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Short Communication

Reactions of selected eggplant cultivars and lines to verticillium wilt caused by *Verticillium dahliae* Kleb.

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Verticillium wilt caused by the fungus *Verticillium dahliae* Kleb. leads to decrease in the yield and quality of eggplant, an economically important vegetable in Turkey. To develop eggplant lines that are resistant to or tolerant to verticillium wilt, this study used cultivars K-1, K-2, K-3, K-4, K-5, K-6, K-7 and K-8 in the studies presented here. The wild species *Solanum torvum* and *Solanum sodemeum*; the resistant cultivated forms DK-1, DK-2, DK-3, DK-4 and DK-6; and the cultivar DK-5 were also included in the study. For the pathogenicity tests, an isolate of *V. dahliae* used was identified by PCR at the Plant Protection Department of the Faculty of Agriculture, A. Menderes University. All the plant materials were tested with this isolate in 2004 and again in 2005. Results from the two years were similar; the lowest disease ratio was obtained from the DK-5 line in both years. F_1 plants were obtained through hybridisation of cultivar K-1 and line DK-5, which were determined to be respectively susceptible and tolerant to *V. dahliae*. Next, F_2 plants were obtained by selfing F_1. In 2006, cultivar K-1, line DK-5, and F_1 and F_2 plants underwent pathogenicity tests. At the end of the pathogenity test, the disease severity in cultivar K-1 and line DK-5 was similar to that seen in previous years, whereas the yellowing and wilting values of F_1 plant leaves, as well as stem isolation and reisolation results, were higher than those of F_2 plants. Using a 0 to 5 scale and considering the yellowing area on leaves, the disease severity in F_1 plants was determined to be 38%; the severity value ranged from 2 to 6% in 55% of 200 F_1 plants and from 36 to 44% in the remaining 45%.

Key words: *Solanum melongena*, *Verticillium dahliae*, resistance, hybridisation.

INTRODUCTION

Verticillium wilts occur worldwide but are most important in temperate regions. Verticillium attacks more than 200 species of plants, including most vegetables, flowers, fruit trees, strawberries, field crops and shade and forest trees (Agrios, 2005). Although, the consumption of eggplant (*Solanum melongena* L.) has increased in Turkey, there has not been a significant increase in its production; indeed, its production has occasionally decreased. One of the most important reasons for the decline in production of eggplant is verticillium wilt caused by *Verticillium dahliae*. This disease is particularly prevalent in greenhouse production areas.

The maintenance of resistance, or at least tolerance, to diseases in vegetable cultivars and lines and to environmental stress factors is detrimental to successful production. Common soil-borne diseases, harmful factors and the intensive use of chemicals to fight plant pathogens have had considerable negative effects on the environment and on human health. To be successful in the market, in addition to high quality and yield, developed cultivars should also possess resistance to diseases and pesticides. Thus, to identify and propagate eggplant lines that are resistant or tolerant to verticillium wilt, *V. dahliae* pathogenicity tests was conducted on several solanum species, cultivars and lines.

MATERIALS AND METHODS

Sensitive cultivars K-1, K-2, K-3, K-4, K-5, K-6, K-7 and K-8 were used in the studies presented here, as well as the wild species *Solanum torvum* and *Solanum sodemeum* and the resistant cultivated forms DK-1, DK-2, DK-3, DK-4 and DK-6. The cultivar

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Table 1. Scale used to determine severity of *V. dahliae* infection in eggplants.

Scale value	Number of plant		The definition of disease severity	The degree of susceptibility/resistance
	2004	2005		
0	D-1, D-2	D-1, D-2	All of the leaves are green	Highly resistant
(20) 1	-	-	30% of the leaves are yellow	Resistant
(40) 2	DK-5	DK-5	50% of the leaves are yellow	Moderate-level resistant
(60) 3	-	-	50%–70% of the leaves are yellow	Moderate-level susceptible
(80) 4	K-2,K-4,K-5, DK-2,DK-3, DK-4	K-4,K-6,K-7, DK-2,DK-3,DK-4, DK-6	Only 1 to 2 leaves on the top are green	susceptible
(100) 5	K-1,K-3,K-6, K-7, K-8, DK-1, DK-6	K-1,K-2,K-3,K-5, K-8,DK-1	All of the leaves are yellow	Highly susceptible

DK-5 was also tested. The lines and cultivars that was resistant in field tests (culture types on which no laboratory tests had been carried out previously) underwent pathogenicity tests in 2004, 2005 and 2006.

After PCR analysis, a *V. dahliae* isolate was grown on PDA media at 25°C for 7 to 10 days. For the pathogenicity tests, eight 5 mm diameter mycelium discs were collected from the *V. dahlia* colonies and used to inoculate sand, PDA and corn flour media that had been prepared in 500 ml bottles and autoclaved (Uçkun, 2001). The inoculated bottles were incubated for 15 days at 25°C under 14 h light and 10 h dark conditions. Five percent (1/19) of the inoculum in the bottles was mixed with potting soil (steam-sterilized at 90 to 100°C for 45 to 60 min) to prepare pathogen-infected soil (Turhan and Turhan, 1989). Previously, cultivated seedlings were then planted in the pathogen-infected soil after their roots were trimmed (one seedling per pot). The pathogenicity test was carried out in these pots in the Plant Protection Department greenhouse using a randomized plots experimental design with ten replicates and considering each pot as a replicate.This study determined the severity of the disease in the eggplant seedling based on yellowing of the leaves. Assessments were made four months after cultivation began, and severity was rated on a 0 to 5 scale (Table 1) (Acciarri et al., 2001; Debode et al., 2005; Uslu, 2004). Applying the Townsend–Heuberger formula to the raw data, disease severity was determined as a percentage (Karman, 1971). To further determine the severity of disease, stem evaluations was performed by taking cross sections 5, 10 and 15 cm from the crown and 5, 10 and 15 cm from the roots of the infected plants. Severity was rated on a 0 to 3 scale according to color change in the veins (0, no color change in the veins; 1, very mild color change; 2, moderate color change; and 3, substantial color change). The cross-sections taken near the root then under-went surface disinfection in 0.5% sodium hypochlorite (NaOCl) followed by two washes with sterile water and drying. The cross sections were placed on PDA media-containing Petri dishes (five cross sections per plate) and left to incubation at 25°C. After 20 to 25 days, the disease agent was reisolated from these cross sections.

Variance analysis of the obtained data was made at a $P < 0.05$ significance level and the groups were formed using Duncan's test. The statistical calculations were made in accordance with Bora and Karaca (1970), Karman (1971) and SPSS13.0 for Windows. Results are presented as group means ± standard error.

RESULTS AND DİSCUSSİON

A literature review revealed that, the most sensitive and accurate pathogenicity test results are obtained from pot experiments; furthermore, in the studies carried out by Bejarano-Alcazar et al. (1999) and Bletsos et al. (1999), pot experiments were utilized. Thus, pathogenicity tests were conducted according to a similar protocol. As a result of the pathogenicity tests that was conducted in 2004 and 2005, cultivars D-1 and D-2 were included (reported to be resistant in the literature) in the resistant group.

These cultivars have previously been used to con-fer resistance to hybrids (Acciarri et al., 2001, Sunseri et al., 2003). In the stem evaluation tests, this study did not find any symptom of disease in the stems and veins of wild cultivars. However, color changes in the veins of DK-5 plants at levels of 2, 2, and 3 at a distance of 5, 10 and 15 cm, respectively, from the crown were observe. Thus, line DK-5 appears to have some degree of resistance, because symptoms of verticilium wilt were readily apparent in the veins of DK-5 plants, based on the amount of wilting and yellowing of the leaves disease severity which was determined to be only 38 and 36%, respectively, in 2004 and 2005. However, the other lines that were tested fell into the susceptible or highly susceptible groups. Generally, color change levels were slightly higher in the cultivated lines and cultivars; this was particularly so for cultivars K-1 and K-2. B. For the use of 0 to 5 scale to rate disease severity based on yellowing of the leaves of these cultivars, it was found that these cultivars belonged to the highly susceptible and susceptible groups, respectively. For the reisolation study, disease was removed from all cultivars.

In 2006, this study performed a pathogenicity test on the susceptible cultivar K-1, the tolerant line DK-5 and the

F_1 and F_2 generations of their hybrid offspring. Together with the control and uninfected plants, a total of 390 plants were tested. Using the 0 to 5 scale to rate disease severity based on the yellowing of the leaves, the disease percentages for the control and uninfected plants were determined to be K-1, 10%; DK-5, 0%; F_1, 4%; and F_2, 0%. In the plants that were cultivated in *V. dahliae*-inoculated soil, disease percentages based on yellowing of the leaves were determined to be K-1, 28%; DK-5, 22%; and F_1, 38%. In 55% of 200 F_2 plants, the disease severity varied from 2 to 6%, while in the remaining 45% of the F_2 plants, the disease severity varied from 36 to 44%.

Next, by evaluating the presence of disease in the veins of the stems as revealed by stem cross sections, and rating it from 0 to 3 with increasing values representing increasing susceptibility, 390 plants into two groups were sorted. Plants in the 0 to 1 group were considered to be resistant/tolerant, while the plants in the 2 to 3 group were considered to be susceptible. F_2 generations of offspring were found, 55% were resistant/tolerant, while the remaining 45% were susceptible.

Based on the yellowing and wilting of the leaves and the stem isolation and reisolation results, the F_1 plants were more susceptible to verticillium wilt than the F_2 plants. Because susceptibility to *V. dahliae* is theoretically controlled by recessive genes, this study expected 50% of the F_2 plants to be resistant/tolerant and 50% of them to be susceptible. This result would have been consistent with the results of Demir (1975) and Klug and Cummings (2003). Because many factors can contribute to resistance, in practice, results are not always as precise as they are in the theory. Yet, these results were still very close to the expected result. The 5% deviation between tolerant and susceptible ratios could have been due to an environmental factor such as disparate weather conditions.

The results that were obtained in this study were reached by completely conventional methods. The next step will be to analyze F_2 (which is determined to be 2 to 6% tolerant using conventional testing methods) using molecular techniques to investigate disease-resistance factors, the disease severity of is determined to be in molecular terms.

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