

Article

Resistance of complex interspecific cotton hybrids to wilt

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Abstract: In the article, the results of determining the degree of tolerance to wilt disease in the wilt background of hybrid families obtained using the method of interspecies complex hybridization in the cotton plant are analyzed. In the F₂–F₄ generations of interspecies complex hybrids, a large-scale process of separation according to the sign of tolerance to wilt disease takes place, and among the hybrids, plants that are not damaged by wilt disease, partially damaged plants, and severely damaged plants are separated. With this in mind, it was confirmed that it is possible to isolate lines and varieties with different levels of wilt damage from complex hybrid combinations of interspecies with different genomes by purposefully conducting selection work by the breeder. A number of wilt disease-tolerant families compared to control cultivars have been isolated and are recommended for use as a starting source in genetic selection studies.

Keywords: cotton; variety; hybridization; interspecies complex hybridization; genotype; genome; wilt disease; tolerance; tolerance; family; line

1. Introduction

Cotton (*Gossypium* spp.) stands as one of the world's most essential fiber crops, contributing significantly to the textile industry. Wilt is the disease that causes the most damage to the cotton plant and harvest, and scientists have been researching it for many years. However, the sustained productivity of cotton is continually challenged by various pathogens, with *Verticillium dahliae* Kleb emerging as a formidable adversary.

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae* Kleb, is recognized for its stealthy invasion and its ability to persist in the soil for extended periods of time. This pathogen poses a significant challenge to cotton crops, affecting the vascular system and leading to detrimental consequences for plant health and fiber quality.

The hallmark of *Verticillium* wilt lies in its capacity to invade the xylem vessels, disrupting water and nutrient transport within the plant. This vascular colonization sets the stage for a gradual progression of symptoms, impacting the overall vigor and productivity of cotton plants.

Verticillium wilt induces a range of symptoms in cotton, including wilting of leaves, chlorosis, and vascular discoloration. Understanding the temporal and spatial expression of these symptoms is crucial for early detection and effective management of the disease.

Beyond the immediate impact on yields, *Verticillium* wilt imposes economic burdens on cotton production. Increased costs for disease management, reduced fiber quality, and the necessity for extended crop rotations contribute to the complexity of the disease's impact on the cotton industry.

This manuscript aims to unravel the complexities surrounding *Verticillium* wilt in cotton. Through an in-depth exploration of the pathogenesis, symptomatology, and current management strategies, we seek to provide a comprehensive understanding of the disease. By doing so, we aim to contribute valuable insights to the development of sustainable practices for mitigating the impact of *Verticillium* wilt on cotton crops.

On the basis of long-term research, the laws of inheritance of the disease were studied, and new cotton varieties resistant to the disease were created. It has been demonstrated that resistance genes from wild species can be transferred to cultivated varieties through interspecies hybridization, and great progress has been made. Wilt disease causes and spreads *Verticillium dahliae* Kleb in the majority of field soils where cotton is grown. Information about the existence of different types and races is given in many literary sources. In this case, it is very necessary and useful to create varieties of cotton that have tolerance, that is, blocks of polymer genes. Because such varieties are resistant to several races of wilt, they maintain productivity in different soil and climate regions. In order to develop cultivars resistant to wilt disease, it is important to involve wild cotton species in hybridization and to transfer resistance genes to cultivar genotypes through hybridization. Wild and semi-wild species of cotton are more resistant to the disease: *G. thurberi* Tod. and *G. raimondii* Ulbr. It is reported in the literature that hybrid plants with a high level of tolerance to wilt disease were obtained by involving the species in hybridization. Wilt disease causes and spreads *Verticillium dahliae* Kleb in the soil of most fields where cotton is grown. Information about the existence of different types and races is given in many literary sources. In this case, it is very necessary and useful to create cotton varieties that are tolerant, i.e., polymer gene-linked blocks. Because such varieties are resistant to several races of wilt, they maintain productivity in different soil and climate regions [1–4]. Wilt disease resistance of hybrids obtained on the basis of cross-breeding of long forms between species and within species is dominant and high, giving information about its transmission from generation to generation in the case of dominance [5,6]. In particular, as a result of carrying out domestic and interspecies hybridization, varieties resistant to “A” and “B” races of felt, resistant to felt, belonging to type IV fiber, and belonging to type IV fiber were created. It is 42% [7].

Regions that cause great damage to the cotton crop and economic losses due to the defeat of cotton by Fusarium wilt disease are the southeastern part of the United States, the Nile Valley of Egypt, India, Tanzania, the southeastern part of Lake Victoria, China, Australia, and the Commonwealth of Independent States (CIS), including Central Asian countries [8–11].

In 1968–1972, Fusarium wilt spread to 60% of fine-staple cotton in the fields of Turkmenistan and caused huge crop losses [12].

Since the 1970s, it has been known in Uzbekistan that Fusarium wilt causes yield losses in medium-fiber cotton. In different regions of our country, cotton yields decreased by 18%–28% due to the Fusarium wilt pathogen.

According to scientific sources, during 2007–2011, up to 68% of the crop was lost when medium-fiber cotton fields in the Fergana Valley were infected with Fusarium wilt. In particular, in the Shahriqhan district of the Andijan region, the cotton variety C-6524 is up to 32.5%; in the Altinkol region, it is up to 30%; in the Baghdad

region of the Fergana region, it is up to 64.2%; and in some areas of the Namangan region, the Namangan-77 variety is up to 68% [13].

Once the plant root is saturated with FOV spores, the vascular tissue is accessed. Only when the primary host plant tissue is infected with FOV spores does the plant exhibit wilt symptoms, and the plant dies completely. Plants infected with the FOV fungus exhibit the following symptoms: 1) darkening of leaf veins; 2) leaf chlorosis; 3) leaf necrosis; 4) leaf drop; 5) change in stem color; 6) the appearance of various small spots; 7) complete wilting of the plant. The development of symptoms of this disease in cotton is influenced by a number of factors related to its genotype, the aggressiveness of the FOV fungus, the physical and biological composition of the soil, and the mechanism of sowing seeds and fertilizing cotton fields [10,14–20].

In recent years, scientists have studied the genetic diversity of OPV populations, their harmful activity, resistance criteria, and molecular phylogenetic characteristics in different plants [21–25].

Currently, eight races of FOV populations have been identified in the Americas, Asia, and Africa; races 1, 2, and 4 in the USA; race 3 in Egypt; race 4 in India; race 5 in Sudan; and race 6 in Brazil; the 7th and 8th races in China; and the 3rd, 4th, and 6th races were identified in Uzbekistan [26,27].

In addition, many studies have been carried out to determine the genome structure, gene sequence, damage level, and distribution area of FOV races. Until 2005, only races FOV 1 and 2 were observed in the cotton fields of California, USA, but Kim and his colleagues were the first to prove that races FOV 3, 4, and 8 were also found in this state based on molecular genetic analysis [28].

There is a lot of information about the good practical results of complex cross-breeding with cultural varieties and wild and semi-types available in the cotton collection in the creation of wilt-resistant varieties of cotton [29–31]. In particular, the 3312 varieties of cotton cultivated are wild *G. trilobum*, *G. hirsutum* L. ssp. *punctatum*, and *G. anomalum* species. It was possible to make hybrid plants resistant to wilt disease [32]. *G. thurbery* Tod of cotton, emphasizing that the wild diploid species is resistant to wilt, gommosis, black root rot, insects, salt, and dehydration, as well as the high technological quality of its fiber, it is recommended to use it widely in selection work [33]; or *G. davidsonii* belonging to the D3 genome, the wild type, is highly resistant to wilt disease. Therefore, using this variety as a primary parent in the selection process can give good results.

In recent years, world scientists have been working to identify useful traits in wild types of cotton belonging to different genome groups, find their controlling genes, and transfer them to the genotype of cultivated varieties using modern methods [34–36].

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2. Materials and methods

Taking into account the above, research has been conducted on the synthesis of various complex hybrids of cotton with the participation of species belonging to

different genome groups and on the study of the laws of inheritance, formation, and variation of traits in hybrid generations.

In this article, the 4-genome $\{(G. \textit{thurberi}$ Tod. x $G. \textit{raimondii}$ Ulbr.) x $G. \textit{arboreum}$ L.) x $G. \textit{hirsutum}$ L.} complex interspecies hybrids synthesized on a new genetic basis are resistant to wilt disease in the upper generations. The analysis of studies on the study of tolerance is presented. S-6524 and Omad varieties involved in hybridization belong to the $G. \textit{hirsutum}$ L. type, while the Termiz-31 variety belongs to the $G. \textit{barbadense}$ L. type. F_1 $\{(G. \textit{thurberi}$ Tod. x $G. \textit{raimondii}$ Ulbr.) x $G. \textit{arboreum}$ L.) synthetic amphidiploid hybrid is tentatively designated as K-28.

In determining the degree of wilt tolerance, Omad, S-4727, Tashkent-6, and Namangan-77 varieties of $G. \textit{hirsutum}$ L. species, which have different tolerances to different races of wilt, participated as control varieties. The obtained results were mathematically and statistically processed in the style of B.A. Dospikhov [37]. Wilt damage was studied in a field infested with natural wilt pathogens. The degree of infection was taken into account three times during the growing season: on 15 August, 1 and 15 September. The level of wilt resistance was determined visually and by breaking the plant stem.

3. Results and discussion

In our research, we analyzed the tolerance of cotton families and lines obtained on the basis of intergenome hybridization to wilt disease in comparison with model and indicator varieties. According to the obtained results, the control Omad and Tashkent-6 varieties, in comparison with other varieties, showed relatively low damage by wilt in a general and strong degree in a naturally damaged environment. That is, the total damage of these varieties was 11.7% and 12.3%, respectively, and the severe damage was 5.5%–6.3%. The Namangan-77 variety, which participated in the quality of the control variety, registered 29.4% damage at the general level and recorded 9.2% damage per day. The S-4727 variety was found to be the most affected in total and severe levels, at 30.3% and 15.6%, respectively. It should be noted that K-28 amphidiploid, i.e., hybrid $[F_1 (F_1 G. \textit{thurberi}$ Tod. X $G. \textit{raimondii}$ Ulbr.) x $G. \textit{arboreum}$ L.] created with the participation of diploid species, had a slightly lower level of damage. The combination of interspecific complex hybrids $F_4(F_1K-28$ x S-6524) was found to be relatively weak, i.e., 7.4% of total and 2.2% of severe damage. The index of $F_4 [BC_1 (F_1 K-28$ x S-6524) x Omad] backcross hybrid of $G. \textit{hirsutum}$ L. variety Omad was relatively high; that is, it was 12.9% at the general level and 1.7% at the strong level. $F_4 \{BC_2 [(F_1K-28$ x S-6524) x Omad] x Omad} hybrid obtained from double backcross was significantly positive in overall and severe wilt resistance compared to single backcross hybrids with overall damage of 9.7% and severe wilt resistance. It was found to be 1.4%. These indicators were at the level of the control Omad and Tashkent-6 varieties, while the Namangan-77 and S-4727 varieties recorded a significantly lower infection (**Figure 1**).

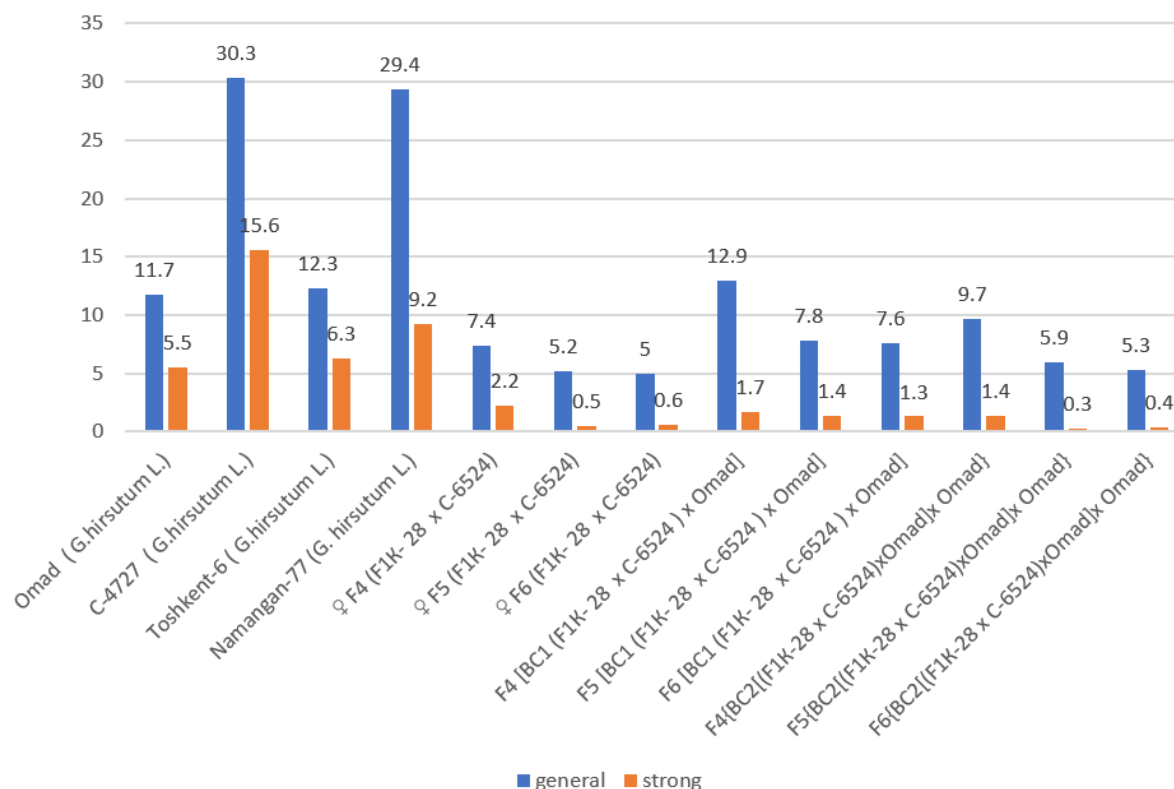


Figure 1. Wilt disease damage rate of interspecies complex and backcross F₄₋₆ plants with 4 types of cotton (%).

The performance of F₅ hybrids was well confirmed by the study of complex and backcrossed F₄ plants of four types obtained with the participation of wild species on a background naturally infested with wilt and the selection of hybrids among them. That is, by this generation of hybrids, the index of hybrids was positive for general and severe damage. Among the studied hybrids, the maternal form F₅ (F₁ K-28 × S-6524) had the best performance, with a total of 5.2% and a strong 0.5% reported damage. It can be seen that the influence of the amphidiploid hybrid was strong in the resistance to wilt of this hybrid, which participated in the maternal form. One and two backcross hybrids obtained by crossing the Omad variety with this wilt-resistant hybrid showed a significant reduction in blight in this generation. Correspondingly, damage in the combination of F₅ BC₁ (F₁ K-28 × S-6524) × Omad is 7.8%; 5.9% (total) in the backcross hybrid F₅ {BC₂ [(F₁ K-28 × S-6524) × Omad] × Omad}, which is 1.4%; 0.3% showed (severe) damage. As a result of the selection of wilt-resistant forms in this generation, the wilt resistance of hybrids in subsequent generations was further increased.

In F₆ plants, the combination of maternal F₆ (F₁ K-28) × S-6524 recorded the least damage, and it was found that 5.0% of the plants were damaged in total, but not a single plant was severely damaged. In the double backcross hybrid with the Omad variety, the rate of infection further decreased to 5.3% in general and 0.4% in severe. The information obtained from the study of the level of tolerance to wilt disease in hybrids was used to create hybrids with the participation of four species with a general and severe degree of wilt disease.

S-6524 and S-4727 varieties belonging to *G. hirsutum* L. have different manifestations depending on the genotype and natural races of wilt. That is, four types

of work were obtained with the participation of the variety S-4727, which is resistant to the first natural race of wilt.

It was found that the hybrids in there were strongly damaged by both levels of wilt compared to the combination obtained from the hybridization with the S-6524 variety, which is considered highly resistant to its second race. In particular, it can be explained by the fact that the total damage of the maternal form $F_4 (F_1 \text{ K-28}) \times \text{S-4727}$ was 19.7% and 3.6% at the severe level. These indicators were 2–3 times higher than the indicator of the combination $F_4 (F_1 \text{ K-28}) \times \text{S-6524}$ in the maternal form. Also, this hybrid index recorded 7.0%–8.0% higher damage than the control Omad and Tashkent-6 varieties. The mother's resistance to wilt disease ensured that the hybrids in her work also had a relatively high level of damage.

Specifically, $F_4 [\text{BC}_1 (F_1 \text{ K-28} \times \text{S-4727}) \times \text{Omad}]$ has a total damage rate of 17.0%, and $F_4 \{ \text{BC}_2 [(F_1 \text{ K-28} \times \text{S-4727}) \times \text{Omad}] \times \text{Omad} \}$ has a 10.9%. Also, the rate of severe damage was also high in these hybrids. It can be seen that in the S-4727 cultivar genotype involved in the maternal hybrid, it also caused resistance to wilt in the hybrids involved. However, by studying these hybrids in a background naturally infested with wilt and selecting uninfected plants, a sharp decrease in the level of wilt damage in subsequent generations was achieved. In particular, the total damage of $F_5 [\text{BC}_1 (F_1 \text{ K-28} \times \text{S-4727}) \times \text{Omad}]$ plants was reduced by 11.2%, and by the F_6 generation, it was reduced by 9.4%. Similarly, the combination of backcross $F_5 \text{ BC}_2 [(F_1 \text{ K-28} \times \text{S-4727}) \times \text{Omad}] \times \text{Omad}$ resulted in a 7.1% reduction in total damage and a 6.8% reduction in F_6 generation.

It can be seen that these indicators are significantly lower than those of the those of the parental and control varieties. From this, it can be concluded that in interspecies complex hybrids with the participation of four species, the separation of different plants in terms of resistance to wilt disease was observed. For this reason, it is possible to isolate forms tolerant to wilt disease by studying them against a background naturally infected with wilt and selecting resistant plants.

It has been established that interspecific complex hybrid plants with five species are more resistant to wilt disease than interspecific complex hybrid plants with four types of cotton. It should be noted here that the resistance to wilt of the hybrids used as maternal hybrids turned out to be different depending on the genotype of the varieties C-6524 and C-4727. The absence of infected plants in general was noted in the Termiz-31 variety, and the severe degree of infection in this variety was 6.8%. Thus, the resistance of the Termiz-31 variety to wilt in the paternal form ensured less wilt damage in hybrids with its participation and increased resistance in hybrid plants. In F_4 plants of interspecific complex hybrids involving 5 species, the level of wilt infection was relatively high, but in subsequent generations, the level of infection decreased. In the complex interspecific hybrid combination $F_4 ((F_1 \text{ K-28} \times \text{S-6524}) \times \text{Termiz-31})$, the degree of wilt damage was 9.8%, while in the following plants F_5 - F_6 disease resistance increased, and the overall incidence was respectively 6.8% and 5.6% (**Table 1**). In accordance with the general level of damage, severely damaged F_4 plants of these hybrids averaged 1.0%, and in subsequent generations, no severely damaged plants were identified among the hybrid plants.

Table 1. Wilt disease damage rate of interspecies complex and backcross F₄₋₆ plants with 5 types of cotton (%).

| No. | Varieties and hybrid combinations | General | | Strong | |
|--|---|------------|------|------------|------|
| | | M ± m | V % | M ± m | V % |
| Control varieties | | | | | |
| 11 | ♂ Термиз-31 variety (<i>G.barbadense</i> L.) | 6.8 ± 0.5 | 9.3 | 0 | 0 |
| 22 | C-4727 variety (<i>G.hirsutum</i> L.) | 30.3 ± 1.6 | 18.2 | 15.6 ± 1.1 | 17.3 |
| 33 | Тошкент-6—Control variety (<i>G.hirsutum</i> L.) | 12.3 ± 1.5 | 19.3 | 6.3 ± 1.0 | 19.5 |
| 44 | Наманган-77—Control variety (<i>G.hirsutum</i> L.) | 29.4 ± 2.1 | 23.4 | 9.2 ± 1.7 | 17.4 |
| Hybrids in maternal form | | | | | |
| 55 | ♀F ₄ (F ₁ K-28) × C-6524 | 7.4 ± 1.0 | 31.2 | 2.2 ± 0.4 | 15.7 |
| 66 | ♀F ₅ (F ₁ K-28) × C-6524 | 5.2 ± 0.9 | 24.3 | 0.5 ± 0.1 | 17.3 |
| 77 | ♀F ₆ (F ₁ K-28) × C-6524 | 5.0 ± 0.4 | 21.7 | 0.6 ± 0.1 | 13.1 |
| Interspecific complex hybrids and backcross hybrids obtained with the participation of 5 types of cotton. | | | | | |
| 88 | F ₄ [(F ₁ K-28 × C-6524) × Термиз-31] | 9.8 ± 1.2 | 33.2 | 1.0 ± 0.3 | 30.9 |
| 99 | F ₅ [(F ₁ K-28 × C-6524) × Термиз-31] | 6.8 ± 0.6 | 27.1 | 0.0 ± 0.0 | 0.0 |
| 110 | F ₆ [(F ₁ K-28 × C-6524) × Термиз-31] | 5.6 ± 0.3 | 20.3 | 0.0 ± 0.0 | 0.0 |
| 111 | F ₄ {BC ₁ [(F ₁ K-28 × C-6524) × Тер-31] × Тер-31} | 8.2 ± 0.9 | 25.4 | 1.9 ± 0.2 | 30.4 |
| 112 | F ₅ {BC ₁ [(F ₁ K-28 × C-6524) × Тер-31] × Тер-31} | 5.2 ± 0.4 | 23.5 | 0.7 ± 0.1 | 13.5 |
| 113 | F ₆ {BC ₁ [(F ₁ K-28 × C-6524) × Тер-31] × Тер-31} | 5.0 ± 0.2 | 16.4 | 0.0 ± 0.0 | 0.0 |

The effect of backcrossing complex interspecific cotton hybrids involving five species with the Termiz-31 variety had a positive effect on the infestation of plants with wilt. That is, wilt infestation in the total degree of backcross F₄ {BC₁ [(F₁ K-28 × S-6524) × Termiz-31] × Termiz-31} was 8.2%, and in the next generations the damage rate decreased and amounted to 5.0%–5.2%.

It was found that this result was lower than that of Termiz-31 participating in the paternal form. Thus, based on the hybridization of wilt-tolerant forms, it is possible to identify forms in hybrid generations that are more resistant to wilt.

Positive results in resistance to wilt were also achieved in interspecific complex hybrids and backcross hybrids obtained with the participation of the amphidiploid F₄ (F₁ K-28) × S-4727, which had a relatively high damage rate.

In particular, it should be noted that these hybrids have a positive effect when they have wild species in their genotype that are highly resistant to wilt. Combination F₄ [(F₁ K-28 × S-4727) × Termiz-31] has an overall wilt hit rate of 10.8% and a strong hit rate of 2.6%. Due to the isolation of wilt-resistant forms in subsequent F₆ generations, the infestation level decreased to 6.5% in total and to 1.4% to a great extent. In addition, wilt infestation in backcross hybrids F₄ {BC₁ [(F₁ K-28 × S-4727) × Termiz-31] × Termiz-31} decreased from 9.0% to 6.1% in F₅ plants and 4% in F₆ plants. The information obtained above on resistance to wilt shows that interspecific complex hybrid plants with the participation of 5 species are 6%–8% less infected than the control varieties Omad and Tashkent-6, and 22%–25% less than the control varieties Namangan-77 and C-4727 varieties.

Thus, it has been established that the resistance to wilt of new interspecific complex hybrids created with the participation of five cotton species belonging to

different genomes depends on the genotype of the parental forms involved in hybridization, and starting from F₄, disease-resistant recombinants appear. As a result of targeted selection of complex interspecific hybrids, a number of families, lines, and varieties resistant to wilt disease have been identified.

Also, conducting field experiments with wilt and focusing on selecting healthy plants will ensure resistance to wilt disease in future generations. It was revealed that the wilting tolerance characteristics of the wild cotton species *G. thurberi* Tod. and *G. raimondii* Ulbr. increase the tolerance of new hybrids obtained with the participation of these species. Research has again demonstrated the importance of using wild and cultivated cotton species belonging to different genomes to improve wilt resistance and isolate rare-tolerant recombinants. Thus, in complex hybrids with different genome structures and the participation of several species, a large-scale process of separation occurs on the basis of resistance to wilt as well as under the influence of the genotype of wild and cultivated species participating in the crossing. Plants not affected by wilt, partially affected, on for this can be explained by the formation of the genotype of the mother, tolerant to wilt disease, or the father, relatively intolerant, and the purpose for which the breeder carried out the breeding work. and severely affected are distinguished. The reason for this can be explained by the formation of the genotype of the mother, tolerant to wilt disease, or the father, relatively intolerant, and the purpose for which the breeder carried out the breeding work. Taking this into account, the possibility of isolating lines and varieties with varying degrees of wilt damage from complex interspecific hybrid combinations with different genomes has been confirmed through targeted breeding work by the breeder.

4. Conclusion

Thus, in complex hybrids with different genome structures, with the participation of several species, a large-scale separation process for wilt disease tolerance occurs. It was found that there is a positive effect of the wild species involved in crossbreeding in increasing tolerance to wilt disease, and among the hybrids there are plants that are not damaged by wilt disease, partially damaged plants, and severely damaged plants. With this in mind, it was confirmed that it is possible to isolate lines and varieties with different levels of wilt damage from complex hybrid combinations of interspecies with different genomes by purposefully conducting selection work by the breeder. A number of wilt disease-tolerant families have been isolated compared to control cultivars and are recommended for use as a starting source in genetic selection studies.

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