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**DEVELOPMENT AND BIOCHEMICAL SCREENING OF F₁ COTTON
HYBRIDS TOLERANT TO VERTICILLIUM DAHLIAE KLEBHAN AS A STRATEGY
FOR CLIMATE-RESILIENT AGRICULTURE**

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Abstract: The abstract is devoted to the analysis of laboratory and field studies aimed at identifying cotton varieties and hybrids tolerant to the pathogen *Verticillium dahliae* Kleb. through the use of biochemical and field assessments. In the research, several cotton varieties of *G. hirsutum* with different genetic origins were used as the initial material for hybridization, namely SB-6, C-6541, Namangan-34, C-6545, and Namangan-77. These varieties, along with their F₁ hybrids, were carefully evaluated for resistance to *V. dahliae* under both laboratory and field conditions. The results of the comparative analysis showed that the two applied methods — biochemical and field assessments — provide practically identical information, which confirms their reliability and indicates that there is no urgent need to repeatedly verify the results of field assessments. Based on the evaluations, the most resistant varieties to *V. dahliae* were classified as Namangan-34, Namangan-77 and C-6541. In addition, among the studied F₁ hybrids, several cross combinations demonstrated promising resistance: 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. The findings clearly demonstrate that the integration of biochemical and field studies is a reliable approach to identify resistant genotypes, and the resistant varieties and hybrids obtained can serve as valuable breeding material for developing cotton with improved tolerance to *V. dahliae*.

Keywords: cotton, medium-fiber, variety, hybrid, resistance, pathogen, biochemistry, antibodies, polyclonal, evaluation, selection.

**IQLIMGA CHIDAMLI QISHLOQ XO`JALIGI STRATEGIYASI SIFATIDA
VERTICILLIUM DAHLIAE KLEBHAN'GA CHIDAMLI F₁ G`O`ZA
DURAGAYLARINI YARATISH VA BIOKIMYOVIY SKRINING QILISH**

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Annotatsiya: Maqolada biokimyoviy va dala baholash usullaridan foydalangan holda *Verticillium dahliae* Kleb. patogeniga chidamli g`o`za navlari va duragaylarini aniqlashga qaratilgan laboratoriya hamda dala tadqiqotlari tahlili keltirilgan. Tadqiqotda duragaylash uchun boshlang`ich material sifatida *G. hirsutum* turining turli genetik kelib chiqishga ega SB-6, S-6541, Namangan-34, S-6545 va Namangan-77 navlari ishlatilgan. Ushbu navlar va ularning F<sub>1</sub> duragaylari laboratoriya hamda dala sharoitida *V. dahliae* ga chidamliligi bo`yicha baholandi. Qiyosiy tahlil natijalari ikkala usul — biokimyoviy va dala baholash — amalda bir xil ma`lumot berishini ko`rsatdi, bu esa ularning ishonchliligini tasdiqlaydi va dala baholash natijalarini qayta tekshirishga zarurat yo`qligini bildiradi. Baholash natijalariga ko`ra, *V. dahliae* ga eng chidamli navlar sifatida Namangan-34, Namangan-77 va S-6541 aniqlandi. Shuningdek, o`rganilgan F₁

duragaylari orasida quyidagi chatishtirish kombinatsiyalari istiqbolli chidamlilik namoyon etdi: Namangan-77 × S-6541, Namangan-34 × SB-6, S-6541 × SB-6, S-6541 × S-6545, S-6545 × S-6541 va SB-6 × S-6541. Olingan natijalar biokimyoviy va dala tadqiqotlarini birgalikda qo‘llash chidamli genotiplarni aniqlashning ishonchli yondashuvi ekanligini, aniqlangan chidamli nav va duragaylar esa *V. dahliae* ga chidamliligi yuqori g‘o‘za yaratishda qimmatli seleksiya materiali bo‘lib xizmat qilishini ko‘rsatadi.

Kalit so‘zlar: g‘o‘za, o‘rta tolali, nav, duragay, chidamlilik, patogen, biokimyo, antitanalar, poliklonal, baholash, seleksiya.

СОЗДАНИЕ И БИОХИМИЧЕСКИЙ СКРИНИНГ ГИБРИДОВ ХЛОПЧАТНИКА F1, ТОЛЕРАНТНЫХ К *VERTICILLIUM DAHLIAE* КЛЕВНАН, КАК СТРАТЕГИЯ КЛИМАТИЧЕСКИ УСТОЙЧИВОГО СЕЛЬСКОГО ХОЗЯЙСТВА

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Аннотация: В статье представлен анализ лабораторных и полевых исследований, направленных на выявление сортов и гибридов хлопчатника, толерантных к возбудителю *Verticillium dahliae* Kleb., с использованием биохимической и полевой оценки. В качестве исходного материала для гибридизации были использованы сорта хлопчатника вида *G. hirsutum* различного генетического происхождения: СБ-6, С-6541, Наманган-34, С-6545 и Наманган-77. Эти сорта и их гибриды F1 были оценены на устойчивость к *V. dahliae* в лабораторных и полевых условиях. Результаты сравнительного анализа показали, что оба применённых метода — биохимическая и полевая оценка — дают практически идентичную информацию, что подтверждает их надёжность и свидетельствует об отсутствии необходимости повторной проверки результатов полевой оценки. По итогам оценки наиболее устойчивыми к *V. dahliae* признаны сорта Наманган-34, Наманган-77 и С-6541. Среди изученных гибридов F1 перспективную устойчивость продемонстрировали комбинации скрещивания: Наманган-77 × С-6541, Наманган-34 × СБ-6, С-6541 × СБ-6, С-6541 × С-6545, С-6545 × С-6541 и СБ-6 × С-6541. Полученные результаты показывают, что интеграция биохимических и полевых исследований является надёжным подходом к выявлению устойчивых генотипов, а выделенные устойчивые сорта и гибриды могут служить ценным селекционным материалом для создания хлопчатника с повышенной толерантностью к *V. dahliae*.

Ключевые слова: хлопчатник, средневолокнистый, сорт, гибрид, устойчивость, патоген, биохимия, антитела, поликлональные, оценка, селекция.

INTRODUCTION

According to FAO and ICAC, global cotton cultivation covers 31–32.6 million hectares, producing 23–25.4 million tons of lint annually. Over 60% of production comes from China, India, and the USA. In Uzbekistan, cotton was sown on 1.032 million hectares in 2023, yielding about 3.5 million tons of raw cotton.

Cotton (*Gossypium hirsutum* L.) remains one of the key strategic crops worldwide and in Uzbekistan, where it plays a vital role in the agricultural economy, provides employment for millions, and serves as a major source of export revenue critical for national food security. However, global climate change intensifies pressure on agriculture: risks of drought are rising, water scarcity worsens due to reduced Amu Darya and Syr Darya flows, soils are degrading and becoming saline, while population growth increases demand for food. All these factors make food security a pressing issue, as cotton cultivation remains a backbone of the economy, directly influencing rural incomes and indirectly affecting food availability.

Under these conditions, crop resistance to biotic stresses becomes critical, particularly to Verticillium wilt (*Verticillium dahliae* Kleb.), which annually causes severe losses in yield and fiber quality in Uzbekistan. Combined with water shortages, the disease creates a double burden on farmers, lowering the efficiency of land and water use. Therefore, the development and adoption of F₁ cotton hybrids with tolerance to wilt, along with high agronomic and fiber traits, is not only a breeding objective but also a strategic measure to strengthen sustainable agriculture.

Leading cotton-producing countries have developed innovative approaches to combat Verticillium wilt. In the USA, resistant lines have been developed and validated in multi-location field trials (Abdelraheem et al., 2020). In Australia, genome sequencing of *V. dahliae* isolates has helped monitor pathogen diversity and identify pathotypes, which is crucial for adapting breeding programs (Gardiner et al., 2024; Webster et al., 2023). In China and India, integrated strategies combine GWAS, transcriptomics, and functional gene validation to accelerate breeding progress (Zhang et al., 2025; Lei et al., 2025). These experiences demonstrate that successful resistance breeding requires both traditional field-based selection and modern molecular tools.

Recent advances in genomics and functional validation have significantly deepened understanding of Verticillium wilt (VW) resistance in upland cotton (*Gossypium hirsutum*). In 2025, researchers constructed a MAGIC population from multiple parents and used GWAS to identify 19 SNPs linked to VW resistance, notably on chromosomes A01 and A02, pinpointing two candidate genes (Ghir_A01G006660 and Ghir_A02G008980) as promising resources for marker-assisted breeding (Ayyaz et al., 2025).

A panoramic integrative study combining GWAS and transcriptome-wide association studies (TWAS) across 290 cotton accessions uncovered 10 stable QTLs whose pyramiding reduced disease index from 70 to 20, mapped eQTL hotspots, and prioritized 15 candidate causal genes, including GhARM regulating ROS homeostasis (Zhang et al., 2025).

Functional genomics further elucidated defense pathways: VIGS and transgenic experiments confirmed GhMYB4's role in downregulating lignin biosynthesis to enhance VW resistance (Xiao et al., 2021), and downstream phenylpropanoid metabolism has been linked to robust immune responses (Lei et al., 2025).

A focused RIL study revealed major QTLs on chromosomes D03 and D09 that significantly influence plant survival and genomic prediction accuracy (~0.73), strengthening genomic selection strategies (Wilson et al., 2024).

Similarly, Kurbonov & Avtonomov (2020) demonstrated that elite local breeding lines could serve as parents for developing F₁ hybrids with improved tolerance. These examples show that both natural and synthetic materials must be included in selection schemes to ensure durable resistance across environments.

Based on the problem being solved, the goal of the research was determined – to evaluate and identify among the source material and F₁ hybrids those with tolerant resistance to *V. dahliae*.

Based on the relevance of the problem being solved and the purpose, the research objectives were defined:

- evaluate and isolate using the test system (Akhunov, Khoshimova, Avtonomov et al., 2012) highly resistant to *V.dahliae* original hybrid material;
- to determine the effects of the GCA (general combining ability) of the original forms and the SCA (specific combining ability). GCA of the original forms and SCA of F1, using the activity of polyclonal antibodies to chitin - specific peroxidase in order to isolate tolerant-resistant to *V.dahliae*;

The studies were conducted in accordance with the laboratory's work program – “Marker associated selection”.

MATERIALS AND METHODS

The field experiment was carried out at the experimental station of the Cotton Breeding, Seed Production and Agrotechnologies Research Institute (CBSPARI), located in the Tashkent region, Republic of Uzbekistan. Field trials were conducted during three consecutive growing seasons (2021–2023). The soil type at the site is classified as typical sierozems, which are characterized by medium fertility and low organic matter content. The groundwater level is generally below 15 meters, ensuring stable conditions without excessive waterlogging. The climate of the region is continental, with hot summers, mild winters, and limited annual precipitation, which is typical for cotton-growing zones of Central Asia. Standard agronomic practices recommended for cotton cultivation in Uzbekistan were followed, including plowing, sowing, irrigation, and pest management, to ensure reliable field performance of the tested materials.

The objects of the study were five cotton varieties of *Gossypium hirsutum* L. differing in their genetic origin and breeding history: CB-6, C-6541, Namangan-34, C-6545, and Namangan-77. These varieties were chosen as they are widely used in breeding programs and demonstrate variability in agronomic and fiber traits, as well as in resistance to *Verticillium dahliae*. They were employed both as parental forms in hybridization schemes and as checks for evaluating resistance. Synthetic selection and hybridization were applied to create initial hybrid materials combining desirable traits such as early maturity, high raw cotton productivity, fiber quality and yield, and tolerance to *V.dahliae*.

Hybridization was performed according to the first method of Griffing (1956), which is based on a full diallel crossing system. This method enables the estimation of general combining ability (GCA) and specific combining ability (SCA) of the parental lines, thus providing insight into the genetic control of target traits. Controlled hand pollination was used, and emasculation of flowers was performed in the evening, followed by pollination with selected pollen the next morning. Each cross was replicated across several plants to ensure the reliability of results. Seeds from successful hybridizations were harvested, labeled, and used for subsequent field evaluation.

Resistance of cotton plants to *V.dahliae* was evaluated under both field and laboratory conditions. In the field, resistance scoring was carried out following the method of Popov, Minko, and Popov (1974), which is based on visual assessment of disease incidence and severity under natural infection pressure. Plants were examined at different growth stages, and a disease index was calculated for each genotype.

For laboratory confirmation, an immune-enzyme assay developed by Akhunov et al. (2012) was applied. This test system allows for the detection of resistance-associated biochemical responses in the plant tissue. Leaf samples were collected at the flowering stage, processed, and

subjected to enzyme-linked immunoassays targeting defense-related proteins. The combined use of field scoring and immunoassays ensured reliable evaluation of resistance levels.

The research followed the breeding and seed production scheme developed by the Cotton Breeding Center (1980), which was included in the national program of breeding and seed production work up to 1990. This scheme involves systematic hybridization, selection of superior plants, and gradual multiplication of elite seed material. The ultimate aim of the program was to obtain hybrid combinations that integrate multiple favorable traits, including ultra-early maturity, high yield, superior fiber quality, and tolerance to major biotic stresses such as *Verticillium* wilt.

Field data included phenological observations (emergence date, flowering time, and boll opening), agronomic performance (plant height, number of bolls per plant, seed cotton yield per hectare), and fiber quality traits (lint percentage, fiber length, strength, and micronaire value). Disease-related traits were assessed using both field scoring and immune-enzyme assays.

Experimental data were processed using analysis of variance (ANOVA) to evaluate the significance of genetic differences among varieties and hybrids. Mean values were compared using least significant difference (LSD) at a probability level of 5%. The ANOVA software package was used for statistical analysis, which allowed testing of genotype $\times$ year interactions. In addition, Griffing's diallel analysis (1956) was applied to partition genetic variance into general combining ability (GCA) and specific combining ability (SCA) effects, enabling identification of superior parental lines and hybrid combinations.

Laboratory analyses were conducted using a spectrophotometer for enzyme assays, standard ELISA kits for immune-enzyme testing, and electronic fiber testing instruments (HVI – High Volume Instrument system) for fiber properties. Field measurements were made using rulers, calipers, and scales to ensure precision in plant height, boll size, and weight. Disease evaluation relied on visual scoring scales and digital data recording for accuracy.

RESULTS

Before proceeding to the determination of the effects of the GCA of the initial forms participating in hybridization and the SCA of the created paired intervarietal hybrids F_1 , based on the results of the biochemical analysis of the average values of the ELISA of polyclonal antibodies, which characterize the object of research for resistance to *V.dahliae*, it is necessary to conduct a one-factor analysis of variance using the ANOVA program.

Based on the analysis of the results of the one-factor analysis of variance, it should be stated:

- The F - statistic is equal to 25.28, which indicates the presence of significant differences between varieties;
- F value indicates that the differences in intervarietal variation exceed those in repetitions;
- The p -value is 9.76×10^{-27} , which is significantly less than the standard significance level (0.05);
- This allows us to reject the null hypothesis, which states that the means in all groups are the same;
- therefore, between varieties and hybrid a F_1 statistically significant differences in mean values are observed;
- which in turn means that the choice of a specific variety or hybrid can significantly influence the magnitude of the above- mentioned trait.

The conducted analysis of the results of the one-factor ANOVA allowed us to proceed to the determination of the magnitude of the effects of the GCA of varieties used in hybridization and SCA in the created direct and reverse hybrids F_1 . The results of the effects of GCA and SCA are presented in Table 1. From which it is evident that the varieties Namangan-34 +0.56 and Namangan-77 +1.41 had the maximum positive effects of GCA.

That is, in the group of varieties involved in hybridization, the best one should be the Namangan-77 variety with GCA +1.40878, which means a significant increase in the value of the analyzed feature compared to the average. In second place is the Namangan-34 variety with the GCA value of +0.56. The above-mentioned varieties should be used to improve resistance to *V.dahliae* by involving in hybridization.

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

No.	Variety/line or hybrid combination	No. of replication				Mean value A_{450}	Result $0,724 \leq D$	GCA	Expected mean	SCA
		I	II	III	IV					
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	2,050	
3	C-6541	0,893	0,896	0,899	0,896	0,896	+	-0,04	0,856	
4	C-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	C-6541 x C-6545	1,544	1,541	1,544	1,547	1,544	+		2,152	-0,61
8	SB-6 x C-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 C-6541	0,98	0,994	0,987	1,002	0,991	+		1,045	-0,05
12	Namangan-34 x C-6545	0,811	0,816	0,815	0,81	0,813	+		0,690	0,12
13	Namangan-34 x Namangan-77	1,532	1,54	1,538	1,53	1,535	+		2,134	-0,60
14	C-6541 x SB-6	0,73	0,724	0,732	0,722	0,727	-		0,518	0,21
15	C-6541 x Namangan-34	0,868	0,862	0,874	0,868	0,868	+		0,800	0,07
16	C-6541 x C-6545	0,521	0,523	0,527	0,525	0,524	-		0,112	0,41
17	C-6541 x Namangan-77	0,838	0,83	0,84	0,832	0,835	+		0,734	0,10
18	C-6545 x SB-6	0,541	0,537	0,56	0,545	0,546	-		0,155	0,39
19	C-6545 x Namangan-34	0,676	0,671	0,676	0,681	0,676	-		0,416	0,26
20	C-6545 x C-6541	0,453	0,46	0,471	0,464	0,462	-		-0,012	0,47
21	C-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 C-6541	0,812	0,81	0,824	0,814	0,815	+		0,694	0,12
25	Namangan-77 x C-6545	0,681	0,69	0,678	0,687	0,684	+		0,432	0,25

As for information on specific combining ability (SCA), the best selection-significant ones include: hybrids F_1 : C-6541 x C-6545, C-6545 x SB-6, C-6545 x Namangan-34, C-6545 x C-6541 and Namangan-77 x C-6545, for which the above-mentioned values are positive and respectively equal to the values: -0.21; 0.41; 0.39; 0.26; 0.47 and 0.25.

As can be seen from the analysis of the research results presented in Table 2, the following varieties had tolerant resistance to *V.dahliae* among the original ones: Namangan-34 and Namangan-77. Among the F_1 hybrids: SB-6 x Namangan-34, SB-6 x C-6541 and Namangan-34 x Namangan-77, both by the activity of monoclonal and polyclonal antibodies. The following were additionally distinguished by the activity of polyclonal antibodies: hybrids F_1 : SB-6 X Namangan-77, Namangan-34 x SB-6, S-6541 x Namangan-34 and C-6545 x Namangan-77.

2-table. Testing of parental pairs and F₁ 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 x Namangan-34	1.699	+	1.557	+
2.	SB-6 x C-6541	1.733	+	1.544	+
3.	SB-6 x C-6545	0.4920	-	0.7760	+
4.	SB-6 x Namangan-77	0.8550	+	1.508	+
5.	Namangan-34 x SB-6	0.8320	+	1.529	+
6.	Namangan-34 x C-6541	0.7650	+	0.9910	+
7.	Namangan-34 x C-6545	0.7920	+	0.813	+
8.	Namangan-34 x Namangan-77	1.755	+	1.535	+
9.	C-6541 x SB-6	0.6160	-	0.7270	-
10.	C-6541 x Namangan-34	0.8800	+	1.568	+
11.	C-6541 x C-6545	0.6550	-	0.524	-
12.	C-6545 x SB-6	0.8120	+	0.5430	-
13.	C-6545 x C-6541	0.6740	-	0.462	-
14.	C-6545 x Namangan-77	0.7250	+	1.487	+
15.	Namangan-77 x SB-6	0.7500	+	1.518	+
16.	Namangan-77 x 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	+

V.dahliae is unstable. According to the activity of mono- and polyclonal antibodies, the variety C-6541 should be classified, and among the F₁ hybrids C-6541 x SB-6, C-6541 x C-6545, C-6545 x SB-6 and C-6545 x C-6541.

As can be seen from Table 3, the most stable original forms include the following varieties: - Namangan-77, Namangan-34 and C-6541 and F₁ hybrids: Namangan-77 x C-6541, Namangan-34 x SB-6, C-6541 x SB-6, C-6541 x C-6545, C-6545 x C-6541, SB-6 x C-6541. Above-mentioned varieties and F₁ hybrids retained their high resistance throughout the entire period of the surveys, and the resistance of the differentiating varieties increased with each survey, with the maximum being set on September 20.

As can be seen from the analysis of the assessment results presented in Table 3, in all variants of the field experiment the incidence of *V.dahliae* increases, which is obviously

associated with the age of the plant, with a drop in air and soil temperature. However, here c
ь and are manifested differences in resistance to the above-mentioned pathogen depending
on the genotype of the source material involved in hybridization.

**3-table. Variability and inheritance of the trait "susceptibility of plants to
V.dahliae " in F₁ varieties and hybrids on September 1 and 20**

No.	Varieties and hybrids Combinations	September 1 st				September 20			
		M+m points	σ	V%	HP	M+m points	σ	V%	HP
1	C-6524 (differentiator)	3.44+0. 10	0.79	22.9 6		4.28+0.1 4	0.53	12.38	
2	C-4727 (differentiator)	4.55+0. 13	0.91	20.0 0		4.66+0.1 0	0.77	16.52	
3	Tashkent-1 (diferenciator)	3.42+0. 09	0.72	21.0 5		4.76+0.0 6	0.49	10.29	
4	Namangan-77	1.75+0. 11	0.75	42.8 5		2.77+0.1 6	1.12	40.43	
5	Namangan-34	3.84+0. 19	1.24	32.2 9		3.92+0.2 1	1.31	33.41	
6	C-6541	1.05+0. 11	0.76	72.3 8		1.85+0.1 8	1.17	63.24	
7	C-6545	2.77+0. 16	1.12	40.4 3		2.98+0.1 1	0.74	24.83	
8	SB-6	2.02+0. 11	0.74	36.6 3		2.81+0.1 8	1.20	42.70	
9	F ₁ Namangan-77 x SB-6	1.82+0. 13	0.76	41.7 6	+0. 4	2.05+0.1 2	0.72	35.12	+37.0
10	F ₁ Namangan-77 x S- 6541	1.28+0. 09	0.73	57.0 3	+0. 3	1.89+0.1 6	1.20	63.49	+0.9
11	F ₁ Namangan-77 x Namangan-34	2.07+0. 15	1.23	59.4 2	+0. 7	2.21+0.1 4	1.12	50.67	+1.9
12	F ₁ Namangan-77 x S- 6545	2.85+0. 17	1.16	40.7 0	- 5.8	2.98+0.1 1	0.77	25.83	-1.1
13	F ₁ Namangan-34 x S- 6541	1.23+0. 10	0.73	59.3 5	+0. 8	2.14+0.1 6	1.16	54.20	+0.7
14	F ₁ Namangan-34 x SB-6	1.74+0.2 4	1.15	66.09	+1. 3	1.91+0.26	1.25	65.44	+2.3
15	F ₁ Namangan-34 X Namangan-77	2.97+0.2 2	1.34	45.11	-0.1	3.11+0.19	1.18	37.94	+0.4
16	F ₁ Namangan-34 x C-6545	1.31+0.1 2	0.73	55.73	+3. 7	1.91+0.20	1.21	63.35	+3.2
17	F ₁ C-6541 x SB-6	1.09+0.2	0.67	61.47	+0.	1.19+0.34	1.14	95.79	+2.3

		0			9				
18	F ₁ C-6541 x C-6545	1.53+0.2 4	0.95	62.09	+0. 4	1.73+0.28	1.11	64.16	+1.2
19	F ₁ C-6541 x Namangan-34	1.90+0.1 6	0.72	37.89	+0. 3	2.52+0.22	0.96	38.09	+0.3
20	F ₁ C-6541 x Namangan-77	1.08+0.2 1	1.12	42.34	-0.6	1.34+0.19	1.21	42.14	-0.8
21	F ₁ C-6545 x Namangan-77	2.94+0.1 6	1.26	42.85	-1.3	3.44+0.11	0.89	25.87	-5.7
22	F ₁ C-6545 x SB-6	1.73+0.2 4	1.06	61.27	+1. 7	2.95+0.17	0.76	25.76	-0.7
23	F ₁ C-6545 x Namangan-34	2.04+0.1 1	0.80	39.21	+5. 4	2.10+0.09	0.70	33.33	+2.8
24	F ₁ C-6545 x C-6541	1.23+0.3 0	1.08	87.80	+0. 8	1.77+0.30	1.08	61.01	+1.1
25	F ₁ SB-6 x Namangan-77	2.76+0.2 2	1.10	39.85	-6.7	2.72+0.13	0.66	24.26	+3.5
26	F ₁ SB-6 x Namangan-34	2.04+0.0 9	0.77	37.74	+0. 9	3.02+0.16	1.31	43.37	+0.6
27	F ₁ SB-6 x C-6541	1.72+0.1 2	1.04	60.46	-0.1	1.86+0.14	1.17	62.90	+0.9
28	F ₁ SB-6 x C-6545	2.12+0.1 2	1.22	57.54	+0. 7	3.08+0.07	0.77	25.00	-2.3

DISCUSSION

Based on the analysis of the experimental data in Tables 1–3, several essential conclusions can be highlighted regarding resistance of cotton to *Verticillium dahliae*.

First, the comparative evaluation of two methods—traditional field scoring and immuno-enzyme assays—demonstrated a very high degree of consistency. Both approaches produced almost identical results, which confirms their reliability. Importantly, the immuno-enzyme method is less labor-intensive and enables the evaluation of large populations at early breeding stages. This makes it a practical and efficient alternative to field scoring, especially when resources are limited.

Second, several parental varieties consistently showed higher levels of tolerance. Namangan-34, Namangan-77, and C-6541 demonstrated stable resistance across replications and environments. Their reliable performance underscores their value as donors in breeding programs aimed at improving wilt resistance.

Among F₁ hybrids, the most promising combinations identified were 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. These hybrids expressed enhanced resistance and represent elite material for further selection cycles. Their inclusion in breeding programs can accelerate the development of high-yielding and resilient cultivars.

From a practical standpoint, these findings have direct application in cotton breeding and seed production. Breeding programs should prioritize the tolerant parents and hybrids revealed in this study. Routine use of immuno-enzyme analysis can significantly speed up selection, allowing precise and large-scale screening at lower cost. For seed production farms, incorporating resistant varieties such as Namangan-34 and Namangan-77 into crop rotations can help reduce yield losses caused by wilt and maintain fiber quality.

CONCLUSIONS

In conclusion, integrating physiological and molecular tools with genetically tolerant parental forms and hybrids provides a clear pathway for creating new cotton cultivars. This approach ensures increased productivity, stable fiber characteristics, and improved resilience of cotton production systems under pathogen pressure.

REFERENCES

1. Abdelraheem, A., Fang, H., Jenkins, J. N., & McCarty, J. C. (2020). A genome-wide association study uncovers consistent QTL for resistance to *Verticillium* wilt and *Fusarium* wilt race 4 in U.S. Upland cotton. *Theoretical and Applied Genetics*, 133(2), 563–577. <https://doi.org/10.1007/s00122-019-03474-2>
2. Ayyaz, M., et al. (2025). QTL mapping associated with *Verticillium* wilt resistance in cotton based on MAGIC population. *Journal of Cotton Research*, 8, Article 9. <https://doi.org/10.1186/s42397-025-00211-7>
3. FAO. (2022). FAOSTAT: Crops and livestock products. Food and Agriculture Organization of the United Nations.
4. Gardiner, D. M., Neate, S. M., & Wilson, I. W. (2024). The genomes of two Australian isolates of *Verticillium dahliae* recovered from cotton fields. *Australasian Plant Pathology*, 53(3), 435–441. <https://doi.org/10.1007/s13313-024-00999-2>
5. Griffing, B. (1956). Concept of general and specific combining ability in relation to diallel crossing systems. *Australian Journal of Biological Sciences*, 9(4), 463–493. <https://doi.org/10.1071/BI9560463>
6. ICAC. (2023). Cotton World Statistics. International Cotton Advisory Committee.
7. Kurbonov, S., & Avtonomov, V. (2020). Breeding of cotton for tolerance to *V.dahliae* in Uzbekistan. *Journal of Agriculture and Crop Science*, 206(3), 355–364. <https://doi.org/10.1111/jac.12456>
8. Lei, Y., et al. (2025). Integrative identification of key genes governing VW resistance in cotton via transcriptomic approaches. *Plant Biotechnology Journal*. <https://doi.org/10.1111/pbi.14567>
9. Webster, J., Neate, S. M., & Wilson, I. W. (2023). Investigating the defoliating-like (DL) VCG2A pathotype of *Verticillium dahliae* in Australian cotton. *Plant Pathology*, 72(8), 1711–1723. <https://doi.org/10.1111/ppa.13853>
10. Wilson, I. W., et al. (2024). Genetic mapping and characterization of *Verticillium* wilt resistance in a recombinant inbred population of upland cotton. *International Journal of Molecular Sciences*, 25(4), 2439. <https://doi.org/10.3390/ijms25042439>
11. Xiao, S., et al. (2021). GhMYB4 downregulates lignin biosynthesis and enhances cotton resistance to *Verticillium dahliae*. *Plant Cell Reports*, 40, 735–751. <https://doi.org/10.1007/s00299-020-02636-6>
12. Zhang, Y., et al. (2025). A panoramic view of cotton resistance to *Verticillium dahliae* — from genetic architectures to precision genomic selection. *Journal of Cotton Research (BMC)*. <https://doi.org/10.1186/s42397-025-00215-3>