^7$ spores/ml with Thoma slide (Çolak et al. 2019, Ozan 2004, Uslu-Kıran et al. 2007).

For VT inoculation, the soil containing roots of 4-5 leaf eggplant seedlings was washed, and than the roots were shaved for seedlings inoculation by immersion in VT spore suspension containing $1x10^7$ spor / ml for 4-5 minutes. Eggplant seedlings inoculated were planted in 3 pots of eggplant seedlings in each pot of 15x15 cm containing steril soil: peat: perlite (1:1:1). The control plants to be used in testing were dipped in sterile water after planting their roots and planted in pots (Çolak et al. 2018). The experiment was conducted with 5 pots for each eggplant genotype with completely randomized design and 3 plants were planted in each pot. The pots were kept in a climatized chamber with 26 ± 2 °C temperature, under 16 hours of light and 8 hours of darkness and a relative humidity of 60-70% conditions. The chamber was located at the Directorate of Biological Control Research Institute, Adana.

The disease severity was calculated using the Townsend-Heuberger formula according to Başay et al. (2011); the disease severity was measured with modified the 0-5 scales where 0: no disease symptoms and no color change in the root veins, 1: 30% of the leaves are yellow and very mild color change in the root veins, 2: 50% of the leaves are yellow and moderate color change in the root veins, 3: 50-70% of the leaves are yellow and substantial color change in the root veins, 4: only 1 to 2 leaves green and 71% color change in the root veins, 5: the plant dies. Plants' symptoms were measured 45 days after inoculations in the pot experiment (Başay et al. 2011, Bora and Karaca 1970, Karman 1971). The VT % disease severity rate was calculated for each eggplant genotype according to Neshev et al. (1997) and Başay et al. (2011)'s evaluation scale. The modified scale for genotypes were: highly resistant (0%: no disease symptom), resistant (0.1-10%), moderate-level resistant (10.1-25%), moderate-level susceptible (25.1-50%), susceptible (50.1-75%), high susceptible (≥75.1%), the resistant level of eggplant genotypes has been demonstrated. The obtained data from inoculation results of the study were conducted by applying variance analysis, comparing average values, LSD (Fisher's Least Significant Difference) test (P: 0.05). These statistics tests were performed and their results were evaluated in Jump Package Program.

RESULTS AND DISCUSSION

As a result of classical testing studies, resistance levels of 42 eggplant genotypes have already determined against one of the most important soil borne fungal *V. dahliae* Kleb. pathogen causing Verticillium wilt disease. For this purpose, disease severity % and resistance status of eggplant genotypes belonging to classical test studies performed according to seedling root dipping method presented in Table 1. The differences between eggplant genotypes (Table 1) in terms of disease severity % values were found statistically significant ($P<0.05$). It was also determined that the severity of the disease against *V. dahliae* varied 8.25-76.53% among the genotypes of different eggplant species. These classical tests unveiled 8 eggplant genotypes from different genus of *Solanum torvum, Solanum incanum, Solanum linnaeanum, Solanum aethiopicum, Solanum sisymbriifolium, Solanum americanum*. The 8 genotypes are belonged to different eggplant species where E4, E5, E7, E8, E10, E24, E33, E42 coded plants were found to be resistant with disease severity between 7.98-9.87%. Additionally, 22 eggplant genotypes with VT disease severity varied from 10.4% to 24.53% were resembled moderately resistant, 4 eggplant genotypes (E1, E12, E15, E41) were moderately susceptible between 25.07% -46.94% disease severity, and 9 eggplant genotypes were over 52% disease severity were in susceptible groups. (Table1, Figure1).

The S. *melongena* accessions have revealed varying levels of sensitivity to Verticillium wilt. However, sources of resistance to *V. dahliae* have been found in some wild *Solanum* species related to eggplants such as *S. torvum, S. linnaeanum, S. aculeatissimum, S. sisymbrifolium* (Collonnier et al. 2001, Liu et al. 2015). *S. torvum* has been reported to be resistant to Verticillium wilt and bacterial wilt, root-knot nematode and mycoplasma. (Collonnier et al. 2001, Kashyap et al. 2003). However, in our conducted tests proved that reactions against different Verticillium isolates were developed 20-27% symptom on *S. torvum* plants whereas other eggplants had 87-100% disease symptoms likely reported by Garibaldi et al. (2005). By screening an area that was naturally infected with the disease between 1998 and 2001, it was observed that the disease resistance of back-crossed *S. linnaeanum* and a cultivated eggplant increased by about 60%. It is reported that *S. linnaeanum* is able to use to increase the resistance against Verticillium wilt (Liu et al. 2015). The most common rootstocks used in eggplant production are *S. torvum, Solanum integrifolium* and *S. sisymbriifolium*. Bletsos et al. (2003) revealed that, eggplant grafted onto *S. torvum* and S. *sisymbriifolium* rootstocks, had positive effects on plant growth, yield and disease incidence of *V. dahliae* without change in fruit quality, and *S. torvum* was more resistant than *S. sisymbriifolium* to *V. dahliae*. Neshev et al. (1997) indicated that reactions of 37 eggplant varieties to Verticillium wilt and compared with Bulgarian varieties Svetlina and Luch. In the study, approximately 35% of the varieties were found to have extremely high resistance and 54% were moderately susceptible. The plant infected with *V. dahliae* in different varieties ranged from 2.5 to 56.8%. The varieties included in the study reported that they were more resistance than the Svetlina variety and could be used as sources of resistance to Verticillium wilt in future breeding programs. In our study, as a result of determining eggplant genotypes in which targeted VT disease resistance status were determined; the breeder will contribute to researches in new hybridization programs and the transfer of the gene of resistance to commercial eggplant varieties.

In vegetable cultivation, in areas where soil-borne diseases are intensive the fact that solarization is insufficient in the

Table 1. Determination of disease severity and resistance status against Verticillium wilt in eggplant genotypes

IC	Genotypes	Species	Disease severity (%)	Status of disease	IC	Genotypes	Species	Disease severity (%)	Status of disease
E1	Topan 374	*Solanum melongena*	40.80g	MS	E23	VI055486*	*Solanum torvum*	21.33bcdef	MR
E2	Aydın Siyahı	*Solanum melongena*	69.47kl	S	E24	VI050329*	*Solanum aethiopicum*	9.04a	R
E3	VI044986*	*Solanum aethiopicum*	22.93def	MR	E25	VI050355*	*Solanum aethiopicum*	20.78bcdef	MR
E4	VI036446*	*Solanum linnaeanum*	9.04a	R	E26	VI050367*	*Solanum aethiopicum*	19.7bcdef	MR
E5	SW	*Solanum torvum*	9.87a	R	E27	VI050371*	*Solanum aethiopicum*	22.40cdef	MR
E6	VI034853*	*Solanum incanum*	10.41a	MR	E28	VI050380*	*Solanum aethiopicum*	21.60bcdef	MR
E7	VI034860*	*Solanum incanum*	9.60a	R	E29	VI050391*	*Solanum aethiopicum*	19.20bcde	MR
E8	VI037466*	*Solanum incanum*	9.07a	R	E30	VI044180*	*Solanum americanum*	60.27j	S
E9	VI042823*	*Solanum incanum*	16.82b	MR	E31	VI047481*	*Solunum americanum*	18.13bcd	MR
E10	VI038170*	*Solanum sisymbriifolium*	9.56a	R	E32	VI042548*	*Solunum americanum*	20.53bcdef	MR
E11	VI037223*	*Solanum inducum*	52.00i	S	E33	VI047620*	*Solanum americanum*	9.82a	R
E12	VI042257*	*Solanum inducum*	40.80g	MS	E34	VI037767*	*Solanum melongena*	24.27ef	MR
E13	VI034894*	*Solanum macrocarpon*	53.33i	S	E35	PI232079*	*Solanum melongena*	66.93k	S
E14	VI047475*	*Solanum macrocarpon*	76.53n	HS	E36	VI042514*	*Solanum melongena*	72.00lmn	S
E15	VI050400*	*Solanum macrocarpon*	46.94h	MS	E37	VI039552*	*Solanum melongena*	21.07bcdef	MR
E16	VI047392*	*Solanum torvum*	22.67cdef	MR	E38	VI042032*A	*Solanum melongena*	24.00ef	MR
E17	VI054894*	*Solanum torvum*	18.94bcde	MR	E39	VI055287*	*Solanum nigrum*	24.53ef	MR
E18	VI055295*	*Solanum melongena*	23.73ef	MR	E40	VI055104*	*Solanum nigrum*	21.60bcdef	MR
E19	VI047482*	*Solanum villosum*	55.21i	S	E41	VI041090*	*Solanum ferox*	25.07f	MS
E20	VI042539*	*Solanum villosum*	20.00bcdef	MR	E42	VI041031*	*Solanum torvum*	8.25a	R
E21	VI037342*	*Solanum xanthocarpum*	17.34bc	MR	Kemer	Variety	*Solanum melongena*	75.07mn	S
E22	VI040261*A	*Solanum xanthocarpum*	24.53ef	MR	Aydın Siyahı	Variety	*Solanum melongena*	60.67klm	S
Köksal	Rootstock	*Solanum melongena X Solanum incanum*	8.77a	R	AGR 703	Rootstock	*Solanum melongena x Solanum aethiopicum*	7.98a	R
Lsd 0.5					4.554				

*:Genotypes were taken from World Vegetable Center in Taiwan. The resistance status, according to % disease severity values of eggplant genotypes has been demonstrated; highly resistant (0%: no disease symptoms, HR), resistant (0.1-10%, R), moderate-level resistant (10.1-25%, MR), moderate-level susceptible (25.1-50%, MS), susceptible (50.1-75%, S), high susceptible (≥75.1%, HS). IC:Institute Code

control alone and the use of disease-resistant varieties or the use of resistant rootstocks has gained importance. Grafting method has started to be preferred especially for varieties with insufficient disease resistance. *Fusarium oxysporum*, *V. dahliae* and *Meloidogyne* sp., in its control, significant successes has been achieved with the use of rootstocks. In the control against soil-borne diseases, it is possible for the rootstocks to provide this resistance by secreting their

Figure 1. An image from classical testing of some eggplant genotypes against Verticillium wilt disease. A diagnostic characteristic of VT is a blotchy yellow leaf color, wilting and distinctive discoloration in the vasculer system in susceptible groups plants (E2, E11, E1, E19), and resistant groups plants (E24, E27, E40) in eggplants genotypes

secretions in the root region. However, in the production of grafted eggplant seedlings, primarily the culture forms, wild forms, close relative species and cross-species hybrids of eggplant; resistance to important biotic and abiotic stress factors should be evaluated in detail in rootstock breeding programs (Çolak et al. 2018, Khah 2005, 2011, Sarıbaş et al. 2019a). In the world, hybrid varieties obtained by hybridization of *S. torvum, S. incanum x S. melongena* and *S. melongena* x *S. aethiopicum* species are used as rootstocks in the production of grafted eggplant seedlings. In the production of grafted eggplant seedlings, *S. torvum* species as rootstock; It has been declared that it provides a high level of resistance to Verticillium wilt, Fusarium wilt, Bacterial wilt and root-knot nematode (Rotino et al. 2002, Sarıbaş et al. 2019b).

Çürük et al. (2009) determined that, in greenhouse soils infected with Verticillium wilt and root-knot nematode; it was determined that the yield values and the quality losses decreased in Fasalis and Pala varieties grafted on the *S. torvum* rootstock. It has been demonstrated that there are genes which provide resistance to soil-borne disease pathogens in the wild forms of eggplant such as *S. torvum*, *S. aethiopicum* and *S. incanum* and in close relative species. However, it has been revealed by research results that *S. melongen*a has a lower level of resistance (Toppino et al. 2008). In the study conducted by Sarıbaş et al. (2019) it was stated that *S. melongena X S. aethiopicum* eggplant rootstock candidates developed in the breeding program were resistant to root-knot nematode (*M. incognita*), Verticillium (*V. dahliae* Kleb.) and Fusarium (*F. oxysporum* f. sp. *melongenae*) wilt diseases as a result of classical testing.

In recent years, rapid screening of disease-resistant lines using molecular markers in breeding studies, saving time, space and reliability by choosing the desired genotypes provides hundreds of plant choices in one day (Barone et al. 2005, Staniaszek et al. 2007). In the studies carried out in this context, studies of resistance to tomato against Verticillium wilt (*V. dahliae* Kleb.) disease have been initiated before, and SNP2827 / Tetraprimer ARMS marker developed in connection with the Ve-2 gene, which provides resistant and susceptible populations, has been developed (Acciarri et al. 2007, Arens et al. 2010). Since eggplant production has been less economical for many years than tomato production, such a study has not been possible until now. In this context, the development of these markers with the *V. dahliae* resistant and susceptible eggplant genotypes obtained as a result of our study contributed to the transfer of the gene of resistance to commercial eggplant varieties in the breeder's new hybridization programs.

The main goal of eggplant breeding is to develop hybrid varieties with high quality and resistant to diseases and pests. The aim of our study is to determine the resistance of Verticillium wilt caused by *V. dahliae* Kleb. disease in 42 eggplant genotypes. As a result of classical tests, in order to increase the effectiveness in the control against soil-borne pathogens such as *V. dahliae*, besides the selection of resistance varieties, the system should be considered as a whole and combined applications should be included in the disease control to protect it in environmental resistance. In this context, with the classical breeding methods, to develop a variety resistant to a single disease pathogen; in terms of productivity and quality, it is not sufficient for areas such as eggplant that have been cultivated in recent years and for a plant species with high number of diseases and pests. In today's conditions, depending on the production area and time, resistance to at least 3 or more diseases-pests is needed. Thus, it has been reported that there is a need to develop varieties resistant to nematode damage with soil-borne pathogens in countries and in Turkey, where eggplant production is intensive (Rotino et al. 2002). As the number of resistance needed increases, the breeding time becomes longer and even impossible. In recent years, while Fusarium wilt resistance has been developed, which has caused significant damage to eggplant with increased production, there is no such study so far for Vetricillium wilt (Goth and Webb 1981). By determining the resistant and susceptible eggplant genotypes obtained as a result of this study, contribution was made to the infrastructure for the development of these markers. With this study results, 42 eggplant genotypes, which can be used as a source of resistance in eggplant breeding, are presented to the breeder. In addition, by identifying the parents who will be resistant to *V. dahlia* Kleb. as a parent or father, it has also contributed to the establishment of alternative projects in terms of obtaining the new varieties through the fruit quality criterion demands which are at the forefront in the world.

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ÖZET

Bu çalışma, patlıcan üretimini sınırlayan *Verticillium dahliae* hastalık etmeninin neden olduğu Verticillium solgunluğu hastalığına karşı dayanıklılık ıslah çalışmalarında kullanılmak üzere 42 adet patlıcan genotipinin reaksiyon durumlarının belirlenmesi amacıyla 2015 yılında yapılmıştır. Patlıcan genotiplerinin hastalığa karşı dayanıklılık durumları klasik testleme ile tespit edilmiştir. Çalışmada farklı patlıcan türlerine ait genotipler arasında *V. dahliae*'ya karşı hastalık şiddetinin %8.25-76.53 arasında değiştiği tespit edilmiştir. Klasik testleme sonucunda E4, E5, E7, E8, E10, E24, E33, E42 kodlu farklı türlere ait patlıcan genotipleri; *Solanum torvum*, *Solanum incanum*, *Solanum linnaeanum*, *Solanum aethiopicum*, *Solanum sisymbriifolium*, *Solanum americanum*'un %7.98-%9.87 hastalık şiddeti ile dayanıklı olduğu tespit edilmiştir. Çalışmada, 22 adet patlıcan genotipinin orta dayanıklı ve 13 adet patlıcan genotipinin hassas gruplar içerisinde yer aldığı tespit edilmiştir. Çalışmada elde edilen Verticillium solgunluğu'na dayanıklılık durumlarının belirlendiği patlıcan genotipleri gelecekte yeni hibrit patlıcan çeşidi geliştirmeye katkı sağlayacaktır.

Anahtar sözcükler: *Solanum melongena* L., Verticillium solgunluğu, dayanıklılık

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