

Mustafa YAŞAR<sup>1a\*</sup>

<sup>1</sup>Muş Alparslan University, Plant Production and Technologies Department, Faculty of Applied Sciences, Muş

<sup>1a</sup>ORCID: 0000-0001-9348-7978

\*Corresponding author:

mustafa.yasar@alparslan.edu.tr

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**Evaluation of Some New Cotton Genotypes Against Verticillium Disease (*Verticillium dahliae* Kleb.)**

**Abstract**

This study was carried out to determine the responses of some new cotton (*Gossypium hirsutum* L.) lines to Verticillium wilt disease (disease agent is *Verticillium dahliae* Kleb.) and to enable the use of disease resistant or tolerant lines in future studies. The field trials were conducted at the trial field, Dicle University, Diyarbakir, Turkey in 2019. The trial field was a naturally contaminated with disease agent *V. dahliae*. Nine new advanced lines of cotton were used as material. Verticillium wilt resistant/tolerant cotton cultivars DP-396, BA-119 and STV-468 were used as control cultivars. Disease index and disease incidence (%) were examined in the study. The experiment was set up in a randomized block design with four replications. According to the variance analysis, significant results were obtained in terms of disease index and the rate of infection (%). Based on disease index values, the STV-468 standard genotype and Hat-1, Hat-2, Hat-3, Hat-4, Hat-5 and Hat-9 candidate lines were the most disease tolerant genotypes. In terms of disease incidence (%), the STV-468 and DP-396 standard genotypes and Hat-1, Hat-2, Hat-5, Hat-7 and Hat-9 advanced lines were determined as the most tolerant genotypes. It was concluded that the cotton genotypes STV-468, Hat-1, Hat-2, Hat-5 and Hat-9 can be used in *V. dahliae* infected cotton cultivation areas.

## INTRODUCTION

Turkey, cotton cultivation area Eleventh in the world in terms of unit area in terms of fiber cotton yield Fifth, in terms of cotton production sixth; in terms of cotton consumption is in fourth place (Çeçen and Karademir, 2021). Cotton (*Gossypium hirsutum* L.) is a globally important cash crop and is a significant source of fiber, oil, feed and biofuel products (Gao et al., 2011). As a natural fiber crop, it is very important for textile industry, and is a backbone in the economy of some developing countries (Shaban et al., 2018). As the largest cultivated fiber crop in the world, cotton is frequently exposed to diversified biotic stresses during its growth (Li et al., 2019). *Verticillium dahliae* Kleb. is a destructive, soil-borne fungal pathogen that causes vascular wilt disease in many economically important crops worldwide (Mo et al., 2015). It is a major threat to more than 400 plant species (Zhang et al., 2016). *V. dahliae* is a poorly understood necrotrophic plant pathogen (Xu et al., 2011). During infection, *V. dahliae* secretes numerous secondary metabolites, which act as toxic factors to promote the infection process (Wang et al., 2021). Verticillium wilt diseases caused by infection by *Verticillium dahliae* is one of the most yield-limiting diseases in cotton (Li et al., 2017). This soil-borne disease significantly affect cotton production (Lang et al., 2012). The fungus survives in the soil for up to 14 years by producing melanized microsclerotia (Li et al., 2019). The infection significantly reduces cotton yield and fiber quality due to leaf chlorosis, necrosis or wilting, leaf or boll abscission, and even plant death (Wang et al., 2016). It is very difficult to combat this pathogen (Song et al., 2018). It colonizes vascular cylinder of the plant (Land et al., 2017). After colonizing the root of cotton, and invade into vascular bundles, causing yellowing and wilting of cotton leaves, and in serious cases, leading to plant death (Zhu et al., 2021). Control by conventional mechanisms is not possible due to a wide

host range and the longevity of dormant fungi in the soil in the case of absence of a suitable host (Dong et al., 2019). The interaction between fungal pathogen *V. dahliae* and cotton is a complicated process (Zhang et al., 2013). Still, most of the molecular components and mechanisms of cotton defense against Verticillium wilt are poorly understood. However, it is known that several effector proteins and cell wall degrading enzymes facilitate the colonization of *V. dahliae*. Initial defense responses against *V. dahliae* include thick cuticle and synthesis of phenolic compounds. Investigation of these defense tactics provide valuable information about the improvement of cotton breeding strategies for the development of durable, cost effective, and broad spectrum resistant varieties. Consequently, this management approach will help to reduce the use of fungicides and also minimize other environmental hazards (Shaban et al., 2018). Exoproteome of *V. dahliae* plays a significant role in this pathogenic process (Chen et al., 2016). Accumulating evidence indicates that chitinases are crucial hydrolytic enzymes, which attack fungal pathogens by catalyzing the fungal cell wall degradation (Xu et al., 2016). Accumulating evidence indicates that plant cell wall-associated receptor-like kinases involve in defense against pathogen attack (Feng et al., 2021). Verticillium pathogens secrete various disease-causing effectors in cotton (Duan et al., 2016). Polygalacturonase-inhibiting protein, belonging to a group of plant defence proteins, specifically inhibits endopolygalacturonases secreted by pathogens (Liu et al., 2017). The lignification of cell wall appositions is a conserved basal defence mechanism in the plant innate immune response (Zhang et al., 2019). Bu et al., (2014) found that an elicitor triggered innate immunity in cotton, which plays an important role in future cotton wilt disease control. Lysin motif-containing proteins are important pattern recognition receptors in plants, which function in the perception of microbe-

associated molecular patterns and in the defense against pathogenic attack (Xu et al., 2017). Only a few genes have been identified that exhibit critical roles in disease resistance (Jun et al., 2015). Because there is no effective chemical means to combat the disease, the only effective way to control *Verticillium* wilt is through genetic improvement (Yang et al., 2018). Also increased concerns about the side effects of synthetic pesticides have resulted in greater interest in developing biocontrol strategies against *Verticillium* wilt (Wei et al., 2019). Development of *Verticillium* wilt-resistant cultivars remains the only economic option for controlling the disease (Zhang et al., 2014). Considering the above facts, this study was carried out to determine the reactions of new advanced cotton lines in Turkey, against *Verticillium* wilt disease and to assist future studies on this subject.

#### MATERIALS and METHOD

In this study, nine new cotton advanced lines and DP-396, BA-119, STV-468 control varieties of *Gossypium hirsutum* L. species were used as material. The trials were carried out at the Department trial field, Faculty of Agriculture, Dicle University, Turkey in 2019 and the field is naturally contaminated with the disease agent *V. dahliae*. The experiment was carried out in a randomized complete block design with four replications. The parcel sizes were 33,6 m<sup>2</sup> (4 rows x 0,7 m x 12 m). Sowing was done with a trial seeder, and two meters of isolation distance was left between the blocks. Before planting, 60 kg/ha of N and 60 kg/ha of P<sub>2</sub>O<sub>5</sub> was applied to the experimental area in 20-20-0 fertilizer form. Just before flowering, prior to first irrigation, pure 60 kg/ha N applied in the form of urea (46% N) fertilizer was banded underground between the crop rows. The experiment field was irrigated nine times in total by furrow irrigation. The methods of the "Disease Index" and "Infection Rate"

features examined within the scope of the study are given below.

#### Disease index

In order to determine the disease index of cotton plants, stem section readings were made after harvest in all plots. For the stem section readings, at least 30 plants were selected from the middle two rows after removing one meter from each row ends, then selected plants were cut from 3-5 cm above the soil level with pruning shears and sectioned. For determination of the disease index, Barrow's (1970) "0-3 Wilt Scale" was used where evaluation was made according to Karman (1971) method. "0 scale value" indicates that the stem section is clean and white in color, "1 scale value" indicates that there are relatively little darkening in the stem section, "2 scale value" indicates that there is darkening in the stem section, but it does not cover the whole section, "3 scale value" indicates darkening in the stem section quite a lot and it represents that it has almost completely covered the body section. The disease index was determined with the help of the following equation (equation 1) where "Number of plants in scale 0"=a; "Number of plants in scale 1"=b; "Number of plants in scale 2"=c; and "number of plants in the 3 scale"=d.

$$\text{Disease Index} = (0*a + 1*b + 2*c + 3*d) / (a + b + c + d) \quad (1)$$

#### Infection rate (%)

The plants whose stem section readings were obtained, counted as infected or healthy according to the disease symptoms in the vascular bundles. The number of infected plants (i) and the number of healthy plants (h) were determined with the help of "equation 2".

$$\text{Infection Rate (\%)} = (100*i) / (i+h) \quad (2)$$

The % expressions obtained here were subjected to angle transformation. The incidence of disease data were analyzed statistically using the JMP 7.0 (SAS Institute Inc.) statistical package program,

together with the disease index values, after being subjected to angle transformation. The results were analyzed with the F test, and the means were grouped according to the LSD test.

## RESULTS and DISCUSSION

### Disease index

The variance analysis results of the average values of the "Disease Index" and "Disease Rate (%)" of cotton genotypes are given in Table 1.

**Table 1.** Variance Analysis results regarding the Disease Index and Infection Rate (%) average values of cotton genotypes

Sources of Variation	Degree of Freedom	Disease Index		Infection Rate (%)	
		Square means	Prob > F	Square means	Prob > F
Genotype	11	0.143254	<.0001**	84.0036	0.002**
Recurrence	3	0.084296	0.0117	59.3305	0.0718
Error	33	0.019704		23.1857	
Overall	47				
CV (%)	6.19			6.05	

Table 1 shows that the genotypes show statistically significant differences at the level of 1% ( $p < 0.0001$ ) from each other in terms of disease index characteristics. The mean values

of the "Disease Index" and the "Infection Rate (%)" and the groups formed according to the LSD test are given in Table 2.

**Table 2.** Disease Index and Infection Rate (%) average values for cotton genotypes and groups formed by LSD Test

Genotypes	Disease Index			Infection Rates (%)		
	Average	Std Error		Average	Std Error	
BA-119 (standard)	2.31	0.076	bc	97.15	1.208	ab
DP-396 (standard)	2.31	0.082	bc	94.55	1.377	bcd
ST-468 (standard)	2.04	0.064	d	91.85	1.356	d
Hat-1	2.10	0.041	d	92.85	0.563	cd
Hat-2	2.20	0.123	cd	94.77	0.949	bcd
Hat-3	2.10	0.071	d	96.45	1.913	abc
Hat-4	2.22	0.066	cd	95.49	0.838	bc
Hat-5	2.11	0.011	d	95.15	2.087	bcd
Hat-6	2.47	0.037	b	98.25	0.726	ab
Hat-7	2.38	0.046	bc	95.64	1.283	bcd
Hat-8	2.70	0.042	a	99.35	0.617	a
Hat-9	2.20	0.071	cd	95.16	1.345	bcd
Average	2.26			95.63		
LSD <sub>5</sub>	0.2019			3.83		

Table 2 shows that the mean disease index values of genotypes vary between 2.04 (STV-468) and 2.70 (Hat-8). The mean disease index was 2.26. The lowest mean

disease index values were recorded at STV-468, Hat-1, Hat-2, Hat-3, Hat-4, Hat-5 and Hat-9 genotypes, whereas the highest mean disease index value was at Hat-8 genotype.

It is noteworthy that there were differences between genotypes in terms of disease index characteristics. The STV-468 standard genotype and Hat-1, Hat-2, Hat-3, Hat-4, Hat-5 and Hat-9 candidate lines were found to be the most disease tolerant genotypes due to showing the lowest disease index values. BA-119, DP-396, Hat-6 and Hat-7 genotypes were moderately tolerant genotypes. The Hat-8 was found to be the most susceptible genotype to the disease, as received highest disease index value.

### Infection rate

It was seen that cotton genotypes showed statistically significant differences at the level of 1% ( $p=0.002$ ) in terms of disease incidence (%) (Table 1). Mean values of disease incidence of genotypes varied between 91.85% (STV-468) and 99.35% (Hat-8) (Table 2). It was determined that the average rate of infection was 95,63%. The highest infection rates were found at Hat-8, Hat-6, Hat-3 and BA-119 genotypes, where the lowest infection rates were observed at the STV-468, DP-396, Hat-1, Hat-2, Hat-5, Hat-7 and Hat-9 genotypes. It was noteworthy that there was a difference between genotypes in terms of disease incidence rates. Our findings support the findings of Korkmaz (2005) and Erdoğan (2009), who stated that cotton varieties have different susceptibility to diseases. Two distinct defense strategies provide a host with survival to infectious diseases: resistance and tolerance. Resistance is dependent on the ability of the host to kill pathogens. Tolerance promotes host health while having a neutral to positive impact of pathogen fitness (McCarville and Ayres, 2018). In our study, existence of high levels of disease incidence of genotypes (varied between 91.85 and 99.35%) reveals that there is no resistance for *Verticillium Disease* (*Verticillium Dahliae* Kleb.) among tested genotypes. But, diversified reaction of genotypes to this fungus also (revealed by different "Disease Index" and the "Infection Rates) shows that there exist tolerance in some

genotypes. Tolerance, the ability of a crop to maintain yield in the presence of disease, is a difficult characteristic to measure (Newton, 2016). Disease tolerance protects the host from infection without targeting pathogens. Tissue damage control is a central underlying mechanism of disease tolerance (Soares et al., 2014). Natural populations show striking heterogeneity in their ability to transmit disease. For example, a minority of infected individuals known as superspreaders carries out the majority of pathogen transmission events (Gopinath et al., 2014).

### CONCLUSIONS

The lowest average values of the disease index and infection rate were recorded at STV-468 standard genotype, and Hat-1, Hat-2, Hat-5 and Hat-9 advanced lines. Diversified reaction of genotypes to this fungus revealed by different disease index and the infection rates shows that there is tolerance in some genotypes. These genotypes may be further utilised in breeding studies and may be further tested in multi-location-season yield trials.

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