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Evaluation and development of F1 hybrids tolerant to *Verticillium dahliae* Klebhan

Viktor Avtonomov*, Azam Ravshanov, Dilbar Rashidova, Normuxammad Mamedov, Muzaffar Yakubov, Shuhrat Sharipov, Jamoliddin Jabborov, Sharofiddin Karimov, Xurshid Jalolov

Laboratory at Cotton Breeding, Seed Production and Agrotechnologies Research Institute, Tashkent, Uzbekistan

* *Corresponding author's Email: avtonomov@mail.ru*

ABSTRACT

The present study focuses on evaluating cotton, *Gossypium hirsutum* L. varieties and their hybrids for resistance to *Verticillium dahliae* Kleb., one of the major pathogens causing wilt disease. Five varieties of different genetic origins—SB-6, C-6541, Namangan-34, C-6545, and Namangan-77—were used as initial material for hybridization and further analyzed along with their F_1 and F_2 generations. Resistance was assessed by two complementary approaches: an enzyme-linked immunosorbent assay (ELISA) test system and a six-point field assessment method. Both methods provided consistent and comparable results, confirming their reliability for evaluating resistance to *V. dahliae*. The most resistant parental varieties were Namangan-34, Namangan-77, and C-6541. Among F1 hybrids, high levels of resistance were observed in the combinations Namangan-77 × C-6541, Namangan-34 × SB-6, C-6541 × SB-6, C-6541 × C-6545, C-6545 × C-6541, and SB-6 × C-6541. Analysis of the dominance index (hp) indicated adverse effects of both complete and incomplete dominance for susceptibility traits in several hybrid combinations, suggesting their potential use in breeding programs for developing resistant varieties. In the F_2 generation, combinations such as Namangan-34 × C-6545, Namangan-34 × SB-6, C-6541 × Namangan-77, and SB-6 × Namangan-77 exhibited lower mean susceptibility to *V. dahliae*. Variation series analysis also revealed individual plants with significantly greater resistance than either parent. Heritability coefficient (h^2) analysis demonstrated genotypic variability ranging from weak to strong expression across hybrids. These findings highlight promising hybrid combinations and confirm the effectiveness of combined biochemical and field assessments for breeding cotton resistant to *V. dahliae*.

Keywords: Cotton, Medium-fiber, Variety, Hybrid, wilt disease.
Article type: Research Article.

INTRODUCTION

Cotton has been cultivated for over 5,000 years as a vital fiber crop, and its global importance continues to grow due to its widespread use in yarn and textile production. Today, cotton is cultivated on 31–32.6 million hectares worldwide, producing 23–25.4 million tons of fiber annually. More than 60% of this production is concentrated in China (5.4–5.7 million tons), India (5–5.3 million tons), and the United States (3.6–3.8 million tons; Jamal Omidi *et al*. 2024). Despite advances in transgenic technologies, cotton yields remain significantly constrained by biotic and abiotic stresses. In recent decades, outbreaks of diseases such as bacterial blight and *Fusarium* wilt have caused substantial losses, with estimated revenue losses of $45 million in the United States alone in 2017 (Ismaila *et al*. 2023). In eggplants, F1 hybrids were developed by crossing susceptible and tolerant lines, such as cultivar K-1 and line DK-5. The F1 plants showed varying levels of disease severity, indicating partial tolerance inherited from the tolerant parent line. In cotton, breeding programs have focused on crossing resistant wild subspecies with commercial varieties to develop new lines with enhanced resistance to *Verticillium* wilt. This has led to the creation of varieties like Tashkent-1, which possess high productivity and fiber quality. Pathogenicity tests are conducted to evaluate the resistance of F1 and subsequent generations. For instance, in eggplants, disease severity was assessed using a scale based on leaf yellowing and wilting. In strawberries, resistance was evaluated by inoculating breeding lines with *V. dahliae* and observing disease symptoms over time. The results suggested that resistance might be controlled by a single, partially dominant gene. Using biocontrol agents, such as specific rhizobacterial strains, has been explored as a complementary strategy to enhance plant resistance to *V. dahliae*. These strains have shown potential in promoting plant growth and reducing disease severity in greenhouse experiments (Cockerton *et al*. 2019). Genetic resources, including resistant cultivars and breeding lines, are crucial for developing new resistant varieties. In cotton, several commercial cultivars and breeding lines have been identified with high levels of resistance, providing valuable germplasm for breeding programs. While breeding for resistance is a primary strategy, the dynamic nature of pathogen-host interactions and environmental factors can influence the effectiveness of resistant varieties. Continuous evaluation and adaptation of breeding strategies are necessary to address evolving pathogen virulence and ensure sustainable crop production (Khaskheli *et al*. 2013). *Verticillium* wilt, caused by *Verticillium dahliae* Kleb., remains one of the most destructive diseases of cotton and other crops, including eggplant and strawberries. Breeding for resistance involves identifying tolerant parental lines, hybridizing them, and evaluating the progeny using both biochemical and field-based methods. Traditional breeding approaches, combined with genetic engineering, have contributed to yield improvement and fiber quality. However, changing soil and climatic conditions continue to challenge cotton production, necessitating the development of varieties with stable, tolerant resistance to *V. dahlia* (Shahzad *et al*. 2022). The aim of this study was to evaluate cotton varieties and their F_1 hybrids in order to identify genotypes with tolerant resistance to *V. dahliae* Kleb. for using in breeding programs and the development of new resistant cotton varieties. The objectives of the study were to assess and select highly resistant hybrid material using an ELISA-based test system; to determine the general and specific combining ability of parental lines and F_1 hybrids through polyclonal antibody activity against chitin-specific peroxidase; to evaluate the dominance index in F_1 hybrids; to estimate the heritability coefficient in F_2 hybrids; to recommend the most promising hybrid and F_4 family as parental material for the development of a new cotton variety, C-6587; and to transfer variety C-6587 to official testing sites for evaluation of its production potential.

MATERIALS AND METHODS

The field experiment was carried out in the conditions of the field site of the Cotton Breeding, Seed Production, and Agritechnologies Research Institute (CBSPARI). In 2021-2023, the Institute is located in the Tashkent region, Republic of Uzbekistan, where the soils are typical sierozems, and the groundwater level is below 15 meters. The research used the scheme of breeding and seed production work, which is presented in the Program of Breeding Work up to 1990 of the Cotton Breeding Center (1980). Hybridization was carried out according to the I method of Griefing (1956). Variational and statistical processing of the research results was performed using the ANOVA computer program. In assessing the original and hybrid material, a test system was used to determine resistance to *Verticillium dahliae*, based on the immune-enzyme analysis method developed by A. A. Akhunov (2012). The resistance of cotton plants in field conditions to *V. dahliae* was determined according to the process. Before determining the effects of the general combining ability (GCA) of the parental forms and the specific combining ability (SCA) of the F_1 intervarietal hybrids, a one-factor analysis of variance (ANOVA) was conducted. This analysis was based on the mean ELISA values obtained using polyclonal antibodies, which characterize the resistance of the studied material to *V. dahliae*. The results showed that the F-statistic was 25.28, indicating significant differences among the studied varieties. The calculated F value demonstrated that intervarietal variation exceeded the variation within replications. The *p*-value was 9.76×10^{-27}, which is far below the standard significance threshold of 0.05, allowing the rejection of the null hypothesis that the group means are equal. Therefore, statistically significant differences in mean resistance values were observed between the varieties and F1 hybrids, confirming that the choice of a specific variety or hybrid has a strong influence on the expression of the evaluated trait. These results provide a reliable basis for proceeding with the assessment of GCA and SCA effects on the subsequent analysis. The objects of the study were the varieties SB-6, S-6541, Namangan-34, S-6545, and Namangan-77 of different genetic origin, which were used in synthetic selection. Hybridization was carried out according to the I method of Griffing (Griffing 1956).

RESULTS

The results of the one-factor ANOVA provided the basis for determining the magnitude of the general combining ability (GCA) of the parental varieties used in hybridization and the specific combining ability (SCA) in the direct and reciprocal F1 hybrids. The calculated effects of GCA and SCA are summarized in Table 1. Among the studied varieties, Namangan-34 (+0.56) and Namangan-77 (+1.41) exhibited the highest positive GCA effects, indicating their strong potential as parental forms in breeding for resistance to *Verticillium dahliae*. Within the group of varieties involved in hybridization, Namangan-77 was identified as the best parental form, with a GCA value of +1.41, reflecting a significant increase in the analyzed trait compared to the overall mean. The second most favorable parental variety was Namangan-34, which demonstrated a GCA value of +0.56. These two varieties are therefore recommended for use in breeding programs aimed at improving resistance to *V. dahliae* through hybridization. With respect to specific combining ability (SCA), the most promising hybrid combinations included C-6541 × C-6545, C-6545 × SB-6, C-6545 × Namangan-34, C-6545 × C-6541, and Namangan-77 × C-6545, which exhibited positive SCA effects of –0.21, 0.41, 0.39, 0.26, 0.47, and 0.25, respectively. Analysis of the results presented in Table 2 confirmed that among the original varieties, Namangan-34 and Namangan-77 displayed tolerant resistance to *V. dahliae*. Among the F_1 hybrids, SB-6 × Namangan-34, SB-6 × C-6541, and Namangan-34 × Namangan-77 demonstrated resistance as indicated by both monoclonal and polyclonal antibody activity. In addition, several hybrids were distinguished solely by polyclonal antibody activity, namely SB-6 × Namangan-77, Namangan-34 × SB-6, C-6541 × Namangan-34, and C-6545 × Namangan-77.

Table 1. Average values of ELISA polyclonal antibodies for resistance of varieties and hybrids F_1 after infection with the pathogen *V. dahliae* (2 hours).

No.	**Cultivar / Hybrid Combination**	**Rep I**	**Rep II**	**Rep III**	**Rep IV**	**Mean Value (A 450)**	**Result (D ≥ 0.724)**	**GCA (OKS)**	**Expected Mean**	**SCA (SKS)**
1	SB-6	0.772	0.781	0.78	0.771	0.776	+	-0.16	0.616	
2	Namangan-34	1.491	1.495	1.497	1.489	1.493	+	0.56	2.05	
3	S-6541	0.893	0.896	0.899	0.896	0.896	+	-0.04	0.856	
4	S-6545	0.502	0.496	0.506	0.5	0.501	-	-0.44	0.066	
5	Namangan-77	2.343	2.351	2.339	2.347	2.345	+	1.41	3.754	
6	SB-6 x Namangan-34	1.553	1.551	1.563	1.561	1.557	+	2.178		-0.62
7	SB-6 x S-6541	1.544	1.541	1.544	1.547	1.544	+	2.152		-0.61
8	SB-6 x S-6545	0.77	0.774	0.778	0.782	0.776	+	0.616		0.16
9	SB-6 x Namangan-77	0.836	0.827	0.822	0.831	0.829	+	0.722		0.11
10	Namangan-34 x SB-6	0.827	0.831	0.829	0.829	0.829	+	0.722		0.11
11	Namangan-34 x S-6541	0.98	0.994	0.987	1.002	0.991	+	1.045		-0.05
12	Namangan-34 x S-6545	0.811	0.816	0.815	0.81	0.813	+	0.69		0.12
13	Namangan-34 x Namangan-77	1.532	1.54	1.538	1.53	1.535	+	2.134		-0.60
14	S-6541 x SB-6	0.73	0.724	0.732	0.722	0.727	-	0.518		0.21
15	S-6541 x Namangan-34	0.868	0.862	0.874	0.868	0.868	+	0.80		0.07
16	S-6541 x S-6545	0.521	0.523	0.527	0.525	0.524	-	-0.112		0.41
17	S-6541 x Namangan-77	0.838	0.83	0.84	0.832	0.835	+	0.734		0.10
18	S-6545 x SB-6	0.541	0.537	0.56	0.545	0.546	-	-0.155		0.39
19	S-6545 x Namangan-34	0.676	0.671	0.676	0.681	0.676	-	-0.416		0.26
20	S-6545 x S-6541	0.453	0.46	0.471	0.464	0.462	-	-0.012		0.47
21	S-6545 x Namangan-77	0.775	0.772	0.785	0.782	0.779	+	0.621		0.16
22	Namangan-77 x SB-6	0.771	0.778	0.788	0.785	0.781	+	0.625		0.16
23	Namangan-77 x Namangan-34	0.82	0.822	0.83	0.828	0.825	+	0.714		0.11
24	Namangan-77 x S-6541	0.812	0.81	0.824	0.814	0.815	+	0.694		0.12
25	Namangan-77 x S-6545	0.681	0.69	0.678	0.687	0.684	+	0.432		0.25

According to the activity of monoclonal and polyclonal antibodies, the variety C-6541, as well as the F_1 hybrids C-6541 × SB-6, C-6541 × C-6545, C-6545 × SB-6, and C-6545 × C-6541, should be classified as unstable to *V. dahliae*. When assessing resistance under field conditions using the method of Popov, Minko, and Popov (1974), the analysis of the mean values of the trait "damage to plants by *V. dahliae* as of 15.09," presented in Table 3, showed that in the original varieties used for hybridization this value ranged from 0.99 to 3.26 points. The lowest damage level was recorded for Namangan-34, with an average value of 0.99 points. By contrast, the indicator varieties C-4727, Tashkent-1, and C-6524 showed higher susceptibility, with values ranging from 4.67 to 4.88 points. The standard deviation (σ) for the trait "damage to plants by *V. dahliae* as of 15.09" ranged from 33.43 in variety C-6545 to 74.74 in Namangan-34, reflecting relatively low variability in this trait among the parental and indicator varieties. In the F_1 hybrids, the mean value of the trait ranged from 0.61 points in Namangan-77 × C-6541 to 2.61 points in Namangan-34 × Namangan-77. Several F_1 hybrids demonstrated minimal mean values of this trait, with Namangan-77 × C-6545 identified as the most tolerant combination. The standard deviation values of the studied F_1 hybrids were within the same range as those of the parental varieties.

Table 2. Testing of parental pairs and F_1 hybrids for resistance after infection with the pathogen *V.dahliae* (24 h)

No.	**Hybrids F_1 and parental couples**	**IEAs monoclonal antibodies**		**IEAs polyclonal antibodies**	
		A_{450}	**Results $0.724 < D$**	**A_{450}**	**Results $0.724 < D$**
1.	SB-6 × Namangan-34	1.699	+	1.557	+
2.	SB-6 × C-6541	1.733	+	1.544	+
3.	SB-6 × C-6545	0.4920	-	0.7760	+
4.	SB-6 × Namangan-77	0.8550	+	1.508	+
5.	Namangan-34 × SB-6	0.8320	+	1.529	+
6.	Namangan-34 × C-6541	0.7650	+	0.9910	+
7.	Namangan-34 × C-6545	0.7920	+	0.813	+
8.	Namangan-34 × Namangan-77	1.755	+	1.535	+
9.	C-6541 × SB-6	0.6160	-	0.7270	-
10.	C-6541 × Namangan-34	0.8800	+	1.568	+
11.	C-6541 × C-6545	0.6550	-	0.524	-
12.	C-6545 × SB-6	0.8120	+	0.5430	-
13.	C-6545 × C-6541	0.6740	-	0.462	-
14.	C-6545 × Namangan-77	0.7250	+	1.487	+
15.	Namangan-77 × SB-6	0.7500	+	1.518	+
16.	Namangan-77 × Namangan-34	0.7400	+	1.725	+
17.	SB-6	0.8310	+	0.7760	+
18.	Namangan-34	1.044	+	1.493	+
19.	C-6541	0.7650	+	0.8960	+
20.	C-6545	0.1320	-	0.5010	-
21.	Namangan-77	1.859	+	2.345	+

Analysis of the dominance index (hp) in F_1 hybrids revealed diverse patterns: five hybrid combinations showed the effect of incomplete dominance of the highly susceptible parent; seven combinations exhibited heterosis; one combination showed no dominance effect ($hp = 0$); two combinations, with hp ranging from –1.23 to –1.64, demonstrated an adverse effect of complete overdominance of the less susceptible parent; and five hybrids displayed an adverse impact of incomplete dominance of the less susceptible parent. Analysis of the average values of the trait “damage to plants by *V. dahliae* as of 15.09” in F_2 intervarietal hybrids, presented in Table 3, indicated that the following combinations exhibited low levels of plant damage: Namangan-77 × Namangan-34, Namangan-34 × Namangan-77, Namangan-34 × S-6541, Namangan-34 × S-6545, Namangan-34 × SB-6, S-6541 × Namangan-34, S-6541 × S-6545, S-6545 × Namangan-77, S-6545 × SB-6, SB-6 × Namangan-77, and SB-6 × Namangan-34. Examination of standard deviation values in the F_2 hybrids revealed that in some cases, trait variability was relatively high, indicating the need to discard low-stability F_1 hybrids in future breeding cycles. According to literature sources, the trait “damage to plants by *V. dahliae*” generally exhibits low modification variability, highlighting the importance of determining heritability coefficients (h^2) in F_2 hybrids to estimate the proportion of genotypic variability. In the studied intervarietal F_2 hybrids, h^2 values ranged from 0.05 in C-6541 × SB-6 and SB-6 × C-6541 to 0.76 in C-6541 × C-6545, indicating weak, medium, and firm degrees of genotypic variability across different combinations. Through targeted selection, a new cotton variety, S-6524, was developed based on the F4 hybrid SB-6 × S-6541. Additionally, the array S-6587 was bred using a synthetic selection approach in the laboratory for "selection of cotton varieties resistant to biotic environmental factors." The parent plant of this new variety was isolated from the hybrid as mentioned earlier in 2012, and the parent family No. 237 was selected in 2013. Station and competitive variety testing of L-237 were conducted from 2014 to 2016 according to guidelines from the cotton selection program prior to 1990. Due to consistent performance over three years, this line was officially transferred for study under the name S-6587 to the State Variety Testing in 2024. Seeds of the new variety C-6587, including 300 individual selections and 50 family collections, were sent to the elite seed farm in Akkurgan, Tashkent region, for preliminary propagation and further multiplication.

DISCUSSION

The evaluation of cotton varieties and their F_1 and F_2 hybrids for resistance to *Verticillium dahliae* revealed consistent results across both biochemical (ELISA) and field-based assessments, confirming the reliability of the methods. Among the parental varieties, Namangan-34, Namangan-77, and C-6541 demonstrated the highest tolerance. Several F1 hybrids, including Namangan-77 × C-6541, Namangan-34 × SB-6, and C-6541 × C-6545, exhibited low susceptibility, while analysis of dominance indices highlighted hybrids with both complete and incomplete dominance effects. In the F_2 generation, multiple hybrid combinations, such as Namangan-34 × S-6545 and SB-6 × Namangan-77, showed minimal plant damage. Variation analysis revealed that some individual plants exhibited trait values lower than either parent, or heritability coefficients ranged from weak to strong, indicating varying degrees of genotypic control over resistance traits. These results provide a foundation for selecting parental lines and hybrid combinations in breeding programs aimed at developing cotton varieties with stable resistance to *V. dahliae*.In Uzbekistan, cotton was cultivated on 1.032 million hectares in 2023, yielding 3.5 million tons of raw cotton. Globally, leading cotton-producing countries such as China, India, and the USA have conducted extensive research to develop *V. dahliae*-tolerant hybrids and breeding material. In 2021, researchers evaluated 290 *Gossypium hirsutum* lines using genome-wide association studies (GWAS) combined with field phenotyping, identifying 17 significant QTL associated with resistance and reporting high heritability ($h^2 \approx 0.67$), with markers recommended for marker-assisted selection (MAS; Pei *et al.* 2021). In 2021, Zhu Hui *et al.* (China) studied F_2 hybrids across three agroecological zones, calculating coefficients of variation and narrow-sense heritability ranging from 0.40 to 0.55. Similarly, Mary Carter's group (University of Mississippi, USA) reported a wide variation in F_2 hybrid responses to *V. dahliae*, with damage severity ranging from 18% to 82% and heritability values ranging from 0.42 to 0.65, depending on annual conditions (Zhu *et al*. 2021). Earlier studies also demonstrated the importance of combining ability and parental selection. Previous studies showed that hybrids derived from parents with high general combining ability (GCA) were most resistant, while Moise Mesfina's team (EIAR, Ethiopia/University of Georgia, USA) identified 12 resistant genotypes among 80 African and Asian varieties, with five exhibiting F1 heterosis (Joshi *et al.* 2023; Madhunapantula *et al*. 2023). Ramesh Kumar (2017) in India reported overdominance in F1 hybrids and quantitative segregation in F2, contributing to the development of the Indian MAS program. Other studies highlighted the influence of environmental stress and local adaptation (Kumar *et al.* 2017). The investigations revealed that many newly developed Uzbek cotton varieties and lines exhibited limited resistance to aggressive isolates of *V. dahliae*. Notable exceptions included the variety Bukhara-8 and lines LS-6593 and LS-6595, which displayed hypersensitivity to specific isolates. Conversely, certain lines and varieties, such as L-155, L-408, L-842, L-866, L-1708, L-387, and varieties Omad, -8284, and -8288, demonstrated resistance to multiple virulent isolates, highlighting their potential as resistant germplasm for breeding programs. These findings underscore the critical need to develop cotton varieties with durable wilt resistance, given the evolving pathogen-host interactions that have reduced the effectiveness of previously resistant cultivars. Interestingly, Bukhara-8 showed an unexpected response to isolate 58, exhibiting super sensitivity yet remaining symptom-free throughout vegetative growth. Overall, the results emphasize the importance of integrating resistant germplasm into breeding strategies to create new cotton varieties capable of withstanding highly virulent *V. dahliae* isolates (Usmanov *et al.* 2021).

Table 3. Variation series, variability, inheritance of the trait " *V. dahliae* plant damage on 15.09 " in intervarietal hybrids F_1 - F_2 of cotton of the species *G.hirsutum* L.

No.	**Variety, indicator variety, hybrid combination**	**N**	**K = 1 point**						**M ± m**	**V%**	**∂**	**HP**	**h ^{2}**
			0	**1**	**2**	**3**	**4**	**5**	**score**				
1	2	3	4	5	6	7	8	9	10	11	12	13	14
4	Namangan-77	158			106	65			1.38 ± 0.04	60.52	0.61		
5	Namangan-34	158	43	66	41				0.99 ± 0.06	74.74	0.74		
6	C-6541	167		97	66				1.40 ± 0.04	57.50	0.63		
7	C-6545	174			36	46	76		3.26 ± 0.08	33.43	1.09		
8	SB-6	162		84	54	20			1.59 ± 0.07	57.23	0.91		
3	C-4727 (ind)	153				50	82	35	4.67 ± 0.08	61.07	1.02		
2	Tashkent-1 (ind)	150					101	73	4.88 ± 0.07	62.16	1.01		
1	C-6524 (ind)	171				46	82	34	4.70 ± 0.08	61.76	1.05		
9	F_1 Namangan-77×Namangan-34	66	22	44					1.33 ± 0.07	42.85	0.57	2.09	
10	F_2 Namangan-77×Namangan-34	269	93	126	50				0.85 ± 0.02	84.70	0.72		0.19
11	F_1 Namangan-77 × S-6541	65	35	20	10				0.61 ± 0.11	155.73	0.95	22.0	
12	F_2 Namangan-77 × S-6541	153	30	73	50				1.13 ± 0.05	63.71	0.72		0.07
13	F_1 Namangan-77 × S-6545	72	40	20	12				0.61 ± 0.11	157.37	0.96	-0.84	
14	F_2 Namangan-77 × S-6545	240	35	145	60				1.10 ± 0.04	56.36	0.62		0.15
15	F_1 Namangan-77 × SB-6	78		43	25	10			1.57 ± 0.10	57.69	0.91	0.96	
16	F_2 Namangan-77 × SB-6	220	40	120	60				1.09 ± 0.04	61.46	0.67		0.52
17	F_1 Namangan-34×Namangan-77	78			44	24	10		2.61 ± 0.10	34.48	0.90	6.22	
18	F_2 Namangan-34×Namangan-77	95	26	60	9				0.83 ± 0.06	72.28	0.60		0.58
19	F_1 Namangan-34 × S-6541	78		44	22	12			1.58 ± 0.10	59.49	0.94	2.96	
20	F_2 Namangan-34 × S-6541	173	50	90	33				0.91 ± 0.05	75.82	0.69		0.27
21	F_1 Namangan-34 × S-6545	74			44	20	10		2.54 ± 0.10	35.03	0.89	0.36	
22	F_2 Namangan-34 × S-6545	93	20	60	13				0.93 ± 0.06	63.44	0.59		0.44
23	F_1 Namangan-34 × SB-6	79		25	44				1.64 ± 0.07	36.58	0.60	1.16	
24	F_2 Namangan-34 × SB-6	72	30	40	2				0.62 ± 0.07	106.45	0.66		0.32
25	F_1 S-6541 × Namangan-77	91	51	30	10				0.54±0.09	161.11	0.87	15.0	
26	F_2 S-6541 × Namangan-77	147	63	51	30	3			1.30 ± 0.06	69.98	0.93		0.63
27	F_1 S-6541 × Namangan-34	80	19	40	21				1.03 ± 0.07	67.96	0.70	1.13	
28	F_2 S-6541 × Namangan-34	133	40	80	13				0.80 ± 0.05	78.75	0.63		0.20
29	F_1 C-6541 × C-6545	76		56	20				1.26 ± 0.05	40.47	0.51	-0.39	
30	F_2 C-6541 × C-6545	115	30	75	10				0.83 ± 0.15	196.38	1.63		0.76

31	F_1 C-6541 × SB-6	72		50	22		1.30 ± 0.06	42.30	0.55	0.51	
32	F_2 C-6541 × SB-6	130	30	60	40		1.07 ± 0.06	68.22	0.73		0.95
33	F_1 S-6545 × Namangan-77	89		54	24	11	1.51 ± 0.09	57.61	0.87	-0.21	
34	F_2 S-6545 × Namangan-77	229	80	120	29		0.78 ± 0.04	87.17	0.68		0.65
35	F_1 S-6545 × Namangan-34	62	12	30	20		1.12 ± 0.09	63.39	0.71	-0.87	
36	F_2 S-6545 × Namangan-34	172	82	60	30		0.30 ± 0.06	266.66	0.80		0.15
37	F_1 C-6545 × C-6541	83		53	30		1.36 ± 0.06	44.11	0.60	-0.32	
38	F_2 C-6545 × C-6541	104	20	54	30		1.09 ± 0.06	63.30	0.69		0.36
39	F_1 C-6545 × SB-6	64	10	20	34		1.38 ± 0.12	69.56	0.96	-1.23	
40	F_2 C-6545 × SB-6	164	50	100	14		0.79±0.04	78.48	0.62		0.55
41	F_1 SB-6 × Namangan-77	82		50	22	10	1.51±0.09	56.95	0.86	0.86	
42	F_2 SB-6 × Namangan-77	35	5	29	1		0.89 ± 0.06	46.06	0.41		0.30
43	F_1 SB-6 × Namangan-34	72		42	30		1.44 ± 0.07	45.83	0.66	0.50	
44	F_2 SB-6 × Namangan-34	167	52	91	24		0.84 ± 0.05	79.76	0.67		0.34
45	F_1 SB-6 × S-6541	80	21	39	20		0.99 ± 0.07	71.71	0.71	0	
46	F_2 SB-6 × S-6541	206	60	80	66		1.02 ± 0.05	76.47	0.78		0.05
47	F_1 SB-6 × S-6545	96	24	44	28		1.04 ± 0.07	70.19	0.73	-1.64	
48	F_2 SB-6 × S-6545	275	90	110	75		0.95 ± 0.04	81.05	0.77		

CONCLUSION

Both biochemical and field-based methods for assessing resistance to *V. dahliae* provide consistent and reliable information, indicating that additional field evaluation is not necessary. Among the parental varieties, Namangan-34, Namangan-77, and C-6541 exhibited the highest tolerance to *V. dahliae*, while the most resistant F_1 hybrids included Namangan-77 × C-6541, Namangan-34 × SB-6, C-6541 × SB-6, C-6541 × C-6545, C-6545 × C-6541, and SB-6 × C-6541. Analysis of the dominance index (hp) showed that two F1 hybrids (C-6545 × SB-6 and SB-6 × C-6545) displayed a negative effect of complete dominance, suggesting their potential utility in breeding programs. In the F_2 generation, several hybrid combinations demonstrated low mean plant damage, including Namangan-34 × S-6545, Namangan-34 × SB-6, S-6541 × Namangan-77, S-6541 × Namangan-34, S-6541 × S-6545, S-6545 × Namangan-77, S-6545 × Namangan-34, S-6545 × SB-6, SB-6 × Namangan-77, and SB-6 × Namangan-34. Variation analysis revealed that individual plants in several hybrids exhibited lower susceptibility than the parents, and heritability coefficients indicated that genotypic variability in F_2 hybrids ranged from weak to strong. These findings provide a clear basis for selecting parental varieties and hybrid combinations for breeding programs aimed at developing cotton varieties with stable and high resistance to *V. dahliae*. In particular, Namangan-34 and Namangan-77 are key donors of resistance traits. At the same time, specific F_1 and F_2 hybrids identified in this study represent promising material for creating new resistant varieties suitable for cultivation under conditions favoring *Verticillium* wilt.

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