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# Evaluation of cotton cultivars for resistance to pathotypes of *Verticillium dahliae*

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ABSTRACT

After the recent detection of serious losses caused by Verticillium wilt of cotton, incited by the defoliating pathotype of *Verticillium dahliae* in the Aegean Region of Turkey, 28 of the most commonly grown cotton cultivars (*Gossypium hirsutum* L.) of Turkey, were evaluated for the presence of field resistance to wilt. Six-week-old plants were inoculated with a cotton nondefoliating (ND) or a cotton defoliating (D) pathotype of *V. dahliae* under controlled conditions. Resistance was evaluated on the basis of external symptoms by calculating areas under disease progress curves. The percentage of plants killed and of those which recovered from the disease was used as additional parameters for including a particular cultivar into a defined category. Most of the evaluated cultivars were susceptible, although at different levels, to both pathotypes of *V. dahliae*. All cultivars were more susceptible to the D than to the ND pathotype. The most promising cultivars in the experiments appeared to be Carmen and ST-373. Carmen showed differential resistance: it was susceptible to the D but resistant to the ND pathotype. ST-373 was moderately susceptible to both pathotypes of *V. dahliae*. A resistance related phenotypic reaction to the disease was quantified by using six growth parameters (plant height, number of nodes, leaf weight, stem weight, leaf to stem ratio, and total shoot weight) measured 13 d after inoculation. The percentage decrease in leaf–stem ratio and leaf weight were found to be the best indicators of resistance. Results obtained in this study will be useful to quantify resistance to *V. dahliae* and identify the best parameters to phenotype in genetic studies.



## 1. Introduction

In Turkey, about 547,000 ha of upland cotton (*Gossypium hirsutum* L.) are grown annually under irrigation in three main regions. These include the Aegean, Mediterranean and South-eastern Anatolia. In the 1990s, approximately 740,000 ha of cotton were grown in Turkey. Thereafter, a steady reduction in cotton production has occurred, because of the abandonment of dry land cotton, increased production costs, and losses due to pests and diseases (Özüdoğru, 2006).

Verticillium wilt, incited by the soil inhabiting fungus *Verticillium dahliae* Kleb. is among the most serious diseases of cotton throughout Turkey causing substantial economic losses. It was first reported in Turkey in 1941 (İyriboz, 1941), but was not identified as an important disease under field conditions until 1967 (Karaca et al., 1971). Since 2000, severe Verticillium wilt has progressively increased in many fields, and an unusually high incidence of a severe wilt disease of cotton has been observed in the Aegean region. This high level of disease has been attributed to new races of *V. dahliae*, or to potassium deficiency, or to the change in tolerance to Verticillium wilt in cv. Nazilli 84 (Göre et al., 2007). Results of the pathogenicity and vegetative compatibility tests, performed by Göre (2007), indicate clearly that increased losses caused by severe wilt in cotton fields in the Aegean region are due to the presence of a highly virulent, D pathotype of *V. dahliae* belonging to vegetative compatibility group one (VCG1).

Severity of attacks by *V. dahliae* depends upon virulence (i.e., the amount of disease caused in a host genotype) of the pathogen isolates (Bell, 1994). *V. dahliae* isolates infecting cotton can be classified into D and ND pathotypes, based on their ability to cause defoliation or not of leaves from shoots (Bejarano-Alcázar et al., 1995, 1996). The ND pathotype is moderately severe and D one is highly virulent on cotton (Schnathorst and Mathre, 1966; Schnathorst et al., 1975). In Turkey, both pathotypes of *V. dahliae* were found infecting cotton (Göre, 2007; Göre et al., 2007). They are present in cotton areas planted with the Turkish cultivars BA-119, BA-Gold, M-503, Nazilli 84-S, ST-373, ST-468, ST-488 and Şahin 2000. The spread of D pathotype in Turkey (Göre et al., 2007) and its

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presence in commercial cotton fields (Göre, 2007; Göre et al., 2007) make it necessary to determine the susceptibility of cotton cultivars to *V. dahliae*.

Control of *V. dahliae* is difficult under intensive cropping systems, such as those adopted in the area infested with the disease in Turkey. Currently, no fungicides are registered for control of this disease on cotton. In addition, the ability of sclerotia of the fungus to survive in the soil for seven or more years (Wilhelm, 1955) and the wide host range of the fungus, make cultural control difficult, emphasizing the need for resistant cultivars (Heale, 1988). The level of resistance in commercial cultivars is unknown and potential sources of resistance to the pathogen in cotton have not been studied to date in Turkey, with the exception of the work by Mert et al. (2005).

The purpose of the first large screening study was to determine the level of resistance to the both pathotypes of *V. dahliae* in commercial cotton cultivars under growth chamber conditions in order to provide useful information to local growers as well as to breeders, who could use this information to develop new lines or germplasm resistant to Verticillium wilt and to determine the best plant parameters to indicate resistance.

## 2. Materials and methods

Twenty-eight of the most commonly grown cotton cultivars were evaluated for resistance to *V. dahliae* in controlled conditions. The cultivars; Aksel, BA-119, BA-151, BA-308, BA-525, BA-Gold, Flaş, Şahin 2000 and Tex were kindly supplied by Özbuğday Seed Research Company (Antakya, Turkey), Candia, Carmen, Celia, Flora and Julia by Bayer Crop Science AG (Leverkusen, Germany), Sayar 314 by Çukurova Agricultural Research Institute (ÇARI) (Adana, Turkey), DD-493, Delta Opal, DP-388, DP-419 and SG-125 by Monsanto (St. Louis, MO, USA), Erşan 92 and Maraş 92 by Kahramanmaraş Agricultural Research Institute (KARI) (Kahramanmaraş, Turkey), M-503 and Nazilli 84-S by Nazilli Cotton Research Institute (NCRI) (Aydın, Turkey), ST-373, ST-453, ST-468 and ST-488 by May Çukonar Seed Corporation (Bursa, Turkey). These cultivars comprised approximately 98% of cotton plantings in Turkey in 2007. Plants were inoculated with isolates of *V. dahliae*, I/22 (VCG2B) and Mn/8 (VCG1), from the collection of the Plant Pathology Laboratory of Plant Protection Research Institute, Izmir, Turkey. Isolate I/22 represents a highly virulent, cotton ND pathotype, and Mn/8 a highly virulent, cotton D pathotype (Göre, 2007). Both isolates maintain the same differential pathogenicity in cotton. Two cotton cultivars, Deltapine 15-21 and Çukurova 1518, were included in each experiment because both of these cultivars are susceptible to both the D and ND pathotypes (Mert et al., 2005; Schnathorst and Mathre, 1966).

Plants were inoculated by the stem-injection method (Bejarano-Alcázar et al., 1996). For stem-injection inoculation, disinfested (1% NaOCl for 2.5 min) germinated seeds were sown in 15-cm-diameter pots (one plant per pot) filled with a sterilized potting mixture (sand:clay loam:peat; 1:1:1, vol:vol). The cotton cultivars were randomly divided into two equal groups. Each group was grown in a growth chamber under fluorescent illumination of 216–270 $\mu E\ m^{-2}\ s^{-1}$, 14:10 L:D. Temperature and relative humidity, were 24–27 °C and 50–70% respectively, during the light period, and 18–22 °C and 60–80% during the dark period. Plants were watered as required and fertilized every 2 weeks with a water soluble fertilizer (20-10-20, N:P:K). Six-week-old plants were inoculated with 6 μl of a $4 \times 10^6$ conidia $ml^{-1}$ suspension in sterile distilled water (Bugbee and Presley, 1967). Control plants were treated similarly with sterile distilled water. To evaluate wilt resistance, disease severities were assessed daily for 13 d, starting 7 d after inoculation. A scale 0–4 was used according to the percentage of foliage affected by chlorotic, necrotic and wilt symptoms and/or defoliation, in an acropetal progression (0 = no symptoms; 1 = 1–33% foliage affected; 2 = 34–66% foliage affected; 3 = 67–100% foliage affected; 4 = dead plants). The percentage of dead plants (PDP), recovery from the disease and other symptoms such as marginal spots of leaves and irregular growth of terminal buds were also considered to estimate the severity of reactions. The area under the disease progress curve (AUDPC) was calculated for each treatment from the assessment of disease incidence using the formula:

$$\mathrm{AUDPC} = \sum_{i=1}^{n-1}\left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

where $y_i$ is the disease incidence in percent at $i$th assessment, $t_i$ is the time of the $i$th assessment in days from the first assessment date, and $n$ is the total number of days the disease was assessed (Campbell and Madden, 1990).

The relationship between disease severity and growth was investigated by measuring several growth parameters 2 weeks after inoculation. Plant height was measured from the cotyledonary node to the top of the plant. The number of nodes per plant was counted from the cotyledonary node to the top of the plant. Leaves taken from both the main stem and secondary branches with their petioles attached were used to determine leaf weight. Stem weight was measured by weighing the stem that was cut at the cotyledon node and stripped of leaves and fruits. After these measurements, leaf–stem ratio and shoot weight were calculated from leaf weight and stem weight (Bölek et al., 2005).

Plant infection was verified by the isolation of the fungus from affected shoots during the experiments. Isolations were taken from three randomly selected plants for each cultivar/pathotype combinations. Pieces of affected tissues were washed in running tap water, bark was removed and woody tissues surface disinfected in 0.5% sodium hypochlorite for 1 min. Chips of wood were placed onto PDA. Plates were incubated at 24 °C in the dark and for 5–6 d.

In both experiments, plants were arranged according to a split-plot completely randomized block design with ten replicated plants per cultivar/pathotype combination. The main plot was the *V. dahliae* pathotypes, and cultivars were assigned to sub-plots. Data collected for the six previously mentioned traits to indicate resistance or susceptibility from disease screening were subjected to analysis of variance in order to calculate F-values and correlation coefficients (SAS Institute, 2000). LSD was calculated to separate mean values.

## 3. Results

Symptoms ranging from sudden wilt or apoplexy, to severe chlorosis of leaves and stunting, were observed in plants inoculated with the D or the ND *V. dahliae* pathotype. Chlorosis was the most common symptom observed within 10 d after inoculation when the ND pathotype was used. Leaves became necrotic but remained attached to the stems. In plants inoculated with the D pathotype, chlorosis was associated with cultivars showing certain level of resistance and defoliation was also common. It occurred, in the absence of chlorosis, in most of the susceptible cultivars inoculated with the D pathotype, starting at 7 d, and intensifying from the tenth day, after inoculation. Defoliation ranged from intensive in susceptible cultivars such as BA-151, Celia, Flaş, Maraş 92 and SG-125, to slight and restricted to the middle of the main shoots in moderately susceptible cultivars such as BA-119, ST-373 and Tex. The D pathotype induced a higher incidence of disease and symptom severity than the ND and earlier death of plants. The D