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REVIEW ARTICLE

Molecular mechanisms of the phytoimmune system against *Fusarium oxysporum* f.sp. *vasinfectum* and *Verticillium dahliae* in cotton plants

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Abstract

Fusarium oxysporum f.sp. *vasinfectum* (FOV) and *Verticillium dahliae* occupy a special place among the pathogenic fungi that affect plant productivity, causing annually serious damage to the yield, fiber quality, morpho-biological and agronomic properties of the cotton plants. Therefore, the study of the complex molecular processes that occur between the pathogen and the plant remains one of the most important tasks. This requires molecular geneticists and breeders to fully understand the defense mechanism that has emerged in cotton plants against pathogens and to be able to apply it correctly in practice. To combat pathogenic fungi, a thorough analysis of the natural defense mechanisms of plants, including miRNA, transcription factors (TFs), quantitative trait loci (QTL), regulatory functions of plant cell membranes and proteins, may be of great importance. In this paper, we reviewed the research conducted in recent years to identify miRNAs, TFs and QTLs participating in the defense mechanism against FOV and *V. dahliae*. This review provides insight to understand research aimed at reducing and controlling the future economic damage caused by pathogenic fungi. Studying those factors by using modern genomic technologies together with OMICS studies has accelerated research in this discipline. As a result, the integration of various methods has emerged, developing new approaches such as multi-omics. Integrating these promising methodologies will enhance our comprehension of the molecular mechanisms underlying wilt resistance in cotton plants, leading to the development of novel resistant varieties.

Keywords: CRISPR/Cas system; *Fusarium oxysporum* f.sp. *vasinfectum*; *Gossypium barbadense*; *Gossypium hirsutum*; RNA interference; *Verticillium dahliae*

Introduction

Cotton has a special place among crops used to provide humanity with oil, fiber and other products and its global production in 2022/2023 accounted for approximately 118.29 million 480-pound bales. Uzbekistan is one of the ten cotton bale exporters. In 2022-2023, cotton production in Uzbekistan reached 740000 MT, making it the 8th largest producer in the world (1). According to the latest World Cotton Statistics Committee data, China and India are considered the leading countries producing cotton. Cotton production in these countries suffers from various stresses. Abiotic (drought, heat, soil salinization) and biotic (fungi, bacteria, viruses) stress factors affecting cotton productivity hinder its growth. Pathogenic fungi causing cotton wilt disease are considered economic factors that reduce cotton fiber quality and productivity in many cotton-producing countries globally (2).

Pathogenic fungi causing vascular wilt diseases in cotton are divided into two types, FOV and *V. dahliae* species, which differ significantly from each other (3). Plants severely infected with FOV show symptoms such as discoloration, rotting, or wilting of the stems and leaves (4). Two defoliating and non-defoliating types of *V. dahliae* are distinguished according to the characteristic of causing negative symptoms in cotton plants (5). Also, there are certain similarities between *V. dahliae* and FOV, including the fact that they occur in the plant vascular system and result in wilt symptoms, causing severe damage to the yield of the cotton plant. Moreover, the pathogenic fungi FOV and *V. dahliae* can survive in the form of mycelium, chlamydospores and microsclerotia in roots and plant debris for a certain period (until the pathogenic organism finds its host). Also, according to other research findings, they can maintain their activity by living at the expense of other crops (6).

Cotton has evolved mechanical and chemical defense mechanisms against pathogens, such as cell wall thickening or the production of various chemical components (7, 8). Among the chemicals produced in response to pathogenic organisms,

phytoalexins act as a primary defense mechanism to protect plants from external biotic factors (9). As a phytoalexin, gossypol plays an important role in the defense mechanism against the pathogen *Verticillium* wilt in cotton and shows high expression levels during infection with the pathogen *V. dahliae* (10). These protective barriers protect the plant to a certain extent from the effects of pathogens. In addition, the emergence of complex defense mechanisms against pathogens at the molecular level in the cotton plant has been studied using modern genomic technologies (11, 12).

In recent years, scientists have studied the presence of factors (miRNA, QTL, etc.) related to resistance to the wilt pathogen in cotton and their importance in plant defense mechanisms. As a result, the increasing number of studies on cotton wilt disease requires scientists to summarize this information for readers. As a measure to mitigate the damage caused by wilt disease, we aim to summarize information related to wilt and convey it to readers. This review summarizes modern scientific research outcomes related to the molecular mechanisms involved in cotton-pathogen interactions. It provides essential insights for the future development of pathogen-resistant cultivars and donor lines in cotton.

Fusarium and *Verticillium* wilt disease resistance molecular mechanisms

During evolution, plants have developed various protective functions against *V. dahliae* and FOV pathogens (13, 14). In plants, this defense mechanism occurs in a stepwise manner. In the initial stage, the plant cell's extracellular enzymes and cell membrane participate in creating its first immune defense mechanism against foreign organisms (15). In the next stage, the plant recognizes pathogenic organisms with the help of special proteinaceous substances located in the cell membrane (mainly its surface) and activates a defense mechanism associated with the pathogen (16). Activation of this phytoimmune system depends on the activity of genes, TFs and miRNAs related to pathogenicity in the cell (Table 1). These factors create a complex defense system against the pathogen in the cell.

The role of miRNAs in the defense against *V. dahliae* and FOV pathogens

MicroRNAs (miRNAs) are structures specific to eukaryotic cells that regulate the activity of target genes in plants as positive or negative post-transcriptional regulators. Since the discovery of miRNAs in plants, their involvement in important biological processes such as plant development, abiotic stress and flowering has been studied by many groups (17-19). Furthermore, the functions of siRNAs (mainly 20-25 nt long) and miRNAs (mainly 21-24 nt long) participating in providing the plant immune system against *V. dahliae* and FOV fungi have been sufficiently studied in the last two decades. Many studies related to miRNAs involved in plant-pathogen interaction have been conducted. In one work, the role of sRNAs in the plant defense mechanism was described using resistant (Mebane B-1) and susceptible (11970) lines to wilt belonging to *Gossypium hirsutum*. In the study, a total of 4116 sRNAs were found based on resistant and susceptible lines infected with FOV and their amount was significantly increased in the resistant Mebane B-1 genotype during FOV pathogen infection and conversely, they significantly decreased in the 11970 susceptible line. In addition, the authors identified a novel Ghr-miR-160, which targets auxin signalling proteins, in the Mebane B-1 line (20). Similarly, in one important study, authors showed that the MAPK kinase cascades are controlled by miRNAs. In plant cells, the activity of the GhMKK6 gene was negatively regulated by GHR-MIR5272. When MiR5272 expression was increased in cotton, it affected the expression levels of GhMKK6 and genes related to wilt resistance, causing plants to become more susceptible to FOV (21). In another work, the authors used strains V991 (with strong virulence) and D07038 (with moderate virulence) of the pathogenic fungus *V. dahliae* to infect KV-1 (*G. hirsutum*) cotton cultivars and identified 37 novel miRNAs based on two different sRNA libraries isolated from the infected plant. Moreover, according to the analyses conducted to identify the target genes of these

Table 1. Characterization of genes involved in resistance in cotton

Plant	Gene	Overexpression/ deletion of gene	Change in the phenotype of plants	Resistance to pathogen	References
G. hirsutum	GhTIR1	Deletion	+	Increased	(31)
G. hirsutum	GhLAC4	Deletion	-	Decreased	(32)
G. hirsutum	GhTCP4	Deletion	-	Decreased	(33)
G. hirsutum	GhGLP2	Overexpression	+	Increased	(35)
G. barbadense	GbSOT4	Deletion	-	Decreased	(36)
G. hirsutum	GhTLP19	Deletion	-	Decreased	(37)
G. barbadense	GbEDS1	Overexpression	+	Decreased	(38)
G. hirsutum	GhDIR1	Overexpression	+	Decreased	(39)
G. barbadense	GbChi23/32/47	Deletion	-	Decreased	(41)
G. barbadense	GbCRR1	Deletion	-	Decreased	(42)
G. barbadense	GbLyp1/7 and LysMe3	Deletion	-	Decreased	(43)
G. hirsutum	GhMYB36	Overexpression	+	Increased	(2)
G. hirsutum	GhMYB4	Overexpression	+	Increased	(45)
G. hirsutum	GhGT-3b_A04	Overexpression	+	Increased	(46)
G. hirsutum	GhWRKY70DB	Deletion	+	Increased	(47)
G. hirsutum	GhWRKY53	Deletion	-	Decreased	(50)
G. hirsutum	GhWRKY48	Deletion	+	Increased	(51)
G. hirsutum	GhWRKY1	Overexpression	+	Increased	(52)
G. barbadense	GbaVd1/2	Deletion	-	Decreased	(55)
G. hirsutum	GhCBP60b	Deletion	-	Decreased	(56)
G. barbadense	GbNAC1	Deletion	-	Decreased	(58)
G. hirsutum	GhCNGC13/32	Deletion	-	Decreased	(62)

Note: in the table + means positive; - means negative

miRNAs, 49 target genes participating in secondary metabolism synthesis, TF and other processes in plants were identified for 24 miRNAs (22).

Two Upland cotton cultivars, Jimian 11, sensitive to *Verticillium* wilt (VW) and Zhongzhimian 2, resistant to VW, were used to dissect the connection between mRNA and miRNA in cotton inoculated with wilt (*V. dahliae*). The authors identified 383 miRNAs - 330 known and 53 novel. After wilt inoculation, 31 miRNA-mRNA pairs showed different expression levels. They concluded the significance of the up-regulation of GhmiR39 and the down-regulation of GhmiR165 for the plant defense mechanism that affects important processes in the cell (23). To identify novel miRNAs in the wilt-susceptible Yi-11 (Upland cotton) and resistant Hai-7124 (Pima cotton) cotton cultivars and clarify their molecular mechanisms in plant defense, the authors constructed four different small RNA (sRNA) libraries from infected plants and identified 140 known and 58 novel miRNAs. Also, based on degradome analysis, variously expressed microRNAs [45] and their target genes [107] were identified under pathogenic *V. dahliae* attack (24). In another interesting work, the authors identified 215 miRNAs based on the wilt-resistant Hai-7124 and non-resistant Yi-11 cotton varieties infected with the pathogen *V. dahliae*. They also identified 14 novel miRNAs as well as two trans-acting sRNAs (TAS3 D8(+) and TAS3 D7(+)) and thousands of endogenous sRNA candidate genes. In addition, after infection with *V. dahliae*, a decrease in the levels of miR482 and miR1448 was observed in cotton. The authors concluded that miR482 and miR1448 are involved as positive regulators of plant defense mechanisms against pathogens (25).

The miR171a-SCL6 is another module system involved in the plant defense against *V. dahliae* in cotton. The overexpression of Ghr-miR171 significantly increased the disease index in plants compared to that of control plants and conversely, silencing of Ghr-miR171 improved the plant defense mechanism by increasing the activity of GhSCL6 and GhPR1 genes (26). In cotton, Ghr-miR482b was found to suppress the plant immune system by negatively regulating the activity of the pathogen-related gene GhRSG2. The disease index in miR482b-silenced plants was significantly reduced when the virus-induced gene silencing (VIGS) method was applied. In contrast, it caused a 0.46-fold increase in disease symptoms in overexpressed plants. Furthermore, the activity of two wilt-related genes, GhPR1 and GhPR2, was observed to be expressed in a GhRSG2-dependent manner in GhRSG2-deleted plants (27). The *V. dahliae* infection was found to affect the miR164, a positive regulator in the plant immune system that blocks the transcription product of GhNAC100. While plants silencing miR164 exhibited severe morphological changes (wilting, yellowing of leaves, increased disease index) under the pathogenic fungus attack, miR164-overexpressed plants showed the reverse outcome, exhibiting high resistance to the pathogenic fungus. An *in vitro* study confirmed the above results, with miR164-deleted plants producing more fungal colonies on a special medium than controls (28). Also, the miR477 performed a similar function to the miR164, indicating a positive correlation between plant resistance and this miRNA. After inoculation with *V. dahliae*, the authors identified 449 microRNAs - 71 known and 378 novel ones based on root samples, 176 miRNAs (28 knowns, 148 novels) showing different expression levels under fungal attack. The loss of Ghr-miR477 strongly impacted the plant chemical composition, with the level of CBP60A increasing significantly. In contrast, plants silencing CBP60A exhibited a positive phenotype similar to control plants, with their disease index making up nearly 20 % (29).

The miRNAs that appear in response to pathogens provide a plant defense mechanism by blocking the transcription of an important gene in the fungal cell. In one comprehensive study, the authors isolated 28 different sRNAs belonging to Upland cotton from the *V. dahliae* V592 strain to study the miRNAs exported by the cotton plant into the fungal cell. This indicated that these miRNAs targeted the Clp-1 and HiC-15 genes of the fungus, which are important for the plant defense system against VW. After inoculation with *V. dahliae* V592, the mRNA levels of these genes in the pathogenic fungi isolated from inoculated cotton were significantly reduced compared to the control pathogenic fungus. The authors confirmed the significance of Clp-1 and HiC-15 genes for virulence by using transgenic fungal strains VdaΔclp-1 and VdaΔhic-15, in which these two genes were deleted, as well as VdaClp-1m and VdaHiC-15m, miRNA-resistant fungi. While the VdaΔclp-1 and VdaΔhic-15 mutants did not induce wilt symptoms in plants, plants infected with the VdaClp-1m and VdaHiC-15m mutants exhibited a strong phenotypic change, showing wilting and yellowing symptoms compared to controls (30).

Transport Inhibitor Response-1 (TIR1) is one of the essential nuclear proteins in the extension of auxin hormone signalling. The expression of this gene, controlled by auxin and miR393 molecules, regulates its activity during *V. dahliae* infection. The loss of GhTIR1 was found to have a profound effect on the chemical composition of plants, with a significant decrease in the transcript levels of genes involved in auxin synthesis. At the same time, plants overexpressing miR393 exhibited a positive phenotype, showing strong resistance to the pathogen *V. dahliae*. The authors concluded that there is a negative correlation between TIR1 and plant resistance, indicating that the TIR1 gene impairs the cotton plant's immune system against VW (31).

Lignin is a part of the plant cell wall, essential in the management of many physiological processes in plant development and the protection mechanism against biotic stresses. The lignin synthesis in plants depends on the activity of the GhLAC4 gene. However, the activity of GhLAC4 in the cell is negatively controlled by the Ghr-miR397 molecule. GhLAC4 knockdown significantly reduced the amount of lignin and the plant resistance properties compared to control plants and conversely, deletion of the Ghr-miR397 molecule increased the amount of lignin. In cotton, the GhLAC4 gene was concluded to be a positive factor in providing a plant defense system against the pathogen *V. dahliae* (32). Teosinte branched1/Cincinnata/proliferating (TCP) transcription factors are responsible for the protective response of plants to external stress factors, such as *V. dahliae*, which depends on the activity of the Ghr-miR319b molecule. It was found that Ghr-miR319b-silenced plants showed a lower disease index under the influence of the pathogen *V. dahliae* compared to control plants and conversely, deletion of the GhTCP4 gene resulted in significantly increased susceptibility to pathogens (33). The plant immune system is supported by a complex chemical process occurring among various microRNAs

belonging to the miR482/miR482.2 family and nucleotide-binding leucine-rich repeat (NB-LRR) genes in cotton species, controlled by the downregulation of miR482/miR482.2 levels and upregulation of NB-LRR transcript levels. *V. dahliae* inoculation significantly decreased almost all types of miR482/miR482.2 in the leaves and roots, while the transcript levels of NB-LRR genes increased significantly. The authors concluded that there is a positive correlation between miRNAs belonging to the mir482/miR482.2 family and NBS-LRR genes, which is essential in ensuring the defense mechanism of plants (34).

The role of proteins in the resistance mechanism to FOV and *V. dahliae*

GhGLP2 belongs to the cupin superfamily proteins and has been shown to participate in pathogen-associated molecular patterns by enhancing the activity of genes (PDF1.2, LOX2 and VSP1) responsible for cellular defense mechanisms against pathogens. Silencing GhGLP2 in cotton using the VIGS method increased the susceptibility of plants to *V. dahliae* and FOV and increased symptoms such as severe wilting and reddening of leaf veins in the plant. In contrast, overexpression of the GhGLP2 gene in cotton showed significant resistance to pathogens, with reduced mycelial growth of fungi and increased cell wall lignification in leaves (35). Sulfotransferases (SOTs) are also sulfate-regulating proteins in various organisms. In *Gossypium barbadense,* the GBSOT4 gene is important in conferring cotton resistance to FOV. Deletion of the GBSOT4 gene by VIGS significantly increased plant susceptibility to pathogens (36). Thaumatin-like proteins (TLPs) have been identified as a defense mechanism for plants against pathogenic organisms. Knockdown of GhTLP19 by VIGS in cotton significantly reduced plant resistance to *V. dahliae*, with increased malondialdehyde (MDA) levels, disease index (DI) levels and decreased catalase (CAT) levels (37). Lipase-like proteins are synthesized in the cell by the GbEDS1 gene and their activity is increased by *V. dahliae*. It was found that disease symptoms and susceptibility in GbEDS1-overexpressed plants had significantly reduced compared to those of control plants. Conversely, knockout of this gene in *G. barbadense* was found to reduce the resistance of plants to wilt disease due to decreased salicylic acid (SA) and H2O2 in the cell (38). Dirigent superfamily proteins are highly expressed in the hypocotyl of Upland cotton after infection with *V. dahliae*. When the GhDIR1 gene was overexpressed, the lignin content changed positively, with lines 1, 8, 9 and 11 synthesizing the highest amount of lignin compared to the remaining lines and the control. Also, the P-value of lignin in these lines (L_1, L_8, L_9 and L_{11}) was significantly less than 0.05. In addition, a significant change was observed in the content of monosaccharides (rhamnose, arabinose, xylose, mannose, galactose, glucose) in the cell, with xylose showing the highest value among the remaining monosaccharides (39). Plant receptor-like kinases (RLKs) are important components of the plant cell wall. The GhWAK7A protein is an important component in the formation of a complex with the chitin of the fungal cell wall in cotton, interacting with GhLYK5 and GhCERK1 and triggering the cotton defense response against phytopathogens (40).

Enzymes located on the cell surface of plants are essential in the breakdown of the cell walls of pathogenic organisms. For example, chitinases are positive regulators of fungal cell wall degradation. In one important study, the authors identified 47, 49, 92 and 116 chitinase genes in the cotton species *G. raimondii*, *G. arboreum*, *G. hirsutum* and *G. barbadense*, respectively and mapped them to their chromosome locations. Accordingly, in cotton, knockout of the Chi23, Chi32 and Chi47 genes has been shown to negatively affect the plant's defense mechanism against *V. dahliae* (41). In plants, Cys-rich repeat protein 1 (CRR1) is important for protecting chitinase 28, a component of the plant cell wall, from a chemical component – *Verticillium dahliae* secretory Ser protease 1 (VdSSEP1) secreted by *V. dahliae*. Silencing CRR1 in cotton was positively correlated with plant resistance to *V. dahliae*, while its overexpression resulted in reverse outcomes (42). Lysine motif (LysM) proteins are cell membrane receptors that mediate plant defense responses to pathogens. In cotton, Lyp1, Lyk7 and LysMe3 proteins accumulate in large quantities around the plasma membrane of cells under the influence of various external stress factors, including the pathogen fungus V. dahliae. Loss of these genes had a strong effect on the chemical composition of the cell, thereby significantly reducing the amount of SA, JA and ROS related to the synthesis of PR1, PR4, PR5 and PR10 genes (43).

The role of transcription factors in regulating cotton resistance to *V. dahliae* and the FOV pathogen

TFs activated by various external stress factors protect plants from pathogenic organisms and play a crucial role in maintaining their immune system. The transcription factor GhMYB36 is important in providing plant defense against the pathogenic fungus *V. dahliae*. In one study, it was found that the susceptibility of transgenic plants silencing GhMYB36 was significantly increased compared to control plants, while fungal biomass and disease symptoms were significantly reduced in plants overexpressing GhMYB36 (2). The MYB6 transcription factor is important in enhancing the plant's defense function against *V. dahliae*. However, its activity depends on the enzymes PUB25 and PUB26 that cause the degradation of the MYB6 gene transcript. Plants with mutated PUB25 and PUB26 genes showed a lower disease index compared to that of control plants (44). It was considered that there is a positive connection between lignification and resistance in plants. However, the authors showed that reducing the lignin content in the membrane structure improved plant resistance to *V. dahliae*. The variability of the amount of lignin in the cell wall depends on the transcription factor GhMYB4. It was found that the amount of lignin in cotton overexpressing GhMYB4 was significantly reduced compared to control plants. As a result, the plant defense mechanism also increased dramatically due to the increase in oligogalacturonoids in the cell (45). The trihelix transcription factor GhGT-3b_A04 is important in providing a plant defense mechanism against pathogens by improving its root system and highly accumulating in the cell nucleus during infection with the pathogen *V. dahliae*. The study revealed that the defense mechanism against *V. dahliae* was improved in plants overexpressing GhGT-3b_A04 due to increased activity of genes involved in salicylic acid synthesis (46). WRKY transcription factors accumulate at high levels in root and stem tissues to enhance the plant's immune system against pathogens. In the study, the authors demonstrated a negative correlation between

the GhWRKY70D13 gene and the plant immune system. Transgenic plants silencing the GhWRKY70D13 gene exhibited positive morphological characteristics compared to the control without showing severe wilting and vascular browning under the effect of the pathogen *V. dahliae*. In addition, the amount of jasmonic acid in response to this fungus in GhWRKY70D13-deleted plants (Ci1 plant (40.65 ng/g) and Ci2 plant (40.36 ng/g)) was significantly increased compared to the control (WT (31.88 ng/g)) (47). In contrast, the reverse outcome of the above study was found in another study, which showed a positive correlation between GhWRKY70 and plant resistance. When the GhWRKY70 gene was silenced, the morphological and physiological parameters of the plants changed negatively, showing a severe sensitivity to the pathogen. It was found that the jasmonic acid (above 0.5 ng/g), chlorophyll (about 0.5 ng/g) and lignin content in the cell were significantly reduced (48). The activity of the GhWRKY40a in plant cells depends on the activity of the GhMPK9 and GhRAF39 genes. The GhWRKY40a transcription factor simultaneously controls the function of the GhERF1 and GhABF2 genes in plant cells. In cotton, the GhWRKY40a gene is important in conferring plant resistance to wilt disease by positively influencing the transcription of GhERF1b and negatively influencing the activity of GhABF2 (49). The GhWRKY53 is an important factor in providing a plant defense mechanism against *V. dahliae* by regulating the activity of genes involved in plants' jasmonic and salicylic acid synthesis. Salicylic acid content and resistance to pathogenic fungi in transgenic plants, silencing GhWRKY53, were significantly reduced. Conversely, JA content in GhWRKY53-silenced transgenic plants increased compared to control plants (50). The GhWRKY48 gene is strongly expressed in plants' roots, stems and other tissues under the influence of various external factors and activates the plant defense mechanism in response to the external environment. After inoculation with *V. dahliae*, plants silencing GhWRKY48 revealed positive results, with them demonstrating high resistance to this pathogen (51). In *G. hirsutum*, the GhWRKY1 transcription factor is a positive regulator that provides a plant defense mechanism by regulating lignin synthesis in the cell. The GhWRKY1 gene improves the defense function of plants against pathogens by activating the PAL6 and GhCOMT1 genes involved in lignin synthesis in the cell (52).

The transcription factor GhBLH7-D06 is highly expressed in cotton, mainly in vascular tissues during the period of infection with VW. In the study, deletion of the GhBLH7-D06 gene strongly affected JA synthesis and membrane structure-lignin synthesis, thereby increasing plant resistance to the pathogen. The authors concluded that there is a negative correlation between the GhBLH7-D06 gene and plant resistance (53). It was also found that the knockout of GhNDR1 and GhMKK2 transcription factors in cotton using the VIGS method reduced resistance to *V. dahliae* (54). GbaVd1 and GbaVd2, homologues of Ve1 in cotton, are genes that synthesize proteins (receptors) located in the cell membrane and act as activating factors for many chemical processes in plant defense against pathogens. Silencing GbaVd1 and GbaVd2 transcription factors using the VIGS method in plants showed stronger disease symptoms than control plants (55). In plants, the GhCBP60b gene is involved in regulating the activity of pathogen-related genes, but its activity is inhibited by VdSCP41, a secretion product secreted by the pathogen *V. dahliae*. Deletion of the GhCBP60b gene using the VIGS method was observed to dramatically reduce the resistance of plants to this pathogen (56).

NAC proteins are expressed in plant vegetative organs, mainly roots and stems, under the influence of external stress such as pathogenic microorganisms and enhance the plant defense mechanism. The authors infected *V. dahliae*-susceptible Jimian 11" (J11) and -resistant Zhongzhimian 2" (Z2) cotton cultivars with pathogenic fungi to dissect the chemical process of NAC that occurs at the molecular level after *V. dahliae* inoculation and identified 271 GhNAC transcription factors. Moreover, 54 GhNAC transcription factors were identified to be differentially expressed in pathogen-infected plants, less than 70 % of which were upregulated and more than 30 % were downregulated (17, 37, 57). Similarly, another study demonstrated that NAC proteins provide plant defense against *V. dahliae*. The authors found that the disease index and fungal biomass in GbNAC1-silenced plants significantly increased compared to control plants. In contrast, the reverse for GbNAC1-overexpressing plants was true (58).

Negative TATA-less (NOT) proteins revealed that they are important in regulating plant resistance to pathogenic fungi by influencing mRNA metabolism and transcriptional regulation processes in the cell. They are mainly expressed in the NOT2_3_5 form (a fusion of NOT2, NOT3 and NOT5) in plant cells and have been identified in all diploid (G. arboretum va G. raimondii) and tetraploid (*G. hirsutum* va *G. barbadense*) cotton species. According to the qRT-PCR analysis, the authors identified that the NOT2_3_5 genes (GbNOT2_3_5-3/8 and GbNOT2_3_5-4/9) showed relatively high expression levels after wilt infection. While the disease index in GbNOT2_3_5-3/8 -deleted plants made up about 40 %, this level for plants silencing GbNOT2_3_5-4/9 was slightly below 45 %. It was concluded that there is a positive correlation between the GbNOT2_3_5 genes and plant resistance (59). In cotton, REVEILLE2 (RVE2) is an important factor in enhancing plant defense against VW by influencing the synthesis of a specific hormone. It was found that fungal biomass and disease index in RVE2-overexpressing plants were significantly reduced compared to control plants. In contrast, the disease index in RVE2-silenced transgenic plants treated with JA was almost indistinguishable from that of control plants (60).

Cyclic nucleotide-gated ion channels (CNGCs) proteins are also important in plant growth and the immune system. Knockdown of two transcription factors, GhCNGC13 and GhCNGC32 in cotton, has been shown to reduce plant resistance to the pathogen *V. dahliae* by impairing plant growth and apical meristem formation (61). Similarly, the authors showed that early nodulin-like proteins (ENODL) are highly expressed in the plant defense system during wilt infection. When transgenic plants overexpressing GhENODL6 were infected with the pathogen *V. dahliae*, they showed a dramatic increase in resistance to the pathogen by activating chemical processes involved in the defense mechanism. Similarly, the positive participation of phenylalanine ammonia -lyase (PAL) and 4-coumarate-CoA ligase (4CL) genes in plant defense against pathogens was clearly observed in plants in which GhENODL6 was deleted. The authors concluded that the activation of multiple molecular pathways in plant defense depends on the activity of the GhENODL6 gene (62).

Quantitative trait loci (QTLs) responsible for resistance to *V. dahliae* and the FOV pathogen in cotton

The study of QTLs located in the genomic regions of the cotton chromosome is one of the important measures to combat FW and VW diseases. QTL loci conferring resistance to the pathogens FOV and V dahliae in cotton are identified using SSR, SNP and other markers used in marker-based selection. Using SNP markers, the authors used the MAGIC (multilineage hybridization) population of Upland cotton to determine QTLs related to *Verticillium* cotton wilt. They identified a QTL conferring major pathogen resistance in a narrow region of chromosome D02 associated with FOV race 4 (FOV4) based on 550 lines, which allowed the identification of 14 candidate genes (63). Also, 110 RIL populations obtained from the cross between the FOV7-resistant 06-1464 and the susceptible Xinhai -1 lines of cotton (*G. barbadense*) were screened using 933,845 SNP markers. In the study, a total of 9 QTLs and a candidate gene, GB_D03G0217, which synthesizes calmodulin (CaM)-like (CML) protein, were identified. Silencing the GB_D03G0217 gene in cotton was observed to increase the pathogen infection rate in plants (64).

In one unique work, the authors artificially infected 469 lines derived from a cross between resistant and non-resistant varieties of Upland cotton with *V. dahliae* to dissect the molecular mechanism of cotton resistance to VW and identified 8 QTLs conferring resistance to VW. In addition, a candidate gene related to resistance, Gh_CPR30, has been identified in *G. hirsutum*. Gh_CPR30 was knocked out using the VIGS method to determine whether this gene confers resistance to plants. It was found that disease symptoms in transgenic plants silencing Gh_CPR30 were lower than in control plants (65). A total of 163 RIL populations derived from Pima S-6 (resistant to FOV4) and 89590 (sensitive to FOV4) lines of Pima cotton were inoculated with FOV and mapped for FOV resistance. A total of 4 (in the first replicate) and 5 (in the second replicate) QTL loci were identified in the study conducted in two replicates. Interestingly, 3 of the total QTLs identified in the study were found to be located on similar chromosomes in both tests (c17/D03, c24/D08 QTL, c25/D13) (66). In another study, moderately resistant cotton lines belonging to Pima and Upland cotton were crossed and a population of 181 backcross inbred lines (BIL) was obtained based on them to identify QTLs conferring resistance to FOV. The 181 BIL populations were infected with the FOV pathogen and 42 unique QTLs related to FW resistance were identified using 7709 SNP markers (67). While the ZZM2 cultivar shows resistance to VW, the J11 cultivar shows susceptibility. The authors identified two candidate QTLs (qvw-D05-1/2) located on Chr D05 of the cotton genome associated with resistance in their hybrid (ZZM2 × J11) progeny populations based on BSA-Seq analysis and these QTLs were the reason for identifying the candidate gene. Moreover, GhDRP-deleted plants showed severe susceptibility to this pathogen, with the disease index of plants (over 30 % at 20 dpi) increasing significantly compared to controls (approximately 10 % at 20 dpi) (68).

Infecting various lines of Upland cotton hybrids with *V. dahliae* and FOV4 pathogens led to the identification of QTLs related to plant pathogens. Based on the results of the *V. dahliae* pathogen test conducted in four replicates, the authors identified two QTL linked with nine SNPs on Chro8 and Chro10 in the first test, Three QTL linked with 14 SNPs on Chro16, Chro10 and Chro8 in the second test, six QTL on Chr 21, Chr 19, Chro16 and Chro8 in the third test and four QTL on Chr 21, Chro16 and Chro8 in the fourth test. Similarly, for the FOV4 pathogen, 8 QTLs linked with 36 SNPs were determined in Chro8, Chro14, Chro16 and Chro18 in the first test and 5 QTLs linked with 29 SNPs were identified in Chro8, Chro14 and Chro17 in the second test. BLAST analysis identified 30 and 35 genes related to resistance to *V. dahliae* and FOV4, respectively (69). In another interesting work, the authors carried out genetic mapping and characterization of QTLs related to resistance to *V. dahliae* in cotton based on MCU-5 (resistant to VW) and Siokra 1-4 (susceptible to VW) cultivars. The study used 240 RIL populations obtained from these cultivars (MCU-5 × Siokra 1-4) for QTL mapping. It was found that the activity of genes located on Chr D03 and Chr D09 of F7 hybrid generations infected with the *V. dahliae* pathogen increased mainly during the wilt infection period. Also, genes differentially expressed and candidate genes associated with resistance in the parental plants (MCU-5 and Siokra 1-4) were identified (70). Similarly, CSSL (chromosome segment substitution line) populations by crossing sensitive (CCRI36) and resistant (Hai1) varieties were obtained. The study identified 40 QTLs associated with resistance to the pathogen *V. dahliae* based on the CSSL lines. These QTLs were located on all chromosomes of the cotton genome except chromosomes Chr04, Chr08, Chr13 and Chr24 and most of the QTLs were located on chromosomes Chr19 and Chr24 (71).

Pima S-7 (Pima cotton) and Acala NemX (Upland cotton) are two different species and their hybrid (Pima S-7 × NemX) progeny populations showed different resistance to FOV1 and FOV4 pathogens. Six significant QTLs located on five chromosomes (Chr1, Chr2, Chr12, Chr15(2), Chr21) of RIL populations infected with the FOV1 pathogen conferred major resistance to the pathogen. In contrast, two QTLs located on Chr14 and Chr17 of hybrid progeny infected with the FOV4 pathogen were found to confer major resistance to the pathogen. The authors concluded that cotton plants have evolved different defense mechanisms against FOV1 and FOV4 pathogens (72). Another work used the CSIL SuVR043 population derived from a hybrid of Sumian 8 and H7124 to map QTLs and analyzed candidate genes related to resistance to the *V. dahliae* pathogen. The study identified three candidate genes, GbCYP450, GbTMEM214 and GbRLK and two QTLs (qVW-Bp2-1/Bp2-2) in plants infected with the pathogen. The authors knocked out these genes to investigate the relevance of the candidate genes mentioned above to resistance. It was found that the disease index in plants with the deletion of GbCYP450 and GbTMEM214 was significantly higher compared to the control (73).

G. barbadense is a species that is more resistant to wilt disease than *G. hirsutum*. There are also certain difficulties in transferring and integrating the *G. barbadense* resistance trait into the *G. hirsutum* cotton genome. This makes creating varieties resistant to *V. dahliae* in *G. hirsutum* hybrids even more difficult. Despite these complex issues, authors identified 119 QTLs associated with pathogen resistance on 25 chromosomes of the genome of *G. hirsutum* hybrid progeny (0-153 × sGK9708) based on six replicates of one-year

experiments in greenhouse conditions and two replicates of four-year experiments in the field (74). Similarly, hybrid population lines containing multiple genetic origins were evaluated for resistance to FOV4. A QTL Fov4 gene, designated Fov4-C14 1, conferring pathogen resistance in the F2 generation of hybrids, was identified and reported to be located on chromosome 14 of plants (75). The Indian cotton cultivar MCU-5 exhibits high resistance to the Australian FOV strain. In contrast, the Siokra 1-4 cultivar is susceptible to the FOV pathogen. The scientists crossed these resistant and susceptible cultivars to obtain F3 and F4 RIL populations and performed QTL analysis on them for resistance in the progeny. In the study, 3 QTL loci were identified in the F3 generation and 8 in the F4 generation. These QTL loci were located on chromosomes Chr 6/A06, Chr 22/D04 and Chr 25/D06 (76).

In another work, the group of authors created intraspecific F2 generation populations based on resistant and susceptible cultivars of *G. hirsutum* to dissect and map disease-resistance genes in cotton. The resulting hybrid generation was molecularly screened with SSR markers and a gene related to resistance was identified on chromosome Chr17/D03 of the cotton genome. In addition, 4 different QTLs related to resistance (qFW-D1-1/D9-1/D3-1/A7-1) were identified on chromosomes Chr7/A07, Chr15/D01, Chr23/D09(c23) and Chr17D03 of the hybrid lines (77). While Hai1 (Pima cotton) shows strong resistance to VW, CCRI36 (Upland cotton) shows susceptibility. Based on their interspecific backcross lines, the authors identified 48 QTLs related to resistance on 19 chromosomes of the cotton genome (Chr1, Chr3, Chr5, Chr6, Chr7, Chr8, Chr9, Chr10, Chr11, Chr12, Chr13, Chr14, Chr15, Chrda17, Chr19 Chr20, Chr21, Chr22, Chr26) using SSR markers and of these, 33 QTLs were found to be in the A genome and the remaining 15 QTLs in the D genome (78).

Conclusion

In many cotton-growing countries, a large portion of the crop is annually damaged by the pathogens FOV and *V. dahliae*. It is often difficult to overcome this problem using traditional breeding methods. The main reason for this phenomenon is the defense reaction of the pathogen fungi that occur relative to the phytoimmune system of the cotton. During evolution, cotton plants have developed several defense systems that reduce the damage caused by pathogens. As a primary defense, a thick cuticle layer and specially synthesized chemical components (phytoalexins) reduce the negative effects of pathogenic organisms to a certain extent. However, a molecular mechanism -based immune system is important in plants, which is activated by the perception of the effects of pathogens by special receptors located on the cell membrane. Studies conducted over the past two decades have shown that miRNAs, transcription factors and quantitative trait loci are key factors (genes) in protecting cotton from pathogens. It was also found that the modification (deletion/overexpression) of these factors using modern genomic technologies such as RNAi and CRISPR/Cas changed the defense system of the cotton plant against pathogens. It is important to introduce modern genomic technologies for cotton breeding to reduce the economic loss caused by pathogenic organisms. In addition, the integration of modern genomic technologies has shown that it will be possible to understand the new defense systems that have emerged in cotton plants against pathogens and, based on this, to create cotton varieties resistant to FOV and *V. dahliae* pathogens in the future. In this review paper, molecular genetics and modern selection studies enhancing resistance to *V. dahliae* and FOV pathogens in cotton have been extensively highlighted. Furthermore, such generalized research findings will further simplify the development of new methods based on modern genetic engineering for young scientists in the future and combat against FOV and *V. dahliae* pathogens.

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Authors' contributions

IEB wrote the manuscript and contributed to literature analysis. MMD co-wrote the manuscript, analyzed literature, and critically edited the paper while also contributing to subsection preparation. ANA participated in manuscript writing and literature analysis. NNK contributed to analyzing the literature. JKN assisted in literature analysis. RRA was involved in literature analysis. SIM participated in literature analysis. AMA critically reviewed and edited the manuscript and contributed to subsection preparation. ZTB rigorously revised the article and gave final approval. All authors have read and approved the final version of the manuscript.

Compliance with ethical standards

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