

## GENETICS OF VERTICILLIUM WILT RESISTANCE IN COTTON

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### ABSTRACT

**Verticillium wilt, caused by *Verticillium dahliae* Kleb., is a major constraint to cotton production in almost all countries where cotton is cultivated. Developing new cotton cultivars resistant to Verticillium wilt is the most effective and feasible way to combat the problem. Little is known about the inheritance of resistance to Verticillium wilt of cotton, especially that caused by the defoliating and non-defoliating pathotypes of the soilborne fungus *V. dahliae*. The objective of this study was to determine the inheritance of seedling reactions to representative isolates defoliating and non-defoliating of *V. dahliae* in crosses of the resistant cotton Giza 45 and Tex with susceptible Albania. F<sub>1</sub>, F<sub>2</sub>, and backcross-F<sub>1</sub> populations were inoculated to determine the mode of inheritance of seedlings by the stem-injection method. The Chi-square test for goodness of fit was used to analyse segregating populations. Two independent recessive genes appeared to control resistance of Giza 45 and Tex to both defoliating and non-defoliating pathotypes. Depending upon the cross, the two genes cumulatively condition immunity or higher levels of resistance than either parent conditioned by single gene. There was no evidence of maternal influence on the inheritance of resistance. Resistance genes in Giza 45 and Tex were recessive; therefore, breeders should consider the use of relatively large F<sub>2</sub> populations to effectively transfer these genes to cultivars with good agronomic characteristics.**

**Key words:** cotton, Verticillium wilt, disease resistance, genetics.

### INTRODUCTION

Cotton (*Gossypium* spp.) is the most widely cultivated fibre crop. Of the two cultivated tetraploid cotton species, Upland cotton *Gossypium hirsutum* L. accounts for nearly 90 % of cotton production in the world (USDA-NASS 2013), while *Gossypium barbadense* L. (source of Pima cotton) contributes about 9% to world production. Verticillium wilt, caused by *Verticillium dahliae*, is one of the major diseases limiting cotton production worldwide (Bell, 1992). In Turkey, about 547,000 ha of Upland cotton are grown annually under irrigation in three main regions: Aegean, Mediterranean and Southeastern Anatolia (Göre et al., 2009; 2014). Verticillium wilt is among the most

serious diseases of cotton throughout Turkey, causing substantial economic losses (Göre, 2007; 2009; 2011). Fiber quality of plants infected by this fungus can be substantially reduced (Bassett, 1974; Göre et al., 2009; 2011; Pullman and DeVay, 1982; Paplomatas et al., 1992; DeVay et al., 1997; Zhang et al., 2012). The development and cultivation of Verticillium wilt resistant cotton cultivars is considered to be the most effective and economical approach to reduce yield losses resulting from this important fungal disease.

There has been a long-term effort to develop cotton cultivars resistant to Verticillium wilt, but only a few commercial Upland cotton cultivars have moderate levels of resistance (Wheeler and Woodward, 2011; Zhang et al., 2012). Several of the Acala 1517

cultivars released by New Mexico State University in the early 1950s carried Verticillium wilt resistance and this source of resistance was later transferred to other Acala cotton cultivars in California during the 1970s (Smith and Cothren, 1999). The source of Verticillium wilt resistance in Acala cotton cultivars was speculated to be derived from *G. barbadense*; however, no conclusive evidence yet exists to confirm this hypothesis (Zhang et al., 2005a, b, 2012). Even though the exact source of Verticillium wilt resistance in Acala cotton remains unknown, there has still been sustained progress in understanding the genetic basis of Verticillium wilt resistance in Upland cotton (Zhang et al., 2013).

Classical genetic studies were first used to study the inheritance of Verticillium wilt resistance in Upland cotton. Roberts and Staten (1972) showed that Verticillium wilt resistance in Upland cotton under severe field exposure had a broad-sense heritability that ranged from 0 to 0.83, depending on Verticillium wilt exposure level, generation evaluated, and source of resistance. In addition, Verticillium wilt resistance was found to be recessive in the crosses of Upland cotton. In contrast, Barrow (1970, 1973) reported that resistance to a mild, non-defoliating Verticillium wilt strain in two Acala lines was controlled by a single dominant gene. More recently, Mert et al. (2005) showed that resistance to a defoliating Verticillium wilt strain was also mediated by a single dominant gene in several susceptible x resistant F<sub>2</sub> and F<sub>2</sub>:3 Upland cotton populations, while two dominant genes were involved in controlling resistance to a non-defoliating Verticillium wilt strain. On the other hand, Verhalen et al. (1971) and Devey and Roose (1987) concluded that resistance to Verticillium wilt displayed by *G. hirsutum* cultivars was quantitatively inherited with resistance generally being recessive. Barnes and Staten (1961) found that transgressive segregation towards either resistance or susceptibility may occur, and that resistance appears to be quantitative. Moreover, the data analysis showed that disease resistance is mainly affected by additive genes while the dominance effects were practically zero. It is

suspected that inconsistencies in tolerance mechanisms may also occur in the study of resistance to Verticillium in cotton depending on the method of disease evaluation (Lüders et al., 2008). In different studies inheritance of resistance was indicated as multigenic and additive (Wilhelm, 1981), incomplete and variably expressed (Bell and Presley, 1969), and heterozygous in some cultivars and lines (Barrow, 1970). Because of the temperature effects, a plant with a moderate level of resistance to the strains may explain why different investigators have concluded, variously, that resistance is dominant, recessive or additive (Bell 1992). Most studies indicated that resistance in cultivars of *G. hirsutum* is multigenic and can be explained by pooled additive and dominant effects (Devey and Roose, 1987).

The objectives of this investigation were to study inheritance of seedling resistance to isolates of pathotypes defoliating and non-defoliating of *V. dahliae* in the cotton cultivars Giza 45 and Tex and to determine if the maternal parent affects the expression of resistance in subsequent generations.

## MATERIAL AND METHODS

### Plant materials

Giza 45 (*G. barbadense* L.) is a tall, late-maturing cultivar that has a high level of resistance to both pathotypes of *V. dahliae* around the world (apparent immunity from infection by isolates of pathotypes defoliating and non-defoliating) and generally good agronomic characteristics. It is a source of wilt resistance in many countries and is being used extensively in breeding work (Avtonomov et al., 1989; Avtonomov and Ibragimov, 1990; Nawar et al., 1998). Tex (*G. hirsutum* L.) is similar to Giza 45 in resistance. It is medium tall variety with good standability. Albania (*G. hirsutum* L.) is a late maturing cultivar which is susceptible to both pathotypes of *V. dahliae*. Unfortunately, the genetics of wilt resistance in these cultivars have not been investigated (Harem, 2014).

Crosses of Giza 45 and Tex with the susceptible cultivars Albania were studied in the F<sub>1</sub>, F<sub>2</sub>, and backcross-F<sub>1</sub> to determine the mode of inheritance of seedling reaction. The

reciprocals of Albania x Giza 45 were also tested with both pathotypes to determine if maternal effects influenced the inheritance pattern of resistance.

#### ***Verticillium dahliae* isolates and inoculation method**

Plants were inoculated with isolates of *V. dahliae*, I/22 (VCG2B) and Mn/8 (VCG1), obtained from the collection of the Plant Pathology Laboratory of Plant Protection Research Institute, Izmir, Turkey. Isolate I/22 represents a highly virulent, cotton non-defoliating pathotype, and Mn/8 a highly virulent, cotton defoliating pathotype (Göre, 2007; Göre et al., 2009). Both isolates maintain the same differential pathogenicity in cotton. Two cotton cultivars, Deltapine 15-21 and Acala SJ-1, were included as a control in each experiment because both of these cultivars are susceptible to both the defoliating and non-defoliating pathotypes (Mert et al., 2005; Schnathorst and Mathre, 1966).

The experiment had a randomised complete block design with ten replicated plants per isolate/cultivars combination. 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-diam pots (one plant per pot) filled with a sterilized potting mixture (sand : clay loam : peat; in 1:1:1 proportion by volume). Plants were grown in a growth chamber under fluorescent illumination of 216-270  $\mu\text{Em}^{-2}\text{s}^{-1}$ , 14:10 L:D. Temperature and relative humidity, respectively, were 24-27°C and 50-70% 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 when they have 4-6 true leaves were inoculated with 6  $\mu\text{l}$  of a  $4 \times 10^6$  conidia  $\text{ml}^{-1}$  suspension in sterile distilled water. Control plants were treated similarly with sterile distilled water (Bejarano-Alcázar et al., 1996).

#### **Disease scoring and data analysis**

Disease severity in individual plants belonging to the 3 populations was rated on a scale of 0-4 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) 2 weeks after inoculation. Analysis of variance and mean comparisons were performed with data on final disease indexes. The experiment was repeated once.

Data from  $F_2$  plants and reciprocal crosses were analysed from each cross and when no difference was observed between the two only pooled data was presented. Chi-square ( $\chi^2$ ) test for goodness of fit was used to compare the genetic ratios.

## **RESULTS AND DISCUSSION**

### **Defoliating pathotype, isolate Mn/8 Albania x Giza 45**

Giza 45 was completely resistant to defoliating pathotype whereas Albania was completely susceptible. The  $F_2$  observed ratios in different infection classes with the postulated  $F_2$  genotypes are given in Table 1. The  $F_2$  segregated in a ratio of 9 susceptible (disease grade 4) : 3 moderately susceptible (disease grade 2 to 3<sup>+</sup>) : 3 moderately resistant (disease grade; to 1<sup>+</sup>):1 resistant (disease grade 0) ( $p > 0.5$ ), indicating that Giza 45 had two independent recessive genes for resistance. Singly, one of the genes from Giza 45 conditioned infection types 0; to 1<sup>+</sup>, and the second gene conditioned infection types 2 to 3<sup>+</sup>. The two genes in combination interacted to condition infection type 0. In the absence of either recessive gene in the homozygous condition, infection type 4 was observed. Type 4 infection in the  $F_1$  and the backcross- $F_1$  seedlings indicated a dominance of susceptibility over resistance. Aguado et al. (2008) and Devay and Roose (1987) have provided examples of genes for wilt resistance that interact to give an enhanced level of resistance. According to them, complementary interaction, which may be additive, results in a higher level of resistance than that conferred by each gene singly. They further indicated that in this type of complementary gene action recessive genes are usually involved.

### Albania x Tex

Albania developed infection types 4 when inoculated with culture defoliating pathotype. No difference was observed between the F<sub>2</sub> and the reciprocal cross. So, data from these were pooled to test the fit to the postulated 9:3:3:1 ratio. As observed in Table 1, the pooled data was a good fit to this ratio indicating that resistance of Tex is controlled by two independent recessive genes. The genes

arbitrarily designated as *aa* and *bb*, conditioned infection type 0; to 1<sup>+</sup> and 2 to 3<sup>+</sup>, respectively. These genes interacted cumulatively to condition a higher level of resistance (infection type 0) than either gene conditioned singly. These results strongly support the results obtained from the preceding cross, Albania x Giza 45. Results also indicated that segregation of resistance was not affected by the genotypes of the species.

Table 1. Seedling reactions of Albania x Giza 45 and Albania x Tex to defoliating pathotype of *Verticillium dahliae*

Hybrids	Infection types				$\chi^2$	P value
	0 (R)	0; to 1 <sup>+</sup> (MR)	2 to 3 <sup>+</sup> (MS)	4 (S)		
Albania x Giza 45						
Postulated ratio	1	3	3	9		
F <sub>2</sub> (observed)	33	109	111	285	1.83	> 0.5
F <sub>2</sub> genotypes	<i>aabb</i>	<i>aaB_</i>	<i>A_bb</i>	<i>A_B_</i>		
BC <sub>1</sub> /Backcross-F <sub>1</sub>				80		
Albania x Tex						
Postulated ratio	1	3	3	9		
F <sub>2</sub> (observed)	26	83	91	274	1.39	> 0.5
BC <sub>1</sub>				80		
Control						
Deltapine 15-21				40		
Acala SJ-1				40		

### Non-defoliating pathotype, isolate I/22

#### Albania x Giza 45

Albania was highly susceptible to non-defoliating pathotype (infection type 4) whereas Giza 45 was highly resistant (infection type 0). The F<sub>2</sub> segregation pattern of this cross conformed to a 4 resistant: 3 intermediate: 9 susceptible ratio indicative of digenic control (Table 2). We postulated that the *aa* gene pair suppressed the B locus and the *bb* gene pair suppressed the A locus, i.e., recessive epistasis. Thus resistance segregating in the F<sub>2</sub> was attributed to two recessive genes from Giza 45. One of the genes (*aa*) conditioned a 0; infection type and the other gene (*bb*) conditioned 1-3<sup>-</sup> infection types. Because neither *aa* or *bb* singly conditioned an infection type as low as that which developed in Giza 45, we

assumed that a slight cumulative effect was obtained when the two gene pairs occurred together. This hypothesis of cumulative interaction between the *a* and *b* alleles was further supported in this test by the mesothetic reaction of the F<sub>1</sub> plants. However, in the F<sub>2</sub> analysis the mesothetic reactions could not be confidently separated from susceptible ones, whereas the low intermediate reactions (1-3<sup>-</sup>) were distinct; the mesothetic and susceptible classes were combined when the data were analysed. The P value calculated for goodness of fit to the hypothetical ratio of 4:3:9 for seedling reaction of plants in the F<sub>2</sub> was >0.5, and supported the hypothesis that seedling reactions were governed by two factors pairs with susceptibility being dominant.

Table 2. Seedling reactions of Albania x Giza 45 to non-defoliating pathotype of *Verticillium dahliae*

Hybrids	Infection types				$\chi^2$	P value
	0	0	1 to 3 <sup>-</sup>	4		
Albania x Giza 45						
Postulated ratio	4		3	9		
F <sub>2</sub> (observed)		177	128	393	0.34	> 0.8
F <sub>2</sub> genotypes	<i>aabb</i>	<i>aaB_</i>	<i>A_bb</i>	<i>A_B_</i>		
BC <sub>1</sub>		17		63		
Control						
Deltapine 15-21				40		
Acala SJ-1				40		

The above hypothesis for Albania x Giza 45 was confirmed by the backcross-F<sub>1</sub> data (Albania x Giza 45 x Albania). One-fourth of the backcross-F<sub>1</sub> plants had mesothetic infection types like the F<sub>1</sub>, and the remaining plants were susceptible.

Concluding, our analysis indicated that two recessive genes conditioned resistance of the cotton Giza 45 to isolates of pathotypes defoliating and non-defoliating of *V. dahliae*. It was not determined that if the same two genes were effective against both pathotypes. Verhalen et al. (1971) has also reported cases where resistance to *Verticillium* wilt in cotton was controlled by recessive genes. Genes conditioning high levels of resistance were epistatic to those conditioning low levels of resistance in all tests. These results are in agreement with the reports by Devay and Roose (1987), Robert and Staten (1972) and Aguado et al. (2008) who reported that genes conditioning low infection types were epistatic to those conditioning high infection types.

## CONCLUSIONS

On the basis of the obtained results, the maternal inheritance had no influence on resistance shown in a cross of Albania x Giza 45. Although relationships of genes in Giza 45 to genes for resistance described previously was not established, knowledge of their mode of inheritance and interactions should facilitate their utilization in breeding programs. Therefore, breeders should consider the use of relatively large F<sub>2</sub> populations to be

able to select *Verticillium* wilt resistant genotypes from their crosses.

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