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A comprehensive review on elucidating the host disease resistance mechanism from the perspective of the interaction between cotton and *Verticillium dahliae*

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Abstract

Verticillium wilt, caused by the infamous pathogen *Verticillium dahliae*, presents a primary constraint on cotton cultivation worldwide. The complexity of disease resistance in cotton and the largely unexplored interaction dynamics between the cotton plant host and *V. dahliae* pathogen pose a crucial predicament for effectively managing cotton Verticillium wilt. Nevertheless, the most cost-effective approach to controlling this disease involves breeding and cultivating resistant cotton varieties, demanding a meticulous analysis of the mechanisms underlying cotton's resistance to Verticillium wilt and the identification of pivotal genes. These aspects constitute focal points in disease-resistance breeding programs. In this review, we comprehensively discuss genetic inheritance associated with Verticillium wilt resistance in cotton, the advancements in molecular markers for disease resistance, the functional investigation of resistance genes in cotton, the analysis of pathogenicity genes in *V. dahliae*, as well as the intricate interplay between cotton and this fungus. Moreover, we delve into the future prospects of cutting-edge research on cotton Verticillium wilt, aiming to proffer valuable insights for the effective management of this devastating fungus.

Keywords Cotton Verticillium wilt, *Verticillium dahliae*, Resistance inheritance, Disease resistance mechanism, Interaction mechanism

Background

Cotton, a vital natural fiber material and cash crop, plays an indispensable role in the national economy in China (Zhang et al. 2022a). With the rise in labor costs and a growing need for mechanization in China, the cultivation practices of cotton have been optimized and adjusted. Xinjiang Uygur Autonomous Region has grown as the primary hub of cotton production in China. According to the announcement made by National Bureau of Statistics of China in 2023, Xinjiang accounts for an impressive 84.98% of the national total cotton planting area, with its yield contributing to an astounding 90.99%. In recent years, the incidence of cotton Verticillium wilt (CVW) in Xinjiang cotton fields has reached alarming levels,

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affecting an overwhelming 50%–70% of them, posing a significant hindrance to the green and healthy development of cotton in China (Liu et al. 2015a; Palanga et al. 2021). Similarly, CVW stands as a formidable threat to cotton production on a global scale, inflicting average yield losses of 15%–30% and surpassing 50% in severe instances (Zhu et al. 2017). Its notorious pathogenic fungus, *Verticillium dahliae*, is a soil-borne plant pathogen that infests the xylem tissue and instigates Verticillium wilt diseases across a spectrum of over 200 plant species worldwide (Zhang et al. 2022a). Under favorable temperature and humidity conditions, the *V. dahliae* spores germinate and give rise to hyphae that intricately entangle themselves around the delicate root tips of cotton plants and then form the hyphopodium at the infection site, which develops into penetration pegs piercing the root epidermis, leading to penetrating the gaps between the root epidermis layers (Zhao et al. 2016). In particular, penetration peg serves as a crucial tool for *V. dahliae* during host root penetration (Zhang et al. 2022b). The penetration peg subsequently matures into the hyphal neck, establishing a distinct fungal-host interface that elegantly facilitates the transmission and secretion of small secreted proteins in an efficient manner (Zhao et al. 2016; Zhou et al. 2017). Eventually, these structures elongate into the vascular tissues, causing obstruction of the vascular bundle and release of toxins that inflict harm upon host plant cells. Consequently, cotton leaves wither, turn yellow, and ultimately succumb to death (Klosterman et al. 2009) (Fig. 1).

The infection cycle of *V. dahliae* primarily encompasses the germination of microsclerotia within the soil, the subsequent germination of hyphae that infiltrate the cotton plant from the root tip, the colonization of cotton's vascular bundles, and the infection of the foliage, resulting in wilting and chlorosis, followed by the re-isolation of pathogenic fungus from affected leaves. The reproductive cycle of *V. dahliae* chiefly involves mycelial expansion, the formation of branched conidiophores, the production of conidia, and the subsequent enlargement and darkening of the mycelium, resulting in the development of microsclerotia.

Due to the lack of robust disease resistance genes in upland cotton (*Gossypium hirsutum*), understanding the intricate molecular mechanisms underlying cotton's resistance to Verticillium wilt proves to be an arduous task (Chen et al. 2005; Shaban et al. 2018). Furthermore, the substantial variability exhibited by these pathogens and their co-evolution with plants have obscured the interaction mechanism between plants and pathogens, presenting a critical challenge to the effective prevention and control of this disease (Bai et al. 2022a; Yang et al. 2023). Cultivating and planting disease-resistant varieties have been proved as the most economically viable approach for preventing and managing CVW (Li et al. 2019). In particular, delving deeper into the mechanisms underlying cotton's resistance to Verticillium wilt and unraveling key genes involved are pivotal aspects of disease-resistant breeding program. Furthermore, in-depth research on future prospects of cutting-edge studies on

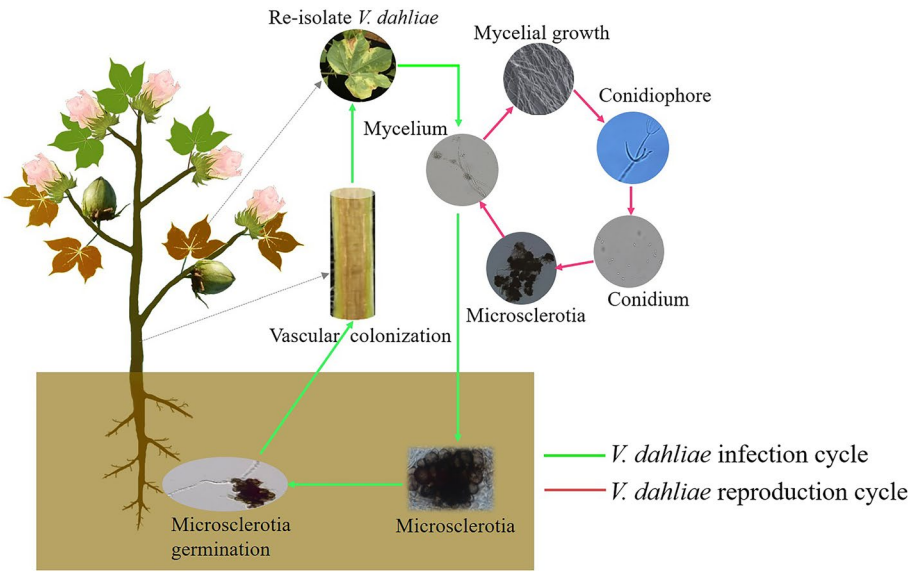

Fig. 1 A concise schematic diagram illustrating the infection and reproduction cycles of *V. dahliae* in cotton

cotton Verticillium wilt can provide valuable insights for effective management of this destructive fungus disease.

Genetic inheritance of cotton resistance to Verticillium wilt

Owning to the dynamic and coevolutionary nature of *V. dahliae* in cotton, significant variations exist in resistance levels across different cotton varieties. Furthermore, the use of various disease grading indicators and statistical methods, along with variations in disease conditions and stages, has led to different research findings. This has resulted in a lack of consensus on this matter (Li et al. 2022). To date, the primary point of contention lies in whether cotton resistance is governed by a singular or major gene, or if it is controlled by multiple micro-genes (Zhao et al. 2021). Additionally, the debate also explores whether disease resistance exhibits dominance or recessiveness in terms of susceptibility (Zhang et al. 2013). Inoculating a single CVW strain under controlled greenhouse or growth chamber conditions minimizes environmental impact and predominantly support the conclusion that resistance to Verticillium wilt is primarily determined by a single gene. Conversely, under field disease conditions, where *V. dahliae* strains are more complex and greatly influenced by the environment, resistance to Verticillium wilt demonstrates polygenic inheritance (Ma et al. 2000). It appears evident that the resistance of island cotton (*Gossypium barbadense*) to Verticillium wilt is mainly determined by a single gene, while demonstrating genetic inheritance associated with quality traits (Zhang et al. 2017). However, for upland cotton, resistance inheritance is more intricate. Typically, conducted inoculations using a single strain of *V. dahliae* in greenhouses tend to result in qualitative trait inheritance (Ma et al. 2000). On the other hand, when identifying resistance within field disease nurseries, quantitative trait inheritance prevails with additive effects being predominant approach utilized (Wang et al. 2012). Based on the aforementioned research reports, it is believed that the inheritance of resistance in upland cotton is significantly influenced by factors such as parental material selection, pathogen type, variations in identification conditions, and standardized disease grading criteria.

Development of molecular markers for cotton Verticillium wilt resistance

In recent years, the advancement of molecular marker technology has greatly facilitated the exploration of the genetic basis and resistance breeding of cotton against Verticillium wilt. Currently, an assortment of molecular markers including simple sequence repeat (SSR), random amplification polymorphism DNA (RAPD), sequence-related amplified polymorphism (SRAP), and amplified fragment length polymorphism (AFLP) are extensively employed in constructing of cotton genetic linkage maps (Zhu et al. 2017; Yuan et al. 2018). The establishment of molecular marker-linked genetic maps serves as a cornerstone for gene localization, cloning, and genomic research. Currently, numerous genetic linkage maps have been developed employing molecular marker technology in the study of cotton (Jiang et al. 2009; Zhao et al. 2021). Researchers have utilized a combination of major gene + polygene inheritance model and multi-generation joint analysis to investigate the genetic characteristics of cotton Verticillium wilt leaves ratio. These findings reveal that disease resistance is governed by two pairs of additive-dominant-epistatic major genes, with the primary genetic effect relying on major effect genes in each segregating generation (Wang et al. 2008). Through quantitative trait locus (QTL) mapping analysis, distinct clusters of disease-resistant QTLs have been identified on chromosomes D7 and D9 (Jiang et al. 2009). In upland cotton, 15 QTLs for Fusarium wilt resistance and 13 QTLs for Verticillium wilt resistance have also been unveiled (Abdelraheem et al. 2020). Institute of Cotton Research of Chinese Academy of Agricultural Sciences has employed segregating populations and natural populations to screen multiple QTLs associated with Verticillium wilt resistance via QTL mapping and association analysis (Zhao et al. 2017). The selection effects of these QTLs can be reliably inherited across different generations. Notably, a predominant QTL cluster linked to Verticillium wilt resistance in cotton was primarily located on chromosome 16 (Zhao et al. 2014, 2021). Furthermore, genome wide association study (GWAS) has identified three loci associated with Verticillium wilt resistance in cotton. Particularly noteworthy, *Gh_D06G0687*, encoding an NB-ARC protein domain, exhibited a significant association with Verticillium wilt resistance (Fang et al. 2017a). Another GWAS result revealed that loci on chromosomes A02, A03, A05, A06, A08, A12, D05, D07, D10, D11, and D12 were found to be linked to Verticillium wilt resistance in cotton. Among them, chromosome D11 harbored the candidate gene cluster of receptor kinases containing L-type lectins (*GhLecRKs-V.9*), which was of particular interest (Zhang et al. 2021). Furthermore, a TIR-NBS-LRR protein was identified as a potential candidate target for disease resistance through GWAS analysis; genome-edited plants showed reduced resistance against *V. dahliae* (Li et al. 2017). To sequence the genomes of a population consisting of 299 upland cotton germplasm resources effectively and accurately assessing their association with disease index of Verticillium wilt, the specific locus amplified fragment sequencing

(SLAF-seq) method was employed. Through the association analysis between approximately 85 000 SNPs and disease index of Verticillium wilt, 17 significantly associated loci were detected (Li et al. 2013).

Although numerous of QTLs associated with pivotal traits in upland cotton have been unveiled, the exploration of genomic interactions remains deficient (Li et al. 2014; Ma et al. 2018). The question of whether intricate epistatic relationships exist among QTLs responsible for disease resistance remains unresolved, and deciphering the functional implications of epistatic QTLs presents formidable challenges (Ma et al. 2018). Furthermore, solely QTLs with major effects have been discovered thus far, while minor effect QTLs largely remain enigmatic (Ma et al. 2021b; Zhao et al. 2023). Consequently, it becomes imperative to establish artificial populations, such as nested association mapping (NAM) and multi-parent advanced generation inter-cross (MAGIC) populations, for uncovering minor effect loci and investigating cumulative effects between loci, thereby delving into exceptional disease-resistant genes.

Functional interpretation of resistance genes in cotton

Upon invasion by *V. dahliae*, cotton undergoes a cascade of physiological and biochemical changes that contribute to the development of resistance in tissue structure. These changes include the thickening of cell walls, accumulation of suberin and lignin, burst of reactive oxygen species (ROS), and increased production of plant antimicrobial compounds, which partially impede the invasion and spread of *V. dahliae* within cotton plants (Chen et al. 2021b; Zhang et al. 2022b, 2022c). However, the pathogen can overcome cotton's physical defenses and cause infection (Liu et al. 2014; Ding et al. 2018). Cotton has evolved intricate mechanisms for disease resistance, primarily involving immune responses regulated by disease-resistant related proteins, extracellular enzymes, membrane receptor proteins, transcription factors, and signaling pathways (Jones et al. 2006; Song et al. 2020; Yang et al. 2020). In recent years, genomics, transcriptomics, and proteomics have provided valuable platforms for dissecting pivotal factors involved in regulating Verticillium wilt resistance in cotton as well as exploring candidate genes (Zhang et al. 2019a; Mo et al. 2021). These key disease-resistance genes are the main players against *V. dahliae*, and their roles are well-established (Table 1).

To date, researchers employed *V. dahliae* to infect various resistant cotton varieties and observed significant disparities in the root phosphoproteome between resistant and susceptible cultivars, with involvement in multiple defense-related metabolic pathways such as lignin biosynthesis, reactive oxygen species generation, and signaling hormones regulation (Mo et al. 2015; Zhang et al. 2016b, 2019b). Subsequent analysis revealed that succinate dehydrogenase GhSDH1-1 and ribosomal protein RPS6 positively regulated cotton's resistance to Verticillium wilt through the salicylic acid (SA) pathway (Zhu et al. 2021). Furthermore, GhWAKL was found to interact with DnaJ protein to activate SA biosynthesis, but mutationed at the Ser 628 site could disrupt this interaction, resulting in weakening cotton's resistance (Feng et al. 2021). These findings provide potential molecular targets and pathways for controlling Verticillium wilt and inducing resistance in cotton. As a hemibiotrophic pathogen, *V. dahliae* exhibits biotrophic tendencies during the early stage of infection, transitioning into a necrotrophic lifestyle as the infection progresses. Thus, the SA signaling pathway was required to confer resistance against *V. dahliae* (Dhar et al. 2020). Numerous plant hormones orchestrate the formation of vascular bundle cell walls to combat pathogen invasion and spread. Among these hormones, ethylene (ET) participates in lignin and suberin biosynthesis, while abscisic acid (ABA) coordinated suberin deposition, jasmonic acid (JA) aided in lignin biosynthesis, with SA counteracting JA's inducing effect on lignin production (Mo et al. 2016; Wang et al. 2020). Moreover, *GhWRKY41* positively regulated the cotton resistance to *V. dahliae* by enhancing the accumulation of flavonoids (Xiao et al. 2023). Besides, the miR530-*GhSAP6* module in cotton leaves responded remotely to *V. dahliae* infection from roots via systemic acquired resistance, and then enlarged SA signaling at locations farther from the injection sites, leading to enhanced resistance of cotton plants to *V. dahliae* (Hu et al. 2023). By analyzing the transcriptome of *G. barbadense* after inoculation with *V. dahliae*, genes involved in the "SA→NPR1→TGA→PR-1→disease resistance" signaling pathway were elucidated, leading to the discovery of numerous defense gene sequences against Verticillium wilt in cotton (Zhang et al. 2013). Through proteomics and virus-induced gene silencing (VIGS) technology, it was revealed that gossypol, brassinosteroids (BR), and JA play a pivotal role in cotton's resistance to Verticillium wilt (Gao et al. 2013). Furthermore, *GbERF1*-like genes could induce upregulation of resistance-related genes, regulate lignin accumulation, and enhance cotton's resistance to Verticillium wilt (Guo et al. 2016). Overexpression of the pathogenesis-related protein gene *GbPR10.5D1* in cotton enhanced the resistance to *V. dahliae*, accompanied by the activation of genes involved in suberin biosynthesis (Guo et al. 2022). Moreover, the analysis of the diploid cotton A2 and D5 subgenomes, along with the tetraploid upland cotton A2D5 genome, has unveiled the crucial role of the cotton NBS-LRR gene family in Verticillium wilt resistance (Li

Table 1 Genes for Verticillium wilt resistance in cotton

Gene name	Annotation	Regulatory mechanism	Cotton species	Disease-resistance characteristics	References
NPR1	Non-expressor of pathogenesis-related genes-1	Elicited systemic acquired resistance	*G. hirsutum*	Increased resistance to *V. dahliae* strain TS2	Parkhi et al. 2010
GhPAO	Polyamine oxidase	Mediation of spermine (Spm) and camalexin signalling	*G. hirsutum*	Increased resistance to *V. dahliae*	Mo et al. 2015
GbNRX1	Thioredoxin	Maintaining ROS balance	*G. barbadense*	Resistance to *V. dahliae* strain V991	Li et al. 2016
HDTF1	Homeodomain transcription factor	Negative regulator of jasmonic acid (JA) pathway	*G. hirsutum*	Enhanced susceptibility to *V. dahliae* strain Vd991	Gao et al. 2016
GhSAMDC	S-adenosylmethionine decarboxylase	Mediated spermine biosynthesis and salicylic acid (SA)- and leucine-correlated signaling	*G. hirsutum*	Increased resistance to *V. dahliae*	Mo et al. 2016
GbEDS1	Enhanced disease susceptibility 1	Regulating SA level and H_2O_2 accumulation	*G. barbadense*	Increased resistance to *V. dahliae*	Zhang et al. 2016
GaRPL18	Ribosomal protein L18	Involved in SA signal transduction pathway	*G. arboreum*	Increased resistance to *V. dahliae* strain Vd07038	Gong et al. 2017
GhHB12	Homeodomain-leucine zipper	Suppressed JA defense signaling	*G. hirsutum*	Enhanced susceptibility to *V. dahliae*	He et al. 2018
GhLAC15	Contained conserve domains of laccases	Induced lignification and arabinose and xylose accumulation	*G. hirsutum*	Increased resistance to *V. dahliae*	Zhang et al. 2019a
GhBLH7-D06	BEL1-like transcription factor	Suppressed t JA defense signaling	*G. hirsutum*	Increased resistance to *V. dahliae* V991	Ma et al. 2020
GhWAKL	Plant cell wall-associated receptor-like kinases	Promoted SA biosynthesis	*G. hirsutum*	Increased resistance to *V. dahliae* strain Vd080	Feng et al. 2021
GhRPS6	Ribosomal protein S6	Activated the SA and JA signaling	*G. hirsutum*	Increased resistance to *V. dahliae* strain Vd080	Zhu et al. 2021
GhBIN2	Protein kinase	Reduced JA content and suppressed the JA signaling	*G. hirsutum*	Enhanced susceptibility to *V. dahliae* strain Vd07038	Song et al. 2021
GhnsLTPsA10	Non-specific lipid transfer protein	Mediated phenylpropanoid metabolism and affected the balance of the downstream metabolic flux of flavonoid and lignin biosynthesis	*G. hirsutum*	Increased resistance to *V. dahliae* strain LX2-1 and V991	Chen et al. 2021
GhRbohD	Respiratory burst oxidase homolog protein D	Activated ROS production	*G. hirsutum*	Increased resistance to *V. dahliae* strain Vd080	Huang et al. 2021
GhSSI2	Conserved stearoyl-ACP desaturase family protein	Activated SA and JA signaling	*G. hirsutum*	Increased resistance to *V. dahliae* strain Linxi 2-1	Mo et al. 2021
GbPR10.5D1	Pathogenesis-related protein	Involved in suberin biosynthesis	*G. barbadense*	Increased resistance to *V. dahliae* V592	Guo et al. 2022
GhWRKY41	WRKY proteins	Modulating the accumulation of lignin and flavonoids	*G. hirsutum*	Increased resistance to *V. dahliae* strain V991 and V592	Xiao et al. 2023
GhSAP6	Stress-associated proteins	Induced systemic acquired resistance	*G. hirsutum*	Increased resistance to *V. dahliae* strain V991	Hu et al. 2023
GbCCD7 and *GbCCD8b*	Carotenoid cleavage dioxygenase	Regulated the accumulation of strigolactone and consequently activated JA and abscisic acid (ABA) signaling	*G. barbadense*	Increased resistance to *V. dahliae* V991	Yi et al. 2023
GhRFS6	Galactosyltransferase	With glycosyltransferase activity and regulation of α-galactosidase activity and raffinose and inositol synthesis	*G. hirsutum*	Increased resistance to *V. dahliae* strain Vd076 and V991	Chang et al. 2023

et al. 2015). Most of the apoplastic proteins secreted by the root in cotton were involved in ROS metabolism and defense response. In a study conducted the silencing and overexpression of the *GbNRX1* gene demonstrated its regulator role in maintaining ROS balance and enhancing cotton's resistance to this disease (Li et al. 2016). Through the utilization of VIGS and transgenic *Arabidopsis*, Gong et al. (2017) revealed that the pivotal role of ribosomal protein GaRPL18 in regulating cotton's resistance to Verticillium wilt through the SA signaling pathway, thereby bolstering its disease resistance; the carotenoid cleavage dioxygenases GbCCD7 and GbCCD8b positively regulated the accumulation of strigolactone, and consequently activated the JA and ABA signaling. This interplay establishes a positive feedback loop involving ABA and a negative feedback loop associated with JA, which collaboratively regulate the homeostasis of strigolactone. Such intricate hormonal balance enhances the resilience of cotton against *V. dahliae* (Yi et al. 2023). Additionally, several genes with negative regulatory effects on plant resistance to Verticillium wilt have been identified. For instance, Song et al. (2021) discovered that GhBIN2 interacted with JAZ proteins to exert a negative regulation on plant resistance. Furthermore, a series of transcription factors including HDTF1, GhHB12, and GhBLH7-D06 were found to exhibit negative regulatory effects as well (Gao et al. 2016; He et al. 2018; Ma et al. 2020). A recent study revealed that GhnsLTPsA10 coordinated plant resistance against this fungus and pests by redirecting the metabolic flux in flavonoid biosynthesis and lignin biosynthesis pathways through the regulation of phenylpropane metabolism (Chen et al. 2021a). This elucidation of functional genes related to resistance serves as the foundation for comprehending the molecular mechanisms underlying cotton resistance and provides an array of high-quality candidate genes for transgenic disease-resistant breeding programs.

Functional annotation of pathogenic genes in *V. dahliae*

The molecular pathogenic mechanism of *V. dahliae* is highly intricate and meticulously connected to a myriad of genes associated with pathogenicity (Cai et al. 2018; Bui et al. 2019). Recently, scholars have extensively investigated the pathogenic-related genes of *V. dahliae* from various perspectives such as genomics, transcriptomics, proteomics, and T-DNA mutant libraries, yielding substantial advancements (Liu et al. 2015b; Qi et al. 2015; Rehman et al. 2016; Wu et al. 2019). Functional analysis of pivotal genes implicated in fungal growth and pathogenicity provides the molecular genetic foundation for comprehending the pathogenic mechanism of *V. dahliae* (Li et al. 2015; Zhang et al. 2016b). Although genes related to growth and development are not inevitably entwined with pathogenicity, those associated with pathogenicity exhibit some degree of correlation with nutritional growth (Luo et al. 2014; Zhang et al. 2015). The remarkable ability of this fungus to finely adapt to the oligotrophic conditions within vascular bundles has captivated the scientific community (Fang et al. 2017b, 2019; Lv et al. 2022). Comparative genomics investigations have uncovered that unlike other non-vascular bundle pathogenic fungi, the genome of *V. dahliae* has undergone intricate modifications to evolve a sophisticated network for degrading plant cell walls, encoding a staggering total of 504 carbohydrate-degrading enzymes. Among these, the members of the pectinase family (including PL1, PL3, and PL9 families) have experienced substantial expansion compared with other plant pathogenic fungi. This expansion directly correlated with the functional prerequisites for degrading host cells rich in pectin during the infection and colonization processes, facilitating entry into vascular bundles and reproduction within them (Klimes et al. 2008, 2015; Chen et al. 2016). Consequently, the expansion of gene family encoding cell wall degradation enzymes and their extracellular secretion by *V. dahliae* represent a significant adaptive strategy for thriving in the oligotrophic environment of vascular bundles (Eynck et al. 2007; El Hadrami et al. 2015). This capacity is further underscored through the involvement of numerous cell wall-degrading enzymes in the infection and colonization processes of *V. dahliae*, thereby exerting a profound influence on the pathogenicity of this pathogen. For instance, VdSSP1, a glycoside hydrolase, contributed to the pathogenicity of *V. dahliae* by breaking down plant cell wall components (Liu et al. 2013); VdEg-1 cellulase played a critical role in early colonization of host wood (Maruthachalam et al. 2011); GH12 (glycoside hydrolase) family proteins such as VdEG1 and VdEG3 acted as pathogenic factors in cotton by actively participating in cellulose degradation (Gui et al. 2017); and the xylanase member VdXyn4 has been definitively implicated in the breakdown of xylan, pectin, and cellulose while instigating collapse of vascular bundle tissue (Cheng et al. 2017; Wang et al. 2021a). Furthermore, multiple signaling factors involved in the regulating cell wall-degrading enzymes also partake in the degradation and utilization of plant cell wall components by *V. dahliae* during infection and colonization processes within vascular bundles. These factors encompass the sucrose non-fermenting protein kinase VdSNF1, transcription factor VdFTF1, and the target of rapamycin protein VdTOR (Tzima et al. 2011; Zhang et al. 2018; Li et al. 2019). The absorption and utilization of nitrogen sources by *V. dahliae* in the oligotrophic environment of vascular bundles played a pivotal role in

its infection and colonization processes. Transcriptome analysis has unveiled distinct regulatory mechanisms employed by *V. dahliae* to discriminate between nitrogen and nitrate utilization, with transcription factors such as the MADS-box transcription factor VdMcm1 and the basic leucine zipper (bZIP) transcription factor VdHapX intricately involved in this intricate process (Xiong et al. 2016; Wang et al. 2018). Furthermore, another bZIP transcription factor VdAtf1 has been discovered to be implicated in response to active nitrogen and regulation of metabolic equilibrium of nitrate and ammonium within *V. dahliae* (Tang et al. 2020). Additionally, *V. dahliae* commonly activates its own pathway for amino acid synthesis in response to the environmental stress caused by nitrogen source deficiency within vascular conduits. This functional mechanism has been validated in the closely related species *Verticillium longisporum*, wherein the transcription factor VlCPC1 played a role in amino acid synthesis, removal of which substantially impeded growth under an amino acid-depleted environment (Timpner et al. 2013). Similarly, these functional genes contribute to vital biological processes that facilitate *V. dahliae*'s adaptation to the nutritionally impoverished milieu within vascular conduits, thereby dictating its colonization and proliferation capabilities within such conduit systems. Consequently, the deletion of those genes led to a marked decrease in the pathogenicity of *V. dahliae*, which was evidenced by varying degrees of pathogenic loss (Zhang et al. 2022a; Wen et al. 2023).

In summary, *V. dahliae* employs a series of signal perception and transduction events to generate specialized structures, such as hyphopodia and infection pegs, facilitating its infiltration into the host and subsequent exploration of vascular bundles (Tran et al. 2014; Zhao et al. 2016; Harting et al. 2020). Simultaneously, it secretes an abundant array of effector proteins and enzymes that degrade the cell wall, thereby carrying out its pathogenic functions (Qi et al. 2016; Qin et al. 2020). Moreover, it engages diverse strategies to adapt to environmental adversity and nutritional scarcities within vascular bundles, thus promoting its growth and propagation (Xiong et al. 2015; Bui et al. 2019; Yang et al. 2020). Collectively, these functional genes constitute the molecular basis for the successful establishment and acclimatization of *V. dahliae* within vascular bundles (Table 2).

The interplay between cotton and *V. dahliae*

Jones et al. (2006) proposed the 'zigzag' model, which presents a systematic interpretation of the dynamic balance between plants and pathogens in the ongoing arms race involving disease resistance and pathogenesis. Plants defend against pathogen invasion by recognizing pathogen-associated molecular patterns (PAMPs) via receptor proteins, thereby triggering PAMPs-triggered immunity (PTI) (Zhou et al. 2017; Gao et al. 2019). Furthermore, plants engaged more intricate immune responses, known as effector-triggered immunity (ETI), by specifically identifying effector proteins secreted by pathogenic fungi using NBS-LRR class proteins (Yang et al. 2010; Leach et al. 2017; Hua et al. 2018). In order to elude or suppress immune responses, pathogens have undergone evolutionary adaptations resulting in a diverse array of effector proteins to successfully infiltrate plants (Gao et al. 2020; Zhu et al. 2023).

Fortunately, cotton has gradually developed its own innate defense system through the protracted coexistence, competition, and coevolution with the fungi pathogen (Yang et al. 2017). The intricate molecular mechanism underlying cotton's resistance to Verticillium wilt is not solely reliant on the functionality of cotton disease resistance genes, but also hinges on the interplay between plant and pathogen (Huang et al. 2021; Zhang et al. 2022a) (Fig. 2). Plant ubiquitin ligases PUBs (PUB25 and PUB26) exerted a negative regulatory control over plant disease resistance. These PUBs not only interacted with VDAL (Asp f 2-like protein) in *V. dahliae*, but also engaged with the plant transcription factor MYB6. As an effector protein, VDAL competed with PUBs to shield MYB6 from ubiquitination degradation, thereby accumulating MYB6 to orchestrate plant resistance against *V. dahliae* effectively (Ma et al. 2021a). Plant calcium-binding protein CBP60g possessed a calcium-binding domain and positively modulated plant resistance to Verticillium wilt while relinquishing its transcriptional activity upon interaction with VdSCP41 protein secreted by *V. dahliae*. *Arabidopsis* mutants lacking CBP60g displayed diminished resistance to this pathogen (Qin et al. 2018). The secreted endopolygalacturonase VdEPG1 positively governed the pathogen's virulence by intricately modulating its growth and development, and effectively suppressed the host immune response elicited by NLP1/INF1. During *V. dahliae* infection process in host, GhOPR9, a plant oxophytodienoate reductase involved in the JA synthesis pathway, interfaced with VdEPG1 on the cell membrane to induce a robust plant immune response that hindered *V. dahliae* infection (Liu et al. 2020, 2023; Chang et al. 2023). The recent study has divulged that GhCML41 through phosphoproteomics analysis, which is a calcium-like protein, positively regulated plant resistance against Verticillium wilt; suppression of the *GhCML41* gene led to diminished lignin synthesis, reduced suberin deposition, lower SA content in cotton leaves, but elevated JA content; the introduction of *GhCML41* into *Arabidopsis* augmented resistance against *V. dahliae* (Zhao 2019). Additionally, the interaction between *V. dahliae*

Table 2 Genes related to pathogenicity in *V. dahliae*

Gene name	Annotation	Regulatory mechanism	Pathogen strain	Pathogenic characteristics	References
VDH1	A class II hydrophobin	Mediated the development of microsclerotia from conidiophores and other hyphal structures	Dvd-T5	Positively regulated the pathogenicity	Klimes et al. 2008
VdEg-1	Endoglucanase-1	Regulating the growth	VdLs17	Positively regulated the pathogenicity	Maruthachalam et al. 2011
VdSNF1	Sucrose non-fermenting-protein kinase	Regulating the degradation of plant cell walls	70wt-r1	Positively regulated the pathogenicity	Tzima et al. 2011
VdSSP1	Specific secreted protein	Regulating the degradation of plant cell walls	VdG1	Positively regulated the pathogenicity	Liu et al. 2013
VlCPC1	Regulator for amino acid biosynthesis	Induced the cross-pathway control of amino acid biosynthesis	VdJR2	Positively regulated the pathogenicity	Timpner et al. 2013
Vta2	Transcription activator of adhesion	Required for fungal growth and conidiation, controled host-plant root infection and H_2O_2 detoxification	VdJR2, Vd52, Vd73 and Va4	Positively regulated the pathogenicity	Tran et al. 2014
VdQase	Cupin domain-containing protein	Involved in the catabolism of quercetin and interference with signaling	Vd9 and Vd21	Positively regulated the pathogenicity	Hadrami et al. 2015
VdMcm1	MADS-box transcription factor	Regulator of conidiation, microsclerotia formation and involved in cell wall integrity	XS11	Positively regulated the pathogenicity	Xiong et al. 2016
VdThit	Thiamine transporter protein	Involved in vegetative growth, reproduction, and invasive hyphal growth	V991	Positively regulated the pathogenicity	Qi et al. 2016
VdNoxB/VdPls1	Tetraspanin, catalytic subunit of membrane-bound NADPH oxidases	Ca^{2+} elevation in hyphopodia, regulated penetration peg formation	V592	Positively regulated the pathogenicity	Zhao et al. 2016
VdEG1 and *VdEG3*	Glycoside hydrolase 12 proteins	Trigger PTI in different ways and together with CBM1-containing proteins manipulate plant immunity	V991	Positively regulated the pathogenicity	Gui et al. 2017
VDECH	Endochitinase	Exhibiting efficient chitinolytic activity	Vd080	Positively regulated the pathogenicity	Cheng et al. 2017
VdFTF1	Transcription factor containing fungal trans domain	Regulating normal vegetative growth, mycelial pigmentation and conidial morphology	V991	Positively regulated the pathogenicity	Zhang et al. 2018
VdHapX	bZip transcription factor	Mediated adaptation to iron starvation and excesses, affects microsclerotium formation	XS11	Positively regulated the pathogenicity	Wang et al. 2018
VdSCP41	Intracellular effector	Directly targeted plant transcription factors to inhibit immunity	V592	Positively regulated the pathogenicity	Qin et al. 2018
VdTOR	Rapamycin binding protein, target of rapamycin	Associated with cell wall degrading enzymes and blocked in the presence of rapamycin	V991	Positively regulated the pathogenicity	Li et al. 2019
Som1 and *Vta3*	Nuclear transcription factors	Required for root penetration and colonisation of the plant host	VdJR2	Positively regulated the pathogenicity	Bui et al. 2019

Table 2 (continued)

Gene name	Annotation	Regulatory mechanism	Pathogen strain	Pathogenic characteristics	References
VdCmr1	Transcription factor	Required for the melanin production and increased survival following UV irradiation	VdLs.17	Positively regulated the pathogenicity	Fang et al. 2019
VdAtf1	bZIP transcription factor	Regulation of nitrosative resistance and nitrogen metabolism	XS11	Positively regulated the pathogenicity	Tang et al. 2020
Vta1	Transcription activator of adhesion 1	Dispensable for root colonization, infection and microsclerotia development	VdJR2	Positively regulated the pathogenicity	Harting et al. 2020
VdTHI20	Thiamine biosynthesis	Involved in vegetative growth and conidiation	V991	Positively regulated the pathogenicity	Qin et al. 2020
VdXyn4	Xylanase	Degraded the plant cell wall and induced immune signaling (in SA-JA pathways)	V991	Positively regulated the pathogenicity	Wang et al. 2021
VDAL	Asp f 2-like protein	As effector protein, competed with MYB6 for binding to PUBs	V991	Positively regulated the pathogenicity	Ma et al. 2021
VdM35-1 and *VdASPF2*	Metalloproteases	Relative to stress adaptation and activated plant immune response	Vd080	Positively regulated the pathogenicity	Lv et al. 2022
VdHP1	Novel hydrophobic protein	Required for development and adaptability	Vd080	Negatively regulated the pathogenicity	Zhang et al. 2022
VdEPG1	Endopolygalacturonase	Regulating mycelial growth, penetration ability, and stress response capability	Vd080	Positively regulated the pathogenicity	Liu et al. 2023
VdGAL4	Glycoside hydrolase family 27	Mediated conidial morphology and yield, formation of microsclerotia, and mycelial penetration	Vd080	Positively regulated the pathogenicity	Wen et al. 2023

VdM35-1, VdASPF2, and cotton GhCML41 through the Yeast two-hybrid system and further analysis revealed that the two genes belong to the metalloproteases M35 family of pathogenic fungi; VdM35-1 and VdASPF2 have been identified as secreted proteins of *V. dahliae*; deletion of those genes resulted in diminished spore production, stunted hyphal branch growth, abnormal spores, and impaired carbon source utilization, significantly attenuating the pathogenicity of this pathogen to host cotton (Lv et al. 2022). Moreover, glutathione-s-transferse (GST) pull-down and luciferase complementation imaging (LCI) technologies have confirmed the interaction between GhCML41 and the *V. dahliae* metalloproteases VdM35-1 and VdASPF2. These investigations provide novel insights into the mechanisms underpinning plant disease resistance while fortifying our theoretical understanding of molecular interactions between plants and pathogens.

Problem and prospect

Cotton, as a tetraploid crop, possesses an intricate genome structure and gene regulatory network (Wang et al. 2021b). With advancements in the field of life sciences, molecular biology, and technologies in cell biology such as *in situ* Hi-C, chromatin conformation capture sequencing, and three-dimensional genome features, a wealth of functional information is now available for a molecular-based disease-resistant breeding program (Pei et al. 2022). Therefore, elucidating the functional roles of non-coding genomic sequences, such as *cis*-regulatory elements, enhancers, and transcribed RNA elements, alongside protein-coding genes, will furnish crucial resources for the development of genomic-assisted breeding programs.

Verticillium wilt, an archetypal soil-borne vascular systemic disease, poses a formidable challenge in terms of field control. Breeding resistant varieties stands out as the most economically viable countermeasure against this disease (Bai et al. 2022b). Significant progress has

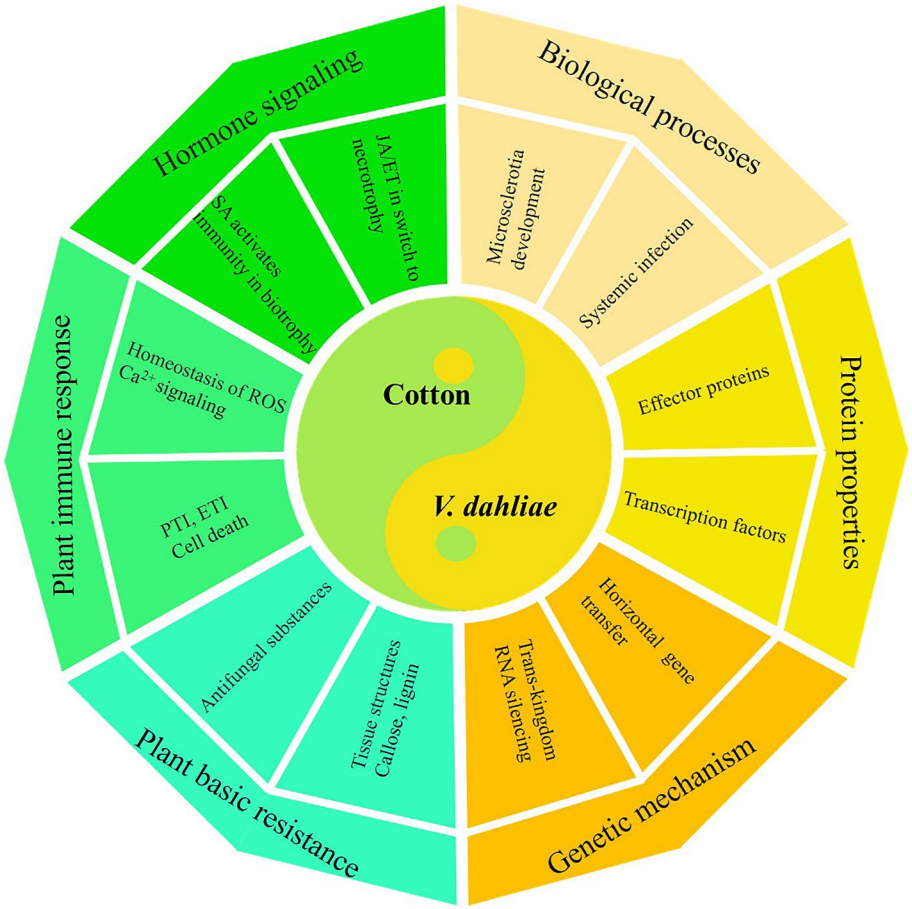

Fig. 2 Strategy diagram of cotton and *V. dahliae* in the arms race between host and pathogen. The diagram presents the ancient Chinese symbol of Taiji, indicating that the attack of *V. dahliae* and the defense of cotton are systematic and complex, with a synergistic evolutionary relationship. *V. dahliae* has evolved a sophisticated pathogenicity mechanism to infect cotton, a cascade of reaction involving microsclerotia development, effector proteins, transcription factors, etc.; on the contrary, cotton has taken a series of defensive measures, including plant basic resistance, PTI/ETI, hormone signaling, etc.

been made in molecular markers, signal transduction pathways, tissue structural resistance, and transgenic breeding for improving cotton Verticillium wilt resistance (Parkhi et al. 2010). Nevertheless, progress in breeding disease-resistant cotton varieties has been slow due to the lack of highly resistant upland cotton germplasm, unclear genetic basis and resistance mechanism, and their linkage with undesirable agronomic traits. As a result, the majority of developed varieties display weak disease resistance (Zhang et al. 2016a). Moreover, the resistance of certain tolerant varieties rapidly wanes after introduction into production due to frequent pathogen variation (Ma et al. 2000). Molecular designed breeding is expected to emerge as the pivotal avenue for future cotton breeding endeavors. The ongoing completion of the diploid A2 and D5 subgenomes of cotton, as well as sketches of the tetraploid upland cotton A2D5 genome, is expected to expedite the selection process for high-quality disease-resistant varieties (Li et al. 2015).

Furthermore, a synergistic coevolution between plants and pathogens exists in nature. Consequently, the elucidation of plant defense mechanisms depends not only on the acquiring of plant resistance genes and deciphering of signaling pathways but also on the understanding pathogen virulence and variation mechanisms (Yang et al. 2020; Zhu et al. 2023). Therefore, studying the interaction between cotton and *V. dahliae* is of paramount importance. Timely communication between these two aspects is essential to unravel the mechanisms behind cotton's resistance to Verticillium wilt and to achieve sustainable, effective control over its incidence in cotton production.

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Authors' contributions
Conceptualization: Ma ZY, Yang J, and Zhu HQ; Wrting-original draft: Zhang YL, Zhao LH, and Li DP; Writing-review: Li ZM, Feng HJ, Feng ZL, Wei F, and Zhou JL.

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Data availability
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Declarations

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References

Abdelraheem AH, Elassbli Y, Zhu V, et al. A genome-wide association study uncovers consistent quantitative trait loci for resistance to Verticillium wilt and Fusarium wilt race 4 in the US upland cotton. Theor Appl Genet. 2020;133:563–77. https://doi.org/10.1007/s00122-019-03487-x.

Bai HY, Feng ZL, Zhao LH, et al. Efficacy evaluation and mechanism of *Bacillus subtilis* EBS03 against cotton Verticillium wilt. J Cotton Res. 2022;5:26. https://doi.org/10.1186/s42397-022-00134-7.

Bai HY, Zhao LH, Pu DD, et al. The control effect and mechanism of *Bacillus velezensis* EBV02 on cotton Verticillium wilt. Cotton Sci. 2022b;34:443–57. https://doi.org/10.11963/cs20220019 (in Chinese with English abstract).

Bui TT, Harting R, Braus-Stromeyer SA, et al. *Verticillium dahliae* transcription factors Som1 and Vta3 control microsclerotia formation and sequential steps of plant root penetration and colonisation to induce disease. New Phytol. 2019;221:2138–59. https://doi.org/10.1111/nph.15514.

Cai Q, Qiao LL, Wang M, et al. Plants send small RNAs in extracellular vesicles to fungal pathogen to silence virulence genes. Science. 2018;360:1126–9. https://doi.org/10.1126/science.aar4142.

Chang BY, Zhao LH, Feng ZL, et al. Galactosyltransferase GhRFS6 interacting with GhOPR9 involved in defense against Verticillium wilt in cotton. Plant Sci. 2023;328:111582. https://doi.org/10.1016/j.plantsci.2022.111582.

Chen JY, Dai XF. Research advance on the resistant mechanism of cotton against Verticillium wilt. Mol Plant Breed. 2005;3(3):427–35 (in Chinese with English abstract).

Chen JY, Xiao HL, Gui YJ, et al. Characterization of the *Verticillium dahliae* exoproteome involves in pathogenicity from cotton-containing medium. Front Microbiol. 2016;7:1709. https://doi.org/10.3389/fmicb.2016.01709.

Chen B, Zhang Y, Sun ZW, et al. Tissue-specific expression of GhnsLTPs identified via GWAS sophisticatedly coordinates disease and insect resistance by regulating metabolic flux redirection in cotton. Plant J. 2021;107:831–46. https://doi.org/10.1111/tpj.15349.

Chen JY, Klosterman SJ, Hu XP, et al. Key insights and research prospects at the dawn of the population genomics era for *Verticillium dahliae*. Annu Rev Phytopathol. 2021;59:31–51. https://doi.org/10.1146/annurev-phyto-020620-121925.

Cheng XX, Zhao LH, Klosterman SJ, et al. The endochitinase VDECH from *Verticillium dahliae* inhibits spore germination and activates plant defense responses. Plant Sci. 2017;259:12–23. https://doi.org/10.1016/j.plantsci.2017.03.002.

Dhar N, Chen JY, Subbarao KV, et al. Hormone signaling and its interplay with development and defense responses in *Verticillium*-plant interactions. Front Plant Sci. 2020;11: 584997. https://doi.org/10.3389/fpls.2020.584997.

Ding PT, Redkar A. Pathogens suppress host transcription factors for rampant proliferation. Trends Plant Sci. 2018;23:950–3. https://doi.org/10.1016/j.tplants.2018.08.010.

El Hadrami A, Islam MR, Adam LR, et al. A cupin domain-containing protein with a quercetinase activity (VdQase) regulates *Verticillium dahliae*'s pathogenicity and contributes to counteracting host defenses. Front Plant Sci. 2015;6:440. https://doi.org/10.3389/fpls.2015.00440.

Eynck C, Koopmann B, Grunewaldt-Stoecker G, et al. Differential interactions of *Verticillium longisporum* and *V. dahliae* with *Brassica napus* detected with molecular and histological techniques. Eur J Plant Pathol. 2007;118:259–74. https://doi.org/10.1007/s10658-007-9144-6.

Fang L, Wang Q, Hu Y, et al. Genomic analyses in cotton identify signatures of selection and loci associated with fiber quality and yield traits. Nat Genet. 2017;49:1089–98. https://doi.org/10.1038/ng.3887.

Fang YL, Xiong DG, Tian LY, et al. Functional characterization of two bZIP transcription factors in *Verticillium dahliae*. Gene. 2017b;626:386–94. https://doi.org/10.1016/j.gene.2017.05.061.

Fang YL, Klosterman SJ, Tian CM, et al. Insights into VdCmr1-mediated protection against high temperature stress and UV irradiation in *Verticillium dahliae*. Environ Microbiol. 2019;21:2977–96. https://doi.org/10.1111/1462-2920.14695.

Feng HJ, Li C, Zhou JL, et al. A cotton WAKL protein interacted with a DnaJ protein and was involved in defense against *Verticillium dahliae*. Int J Biol Macromol. 2021;167:633–43. https://doi.org/10.1016/j.ijbiomac.2020.11.191.

Gao F, Guo HS. The battle and pathogenic intervention between plant and soilborne pathogens. J Nat. 2020;42:499–504. https://doi.org/10.3969/j.issn.0253-9608.2020.00.001 (in Chinese).

Gao W, Long L, Zhu LF, et al. Proteomic and virus-induced gene silencing (VIGS) analyses reveal that gossypol, brassinosteroids, and jasmonic acid contribute to the resistance of cotton to *Verticillium dahliae*. Mol Cell Proteom. 2013;12:3690–703. https://doi.org/10.1074/mcp.M113.031013.

Gao W, Long L, Xu L, et al. Suppression of the homeobox gene *HDTF1* enhances resistance to *Verticillium dahliae* and *Botrytis cinerea* in cotton. J Integr Plant Biol. 2016;58:503–13. https://doi.org/10.1111/jipb.12432.

Gao F, Zhang BS, Zhao JH, et al. Deacetylation of chitin oligomers increases virulence in soil-borne fungal pathogens. Nat Plants. 2019;5:1167–76. https://doi.org/10.1038/s41477-019-0527-4.

Gong Q, Yang ZE, Wang XQ, et al. Salicylic acid-related cotton (*Gossypium arboreum*) ribosomal protein GaRPL18 contributes to resistance to *Verticillium dahliae*. BMC Plant Biol. 2017;17:59. https://doi.org/10.1186/s12870-017-1007-5.

Gui YJ, Chen JY, Zhang DD, et al. *Verticillium dahliae* manipulates plant immunity by glycoside hydrolase 12 proteins in conjunction with carbohydrate-binding module 1. Environ Microbiol. 2017;19:1914–32. https://doi.org/10.1111/1462-2920.13695.

Guo WF, Jin L, Miao YH, et al. An ethylene response-related factor, GbERF1-like, from *Gossypium barbadense* improves resistance to *Verticillium dahliae* via activating lignin synthesis. Plant Mol Biol. 2016;91:305–18. https://doi.org/10.1007/s11103-016-0467-6.

Guo J, Cao PH, Yuan LT, et al. Revealing the contribution of GbPR10.5D1 to resistance against *Verticillium dahliae* and its regulation for structural defense and immune signaling. Plant Genome. 2022;15:e20271. https://doi.org/10.1002/tpg2.20271.

Harting R, Höfer A, Tran VT, et al. The Vta1 transcriptional regulator is required for microsclerotia melanization in *Verticillium dahliae*. Fungal Biol. 2020;124:490–500. https://doi.org/10.1016/j.funbio.2020.01.007.

He X, Wang TY, Zhu W, et al. GhHB12, a HD-ZIP I transcription factor, negatively regulates the cotton resistance to *Verticillium dahliae*. Int J Mol Sci. 2018;19:3997. https://doi.org/10.3390/ijms19123997.

Hu G, Wang BT, Jia P, et al. The cotton miR530-SAP6 module activated by systemic acquired resistance mediates plant defense against *Verticillium dahliae*. Plant Sci. 2023;330:111647. https://doi.org/10.1016/j.plantsci.2023.111647.

Hua CL, Zhao JH, Guo HS. Trans-kingdom RNA silencing in plant-fungal pathogen interactions. Mol Plant. 2018;11:235–44. https://doi.org/10.1016/j.molp.2017.12.001.

Huang WT, Zhang YL, Zhou JL, et al. The respiratory burst oxidase homolog protein D (GhRbohD) positively regulates the cotton resistance to *Verticillium dahliae*. Int J Mol Sci. 2021;22(23):13041. https://doi.org/10.3390/ijms222313041.

Jiang F, Zhao J, Zhou L, et al. Molecular marker localization of resistance genes to Verticillium wilt in upland cotton. Acta Sci. 2009;39:849–61 (in Chinese).

Jones JD, Dangl JL. The plant immune system. Nature. 2006;444:323–9. https://doi.org/10.1038/nature05286.

Klimes A, Amyotte SG, Grant S, et al. Microsclerotia development in *Verticillium dahliae*: regulation and differential expression of the hydrophobin gene *VDH1*. Fungal Genet Biol. 2008;45:1525–32. https://doi.org/10.1016/j.fgb.2008.09.014.

Klimes A, Dobinson KF, Thomma BP, et al. Genomics spurs rapid advances in our understanding of the biology of vascular wilt pathogens in the genus *Verticillium*. Annu Rev Phytopathol. 2015;53:181–98. https://doi.org/10.1146/annurev-phyto-080614-120224.

Klosterman SJ, Atallah ZK, Vallad GE, et al. Diversity, pathogenicity, and management of *Verticillium* species. Annu Rev Phytopathol. 2009;47:39–62. https://doi.org/10.1146/annurev-phyto-080508-081748.

Leach JE, Triplett LR, Argueso CT, et al. Communication in the phytobiome. Cell. 2017;169:587–96. https://doi.org/10.1016/j.cell.2017.04.025.

Li H, Peng ZY, Yang XH, et al. Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. Nat Genet. 2013;45:43–50. https://doi.org/10.1038/ng.2484.

Li FG, Fan GY, Wang KB, et al. Genome sequence of the cultivated cotton *Gossypium arboreum*. Nat Genet. 2014;46:567–72. https://doi.org/10.1038/ng.2987.

Li FG, Fan GY, Lu CR, et al. Genome sequence of cultivated upland cotton (*Gossypium hirsutum* TM-1) provides insights into genome evolution. Nat Biotechnol. 2015;33:524–30. https://doi.org/10.1038/nbt.3208.

Li YB, Han LB, Wang HY, et al. The thioredoxin GbNRX1 plays a crucial role in homeostasis of apoplastic reactive oxygen species in response to *Verticillium dahliae* infection in cotton. Plant Physiol. 2016;170:2392–406. https://doi.org/10.1104/pp.15.01930.

Li TG, Ma XF, Li NY, et al. Genome-wide association study discovered candidate genes of Verticillium wilt resistance in upland cotton (*Gossypium hirsutum* L.). Plant Biotechnol J. 2017;15:1520–32. https://doi.org/10.1111/pbi.12734.

Li LX, Zhu TT, Song Y, et al. Functional characterization of target of rapamycin signaling in *Verticillium dahliae*. Front Microbiol. 2019;10:501. https://doi.org/10.3389/fmicb.2019.00501.

Li TG, Gong DY, Zhang QQ. Genetics of cotton resistance to Verticillium wilt: research progress. J Agric. 2022;12:31–6 (in Chinese with English abstract).

Liu SY, Chen JY, Wang JL, et al. Molecular characterization and functional analysis of a specific secreted protein from highly virulent defoliating *Verticillium dahliae*. Gene. 2013;529:307–16. https://doi.org/10.1016/j.gene.2013.06.089.

Liu TL, Song TQ, Zhang X, et al. Unconventionally secreted effectors of two filamentous pathogens target plant salicylate biosynthesis. Nat Commun. 2014;5:4686. https://doi.org/10.1038/ncomms5686.

Liu HY, Wang W, Zhang RF, et al. Occurrence research of cotton Verticillium wilt and the phylogenetic evolution analysis of *Verticillium dahliae* in Xinjiang. Xinjiang Agric Sci. 2015;52:65–71. https://doi.org/10.6048/j.issn.1001-4330.2015.01.011 (in Chinese with English abstract).

Liu YJ, Li ZF, Feng ZL, et al. Phenotypic analysis of low virulent *Verticillium dahliae* mutants on cotton and cloning of pathogenicity related genes. Acta Phytopathol Sin. 2015b;45:258–69. https://doi.org/10.13926/j.cnki.apps.2015.03.005 (in Chinese with English abstract).

Liu SC, Sun RB, Zhang XJ, et al. Genome-wide analysis of OPR family genes in cotton identified a role for GhOPR9 in *Verticillium dahliae* resistance. Genes. 2020;11:1134. https://doi.org/10.3390/genes11101134.

Liu SC, Liu RB, Lv JY, et al. The glycoside hydrolase 28 member VdEPG1 is a virulence factor of *Verticillium dahliae* and interacts with the jasmonic acid pathway-related gene *GhOPR9*. Mol Plant Pathol. 2023;24:1238–55. https://doi.org/10.1111/mpp.13366.

Luo XM, Xie CJ, Dong JY, et al. Interactions between *Verticillium dahliae* and its host: vegetative growth, pathogenicity, plant immunity. Appl Microbiol Biotechnol. 2014;98:6921–32. https://doi.org/10.1007/s00253-014-5863-8.

Lv JY, Zhou JL, Chang BY, et al. Two metalloproteases VdM35-1 and VdASPF2 from *Verticillium dahliae* are required for fungal pathogenicity, stress adaptation, and activating immune response of host. Microbiol Spectr. 2022;10:e0247722. https://doi.org/10.1128/spectrum.02477-22.

Ma ZY, He SP, Wang XF, et al. Resequencing a core collection of upland cotton identifies genomic variation and loci influencing fiber quality and yield. Nat Genet. 2018;50:803–13. https://doi.org/10.1038/s41588-018-0119-7.

Ma Q, Wang NH, Ma L, et al. The cotton BEL1-like transcription factor GhBLH7-D06 negatively regulates the defense response against *Verticillium dahliae*. Int J Mol Sci. 2020;21:7126. https://doi.org/10.3390/ijms21197126.

Ma AF, Zhang DP, Wang GX, et al. *Verticillium dahliae* effector VDAL protects MYB6 from degradation by interacting with PUB25 and PUB26 E3 ligases to enhance Verticillium wilt resistance. Plant Cell. 2021;33:3675–99. https://doi.org/10.1093/plcell/koab221.

Ma ZY, Zhang Y, Wu LQ, et al. High-quality genome assembly and resequencing of modern cotton cultivars provide resources for crop improvement. Nat Genet. 2021;53:1385–91. https://doi.org/10.1038/s41588-021-00910-2.

Ma ZY, Wang XF, Zhang GY, et al. Genetic studies of Verticillium wilt resistance among different types of sea island cottons. Acta Agron Sin. 2000;26(3):315–21 (in Chinese with English abstract).

Maruthachalam K, Klosterman SJ, Kang S, et al. Identification of pathogenicity-related genes in the vascular wilt fungus *Verticillium dahliae* by *Agrobacterium tumefaciens*-mediated T-DNA insertional mutagenesis. Mol Biotechnol. 2011;49:209–21. https://doi.org/10.1007/s12033-011-9392-8.

Mo HJ, Wang XF, Zhang Y, et al. Cotton polyamine oxidase is required for spermine and camalexin signalling in the defence response to *Verticillium dahliae*. Plant J. 2015;83:962–75. https://doi.org/10.1111/tpj.12941.

Mo HJ, Sun YX, Zhu XL, et al. Cotton S-adenosylmethionine decarboxylase-mediated spermine biosynthesis is required for salicylic acid- and leucine-correlated signaling in the defense response to *Verticillium dahliae*. Planta. 2016;243:1023–39. https://doi.org/10.1007/s00425-015-2463-5.

Mo SJ, Zhang Y, Wang XF, et al. Cotton GhSSI2 isoforms from the stearoyl acyl carrier protein fatty acid desaturase family regulate Verticillium wilt resistance. Mol Plant Pathol. 2021;22:1041–56. https://doi.org/10.1111/mpp.13093.

Palanga KK, Liu RX, Ge Q, et al. Current advances in pathogen-plant interaction between *Verticillium dahliae* and cotton provide new insight in the disease management. J Cotton Res. 2021;4:25. https://doi.org/10.1186/s42397-021-00100-9.

Parkhi V, Kumar V, Campbell LM, et al. Resistance against various fungal pathogens and reniform nematode in transgenic cotton plants expressing *Arabidopsis* NPR1. Transgenic Res. 2010;19:959–75. https://doi.org/10.1007/s11248-010-9374-9.

Pei LL, Huang XH, Liu ZP, et al. Dynamic 3D genome architecture of cotton fiber reveals subgenome-coordinated chromatin topology for 4-staged single-cell differentiation. Genome Biol. 2022;23:45. https://doi.org/10.1186/s13059-022-02616-y.

Qi XL, Su XF, Guo HM, et al. A ku70 null mutant improves gene targeting frequency in the fungal pathogen *Verticillium dahliae*. World J Microbiol Biotechnol. 2015;31:1889–97. https://doi.org/10.1007/s11274-015-1907-1.

Qi XL, Su XF, Guo HM, et al. VdThit, a thiamine transport protein, is required for pathogenicity of the vascular pathogen *Verticillium dahliae*. Mol Plant Microbe Interact. 2016;29:545–59. https://doi.org/10.1094/MPMI-03-16-0057-R.

Qin J, Wang KL, Sun LF, et al. The plant-specific transcription factors CBP60g and SARD1 are targeted by a *Verticillium* secretory protein VdSCP41 to modulate immunity. Elife. 2018;7:e34902. https://doi.org/10.7554/eLife.34902.

Qin TF, Hao W, Sun RR, et al. *Verticillium dahliae* VdTHI20, involved in pyrimidine biosynthesis, is required for DNA repair functions and pathogenicity. Int J Mol Sci. 2020;21:1378. https://doi.org/10.3390/ijms21041378.

Rehman L, Su XF, Guo HM, et al. Protoplast transformation as a potential platform for exploring gene function in *Verticillium dahliae*. BMC Biotechnol. 2016;16:57. https://doi.org/10.1186/s12896-016-0287-4.

Shaban M, Miao YH, Ullah A, et al. Physiological and molecular mechanism of defense in cotton against *Verticillium dahliae*. Plant Physiol Biochem. 2018;125:193–204. https://doi.org/10.1016/j.plaphy.2018.02.011.

Song RR, Li JP, Xie CJ, et al. An overview of the molecular genetics of plant resistance to the Verticillium wilt pathogen *Verticillium dahliae*. Int J Mol Sci. 2020;21:1120. https://doi.org/10.3390/ijms21031120.

Song Y, Zhai Y, Li L, et al. BIN2 negatively regulates plant defence against *Verticillium dahliae* in *Arabidopsis* and cotton. Plant Biotechnol J. 2021;19:2097–112. https://doi.org/10.1111/pbi.13640.

Tang C, Li TY, Klosterman SJ, et al. The bZIP transcription factor VdAtf1 regulates virulence by mediating nitrogen metabolism in *Verticillium dahliae*. New Phytol. 2020;226:1461–79. https://doi.org/10.1111/nph.16481.

Timpner C, Braus-Stromeyer SA, Tran VT, et al. The Cpc1 regulator of the cross-pathway control of amino acid biosynthesis is required for pathogenicity of the vascular pathogen *Verticillium longisporum*. Mol Plant Microbe Interact. 2013;26:1312–24. https://doi.org/10.1094/MPMI-06-13-0181-R.

Tran VT, Braus-Stromeyer SA, Kusch H, et al. *Verticillium* transcription activator of adhesion Vta2 suppresses microsclerotia formation and is required for systemic infection of plant roots. New Phytol. 2014;202:565–81. https://doi.org/10.1111/nph.12671.

Tzima AK, Paplomatas EJ, Rauyaree P, et al. *VdSNF1*, the sucrose nonfermenting protein kinase gene of *Verticillium dahliae*, is required for virulence and expression of genes involved in cell-wall degradation. Mol Plant Microbe Interact. 2011;24:129–42. https://doi.org/10.1094/MPMI-09-09-0217.

Wang HM, Lin ZX, Zhang XL, et al. Mapping and quantitative trait loci analysis of Verticillium wilt resistance genes in cotton. J Integr Plant Biol. 2008;50:174–82. https://doi.org/10.1111/j.1744-7909.2007.00612.x.

Wang GN, Zhao GY, Yue XW, et al. Pathogenicity and ISSR genetic differentiation of *Verticillium dahliae* isolates from cotton growing areas of Hebei Province. Cotton Sci. 2012;24:348–57 (in Chinese with English abstract).

Wang YL, Deng CL, Tian LY, et al. The transcription factor VdHapX controls iron homeostasis and is crucial for virulence in the vascular pathogen *Verticillium dahliae*. mSphere. 2018;3:e00400-18. https://doi.org/10.1128/mSphere.00400-18.

Wang CH, Wang H, Li PX, et al. Developmental programs interact with abscisic acid to coordinate root suberization in *Arabidopsis*. Plant J. 2020;104:241–51. https://doi.org/10.1111/tpj.14920.

Wang D, Chen JY, Song J, et al. Cytotoxic function of xylanase VdXyn4 in the plant vascular wilt pathogen *Verticillium dahliae*. Plant Physiol. 2021;187:409–29. https://doi.org/10.1093/plphys/kiab274.

Wang GN, Wang XF, Zhang Y, et al. Dynamic characteristics and functional analysis provide new insights into long non-coding RNA responsive to *Verticillium dahliae* infection in *Gossypium hirsutum*. BMC Plant Biol. 2021;21:68. https://doi.org/10.1186/s12870-021-02835-8.

Wen Y, Zhou JL, Feng HJ, et al. VdGAL4 modulates microsclerotium formation, conidial morphology, and germination to promote virulence in *Verticillium dahliae*. Microbiol Spectr. 2023;11(1):e03515-22. https://doi.org/10.1128/spectrum.03515-22.

Wu LY, Du GH, Bao R, et al. *De novo* assembly and discovery of genes involved in the response of *Solanum sisymbriifolium* to *Verticillim dahliae*. Physiol Mol Biol Plants. 2019;25:1009–27. https://doi.org/10.1007/s12298-019-00666-4.

Xiao SH, Ming YQ, Hu Q, et al. GhWRKY41 forms a positive feedback regulation loop and increases cotton defence response against *Verticillium dahliae* by regulating phenylpropanoid metabolism. Plant Biotechnol J. 2023;21(5):961–78. https://doi.org/10.1111/pbi.14008.

Xiong DG, Wang YL, Tian CM. Transcriptomic profiles of the smoke tree wilt fungus *Verticillium dahliae* under nutrient starvation stresses. Mol Genet Genomics. 2015;290:1963–77. https://doi.org/10.1007/s00438-015-1052-4.

Xiong DG, Wang YL, Tian LY, et al. MADS-Box transcription factor VdMcm1 regulates conidiation, microsclerotia formation, pathogenicity, and secondary metabolism of *Verticillium dahliae*. Front Microbiol. 2016;7:1192. https://doi.org/10.3389/fmicb.2016.01192.

Yang HB, Shi YT, Liu JY, et al. A mutant CHS3 protein with TIR-NB-LRR-LIM domains modulates growth, cell death and freezing tolerance in a temperature-dependent manner in *Arabidopsis*. Plant J. 2010;63:283–96. https://doi.org/10.1111/j.1365-313X.2010.04241.x.

Yang YH, Lu Y, Wu YJ, et al. Relationship between Verticillium wilt resistance and immune response in upland cotton. J Nucl Agric Sci. 2017;31:588–96. https://doi.org/10.00-8551(2017)03-0588-09 (in Chinese with English abstract).

Yang J, Wang XF, Xie MX, et al. Proteomic analyses on xylem sap provides insights into the defense response of *Gossypium hirsutum* against *Verticillium dahliae*. J Proteom. 2020;213:103599. https://doi.org/10.1016/j.jprot.2019.103599.

Yang ZE, Gao CX, Zhang YH, et al. Recent progression and future perspectives in cotton genomic breeding. J Integr Plant Biol. 2023;65:548–69. https://doi.org/10.1111/jipb.13388.

Yi FF, Song AS, Cheng K, et al. Strigolactones positively regulate Verticillium wilt resistance in cotton via crosstalk with other hormones. Plant Physiol. 2023;192:945–66. https://doi.org/10.1093/plphys/kiad053.

Yuan Y, Feng ZL, Li ZF, et al. Pathogenicity assay and evaluation of *Verticillium dahliae* on cotton. Acta Phytopathol Sin. 2018;48:248–55. https://doi.org/10.13926/j.cnki.apps.000124 (in Chinese with English abstract).

Zhang Y, Wang XF, Ding ZG, et al. Transcriptome profiling of *Gossypium barbadense* inoculated with *Verticillium dahliae* provides a resource for cotton improvement. BMC Genomics. 2013;14:637. https://doi.org/10.1186/1471-2164-14-637.

Zhang YL, Li ZF, Feng ZL, et al. Isolation and functional analysis of the pathogenicity-related gene *VdPR3* from *Verticillium dahliae* on cotton. Curr Genet. 2015;61:555–66. https://doi.org/10.1007/s00294-015-0476-z.

Zhang Y, Wang XF, Rong W, et al. Island cotton enhanced disease susceptibility 1 gene encoding a lipase-like protein plays a crucial role in response to *Verticillium dahliae* by regulating the SA level and H_2O_2 accumulation. Front Plant Sci. 2016;7:1830. https://doi.org/10.3389/fpls.2016.01830.

Zhang T, Zhao YL, Zhao JH, et al. Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. Nat Plants. 2016;2:16153. https://doi.org/10.1038/nplants.2016.153.

Zhang Y, Wang XF, Rong W, et al. Histochemical analyses reveal that stronger intrinsic defenses in *Gossypium barbadense* than in *G. hirsutum* are associated with resistance to *Verticillium dahliae*. Mol Plant Microbe Interact. 2017;30:984–96. https://doi.org/10.1094/MPMI-03-17-0067-R.

Zhang WQ, Gui YJ, Short DPG, et al. *Verticillium dahliae* transcription factor VdFTF1 regulates the expression of multiple secreted virulence factors and is required for full virulence in cotton. Mol Plant Pathol. 2018;19:841–57. https://doi.org/10.1111/mpp.12569.

Zhang Y, Wu LZ, Wang XF, et al. The cotton laccase gene *GhLAC15* enhances Verticillium wilt resistance via an increase in defence-induced lignification and lignin components in the cell walls of plants. Mol Plant Pathol. 2019;20:309–22. https://doi.org/10.1111/mpp.12755.

Zhang YH, Shi YQ, Zhao LH, et al. Phosphoproteomics profiling of cotton (*Gossypium hirsutum* L.) roots in response to *Verticillium dahliae* inoculation. ACS Omega. 2019;4:18434–43. https://doi.org/10.1021/acsomega.9b02634.

Zhang Y, Chen B, Sun ZW, et al. A large-scale genomic association analysis identifies a fragment in Dt11 chromosome conferring cotton Verticillium wilt resistance. Plant Biotechnol J. 2021;19:2126–38. https://doi.org/10.1111/pbi.13650.

Zhang YL, Zhou JL, Zhao LH, et al. A review of the pathogenicity mechanism of *Verticillium dahliae* in cotton. J Cotton Res. 2022;5:3. https://doi.org/10.1186/s42397-021-00111-6.

Zhang DD, Dai XF, Klosterman SJ, et al. The secretome of *Verticillium dahliae* in collusion with plant defence responses modulates Verticillium wilt symptoms. Biol Rev Camb Philos Soc. 2022;97:1810–22. https://doi.org/10.1111/brv.12863.

Zhang XJ, Zhao LH, Liu SC, et al. Identification and functional analysis of a novel hydrophobic protein VdHP1 from *Verticillium dahliae*. Microbiol Spectr. 2022;10:e0247821. https://doi.org/10.1128/spectrum.02478-21.

Zhao P. Molecular mechanism of cotton resistance to Verticillium wilt regulated by GhCML41 protein. Yangling: Northwest A & F University; 2019.

Zhao YL, Wang HM, Chen W, et al. Genetic structure, linkage disequilibrium and association mapping of Verticillium wilt resistance in elite cotton (*Gossypium hirsutum* L.) germplasm population. PLoS ONE. 2014;9:e86308. https://doi.org/10.1371/journal.pone.0086308.

Zhao YL, Zhou TT, Guo HS. Hyphopodium-specific VdNoxB/VdPls1-dependent ROS-Ca^{2+} signaling is required for plant infection by *Verticillium dahliae*. PLoS Pathog. 2016;12:e1005793. https://doi.org/10.1371/journal.ppat.1005793.

Zhao YL, Wang HM, Chen W, et al. Regional association analysis-based fine mapping of three clustered QTL for Verticillium wilt resistance in cotton

(*G. hirsutum* L.). BMC Genomics. 2017;18:661. https://doi.org/10.1186/s12864-017-4074-y.

Zhao YL, Chen W, Cui YL, et al. Detection of candidate genes and development of KASP markers for Verticillium wilt resistance by combining genome-wide association study, QTL-seq and transcriptome sequencing in cotton. Theor Appl Genet. 2021;134:1063–81. https://doi.org/10.1007/s00122-020-03752-4.

Zhao YL, Wang WJ, Chen W, et al. Identification of major QTL for Verticillium wilt resistance in cotton by using F_2 and RIL population. Cotton Sci. 2023;35:101–16. https://doi.org/10.11963/cs20220059 (in Chinese with English abstract).

Zhou TT, Zhao YL, Guo HS. Secretory proteins are delivered to the septin-organized penetration interface during root infection by *Verticillium dahliae*. PLoS Pathog. 2017;13:e1006275. https://doi.org/10.1371/journal.ppat.1006275.

Zhu HQ, Li ZF, Feng ZL, et al. Overview of cotton Verticillium wilt research over the past decade in China and its prospect in future. Cotton Sci. 2017;29:37–50. https://doi.org/10.11963/1002-7807.zhqzhq.20170825 (in Chinese with English abstract).

Zhu DD, Zhang XY, Zhou JL, et al. Genome-wide analysis of ribosomal protein GhRPS6 and its role in cotton Verticillium wilt resistance. Int J Mol Sci. 2021;22:1795. https://doi.org/10.3390/ijms22041795.

Zhu YT, Zhao M, Li TT, et al. Interactions between *Verticillium dahliae* and cotton: pathogenic mechanism and cotton resistance mechanism to Verticillium wilt. Front Plant Sci. 2023;14:1174281. https://doi.org/10.3389/fpls.2023.1174281.