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REVIEW

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# A review of the pathogenicity mechanism of *Verticillium dahliae* in cotton

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## Abstract

Verticillium wilt, caused by the notorious fungal pathogen *Verticillium dahliae*, is one of the main limiting factors for cotton production. Due to the stable dormant structure microsclerotia, long-term variability and co-evolution with host plant, its pathogenicity mechanism is very complicated, and the interaction mechanism between pathogen and host plant is also unclear. So identification and functional analysis of the genes involved in the pathogenicity or virulence of this fungus will benefit to uncover the molecular pathogenic mechanism of *V. dahliae*. In this review, many multifunction genes covering microsclerotia development, pathogen infection, effector proteins, transcription factors, horizontal gene transfer and trans-kingdom RNA silencing have been summarized to provide a theoretical basis to deep understand the molecular pathogenicity mechanism of *V. dahliae* and promote to effectively control Verticillium wilt. Furtherly, these pathogenicity-related genes may be considered as targets for effective control of Verticillium wilt in cotton.

**Keywords:** *Verticillium dahliae*, Cotton, Pathogenicity-related genes, Molecular pathogenic mechanism

## Background

*Verticillium dahliae* Kleb. is a soil-borne hemibiotrophic phytopathogenic fungus with a wide host range and worldwide distribution, which generally causes plant dysplasia, leaf wilting and yellowing, vascular bundles browning, and eventually leading to early death (Klosterman et al. 2009; Zhou et al. 2021). Because of stable dormant structure microsclerotia, this fungus can survive in the soil for more than ten years even under adversity conditions (Fradin and Thomma 2006). Moreover, the pathogen population is rich in genetic diversity and its pathogenicity is prone to variability. In the field, it co-evolves with host plant to appear a strong pathotype (Atallah et al. 2010; Song et al. 2020). Many researches have been made to control Verticillium wilt, such as breeding resistant cultivars, crop rotation, developing soil fumigants, applying chemical fungicides, as well as biological control (Acharya et al. 2020; Ingram et al. 2020; Zhang et al. 2021). Unfortunately, Verticillium wilt still results in extensive economic losses.

Cotton, a primary natural fiber producing crop of great importance to the global textile industry, suffers from tremendous yield losses in *V. dahliae*-infested soil approximately 10%~35% in many countries annually, and the fiber quality by reducing micronaire and span length, which is considered as the cancer of cotton production (Fradin and Thomma 2006; Zhang et al. 2011). The objective of this review is to summarize the molecular pathogenicity mechanism of *V. dahliae*, with major focus on the cotton-*V. dahliae* complicated interaction, and provide a theoretical basis for further effective control of cotton Verticillium wilt.

The plant immunity system has been described as a 'zigzag' model, which exhibits a systematic explanation

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of the dynamic balance between pathogen and host plant in the arms race between pathogenicity and disease resistance (Jones and Dangl 2006). Plants recognize pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs) to trigger immunity responses (PAMPs-triggered immunity, PTI); Plants use R proteins to specifically discern pathogen effectors, thereby activating further complex immunity responses (Effector-triggered immunity, ETI). In order to avoid or suppress plant immune responses, pathogens have evolved more diverse effector proteins to successfully infect plants. As a PAMPs, the xyloglucan hydrolase PsXEG1 secreted by *Phytophthora sojae* plays a virulence factor to help pathogen infection. Meanwhile, soybean can distinguish PsXEG1 to trigger immune reactions, leading to inhibit the infection of *P. sojae*. However, the RXLR effector proteins are recruited by *P. sojae* to suppress these immune responses and successfully infect soybean (Ma et al. 2015). Further study has been shown that PsXEG1 and PsXLP1, which contain conserved domains, coordinately regulate pathogen infection in soybean. PsXLP1 competitively interferes with the suppressor GmGIP1 as a bait, thereby saving PsXEG1 and leading to disease occur (Ma et al. 2017). So far, this new pathogenic mechanism has yet not been found in cotton-*V. dahliae* battle. As a well-known PAMPs, chitin is unique among the major component of fungal cell walls, triggering host immunity and restraining pathogen infection. De Jonge et al. (2010) demonstrated that lysine motif (LysM) domain-containing effector protein Ecp6 of *Cladosporium fulvum* mediated pathogenicity by blocking the chitin-triggered host immunity, which represents a common strategy of host immune suppression. Interestingly, Gao et al. (2019) revealed that deacetylation of chitin avoided host perception by LysM-containing receptor as a major virulence strategy, due to the reason that PDA1 possesses deacetylation activity and pathogenicity traits in both *V. dahliae* and *Fusarium oxysporum*, with conserved domains in Ascomycota. All these evidences showed that deacetylation of chitin to elude host perception is a conserved and common intercellular stealth tactic of soil-borne pathogenic fungi.

The molecular pathogenicity mechanism of *V. dahliae* is very complex, which is related to a large number of pathogenicity-related genes. In recent years, researchers have studied *V. dahliae* pathogenicity-related genes through various perspectives such as genome, transcriptome, proteome and T-DNA mutant library, having made phased progress (Gold et al. 2017; Qi et al. 2015; Rehman et al. 2016; Wu et al. 2019). Functional analysis of these multifunctional genes involved in the fungus growth and pathogenicity is the molecular genetic basis of revealing pathogenesis of *V. dahliae*. Up to now, genes related to growth and development are not necessarily correlated to pathogenicity, on the contrary, genes related to pathogenicity more or less connected with vegetative growth (Luo et al. 2014). Unfortunately, the pathogenicity- or virulence-related genes in wilt fungus, which may be as potential targets for controling plant Verticillium wilt disease, remaining poorly elucidated. In this review, some key findings and pathogenicity-related genes are summarized to provide a theoretical basis for further understanding of the molecular pathogenicity mechanism of *V. dahliae*.

## Biological processes in pathogenicity of *V. dahliae*

### Microsclerotia development

As the long-term survival resting structure, microsclerotia plays a critical role in the disease cycle as primary sources of infection, germination of microsclerotia is the first step in host invasion and initiation of Verticillium wilt disease (Fradin and Thomma 2006; Shaban et al. 2018; Zhang et al. 2016c) (Fig. 1). Although microsclerotia development is not always correlated with virulence, some studies have uncovered evidences that the decrease in microsclerotia and melanin reduce pathogenicity and survival of *V. dahliae* isolates (Rauyaree et al. 2005; Tzima et al. 2010; Zhang et al. 2015). However, *VdPKAC1* (cAMP-dependent protein kinase A), *VGB* (G protein β subunit gene), *VdMsn2* ($C_2H_2$ transcription factor) and *VdPLP* (patatin-like phospholipase gene) negatively regulated the formation of microsclerotia, and the deletion mutants of those genes caused lower fungal pathogenicity with higher production of microsclerotia (Qi et al. 2018; Tian et al. 2017; Tzima et al. 2012). A total of 1 654 genes with differential expression have been identified by analyzing whole genome-wide expression profiles of germinating microsclerotia in *V. dahliae* (Hu et al. 2014). This process was more likely to regulate by transcription factors including $C_2H_2$, bZIP and fungal-specific transcription factors domain-containing proteins. In addition, G-protein receptors, $Ca^{2+}$, small GTPases, and cAMP were involved in this signal transduction (Luo et al. 2019).

Generally, microsclerotia and melanin appear together, thus most genes coordinately regulate the development of microsclerotia and the accumulation of melanin (Li et al. 2019a). *Vayg1* (dihydroxynaphthalene (DHN)-melanin), the common form among fungi and named for the intermediary of melanin biosynthetic pathway, is necessary for melanin and microsclerotia production (Fan et al. 2017). *VdSsk1* (response regulator), transcription factor *VdCmr1*, cluster-specific genes *VdPKS1* (polyketide synthase), and *VdLac1* (laccase) encoding at initial and endpoint steps in DHN melanin production, were required for melanin synthesis, but neither required for

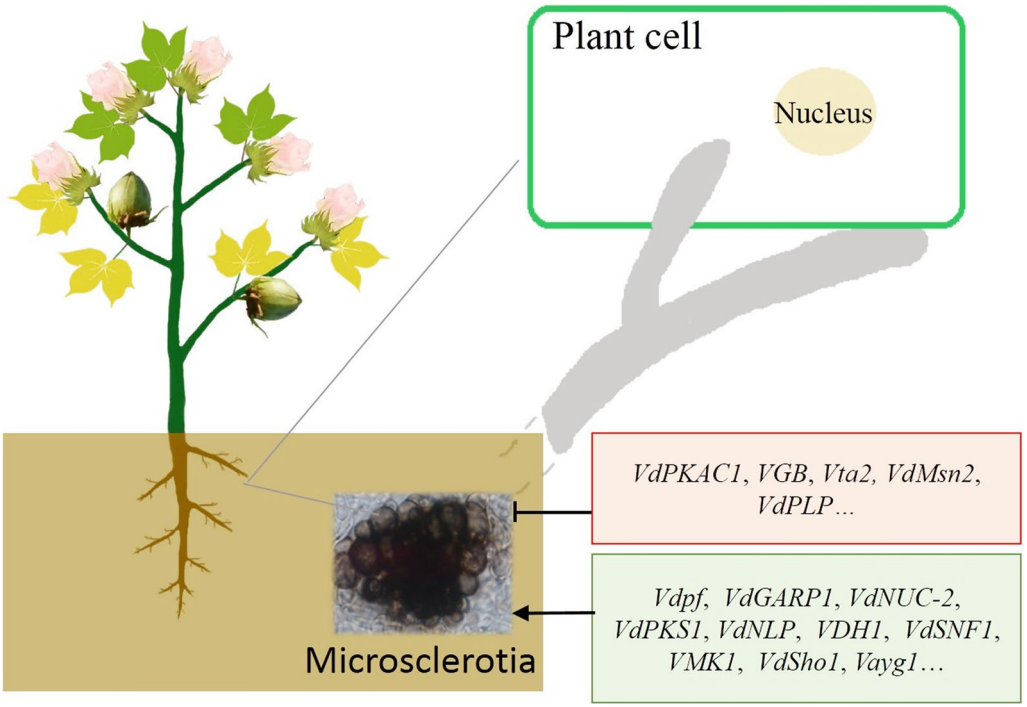


**Fig. 1** The simple schematic diagram of the infection process of *Verticillium dahliae* in cotton. As the initial source of infection, microsclerotia survives in the soil for a long time. Once the conditions are suitable, the microsclerotia germinates to form hyphae and infects cotton root tips. Some postive/negative regulation genes are involved in microsclerotia development

microsclerotia formation (Fang et al. 2019; Wang et al. 2018c; Zheng et al. 2019). Additionally, the high osmolarity glycerol (HOG) pathway is an important regulatory strategy for microsclerotia and melanin synthesis. *VdHog1*, a mitogen-activated protein kinase, controled microsclerotia formation, virulence and stress reaction of *V. dahliae*. *VdPbs2*, the conserved upstream component of *VdHog1*, was a key regulator of microsclerotia formation (Tian et al. 2016; Wang et al. 2016a, b). And *VdMsn2* was predicted to function as a downstream player in the HOG pathway, disruption mutants produced significantly higher microsclerotia (Tian et al. 2017). Furthermore, RNA-Seq used to analyze the response of *V. dahliae* to nutrient starvation indicated that melanin biosynthesis and microsclerotia development were induced under nitrogen starvation, but microsclerotia development was suppressed under carbon starvation (Xiong et al. 2015).

### Systemic infection

The initial interaction of a pathogenic fungus with host is complex and involves numerous metabolic pathways. Verticillium wilt is a typical systemic vascular infection disease, successful adhesion and penetration of host plant root are the first step for *V. dahliae* to systematically infect and cause disease (Du et al. 2017). During colonization, *V. dahliae* spores develop into hyphae surrounding the roots. Only a few hyphae tightly adhere to the root surface and form hyphopodium at the infection site, which further growth into penetration pegs to facilitate infection. Unlike the infestation nail of *Magnaporthe grisea*, *Verticillium* species lack this special structure, and their attachment depends on some molecules, such as hyphopodium and adhesins (Eynck et al. 2007). *Vta1* (transcription activator of adhesion 1) rescued adhesion in non-adhesive *Saccharomyces cerevisiae* cells, *Vta2* and *Vta3* were required for early steps in plant colonization and infection (Tran et al. 2014). However, Harting et al. (2020) revealed that *Vta1* function was dispensable for root colonization and infection, but required for melanin production and activated transcription of melanin biosynthesis genes including the polyketide synthase encoding *PKS1* and the laccase LAC1. *VdSho1* (tetraspan transmembrane protein), as an osmosensor, was necessary for plant penetration and melanin synthesis contributing to virulence (Li et al. 2019a). Besides, two transcription factors *Vph1* (VDAG_06555) and *Vhb1* (VDAG_08786) were also participated in penetration (Sarmiento-Villamil et al. 2018b). *Msb* encoded a transmembrane mucin with highly conservative in the Mitogen-activated protein kinase (MAPK) signal pathway, the

invasive growth and adhesive capacity of *VdMsb* deletion mutants were significantly decreasing (Tian et al. 2014). *VdPls1* (tetraspanin) and *VdNoxB* (catalytic subunit of membrane-bound NADPH (nicotinamide adenine dinucleotide phosphate) oxidase) colocated in the plasma membrane at the base of hyphopodium were specific expression in hyphopodium, indicating that NADPH oxidase regulated septin ring organization (Zhao et al. 2016). As a regulator of hyphopodium formation and pathogenicity, *VdCSIN1* (cellophane surface-induced gene) regulated hyphopodium formation via cAMP-mediated signal pathway to promote host colonization of *V. dahliae* (Sun et al. 2019). After hyphopodium infected cotton root, hyphal neck was an important site for directly/indirectly pathogenic related genes during plant root infection. A septin ring, requiring *VdSep5*, partitioned the hyphopodium, the invasive hyphae and further formed the specialized fungi-host interface. Moreover, *VdSec22*, *VdSyn8*, and *VdExo70* (exocyst subunit) also inhibited transmission of the secreted proteins inside the hyphopodium and regulated virulence (Zhou et al. 2017b).

As the first physical barrier of plants, the plant cell wall plays a basic role in preventing the invasion of pathogenic fungi. If pathogens successfully infect plants, they have to break through this barrier. Analysis of the genome sequence of *V. dahliae* demonstrated that it contained a large number of genes encoding carbohydrate-active-enzymes and cell wall degrading enzymes (CWDE), which may be one of the reasons why that fungus could colonize lots of host plants. The typical secretion of CWDE include pectinases, xylanases, cellulases, and proteases (Chen et al. 2016). Pectin as the main element of the primary plant cell wall plays a key role in defence mechanism against plant pathogens. The pectate lyase (VdPEL1) induced cell death in several plants and triggered defense responses depending on enzymatic activity (Yang et al. 2018). *VdSNF1* (Sucrose non-fermenting protein kinase) regulated the activity of pectinase and galactase, the pathogenicity of knockout mutants was significantly reduced during infection in tomato (Tzima et al. 2011). Besides, *VdSSP1*, encoding a secreted protein, has also been implicated in virulence of *V. dahliae* in cotton. *VdSSP1* was essential for cell wall degradation and the absence of this gene significantly mitigated virulence (Liu et al. 2013). In addition, pathogenesis related genes *VdPR1* and *VdPR3* affected the infectivity via regulating the activity of cellulase (Zhang et al. 2015, 2016c). The MADS-box transcription factor *VdMcm1* was a key regulator of cell wall integrity (Xiong et al. 2016).

When *V. dahliae* arrives the xylem from the intercellular space of the plant root cortex, the fungus has to adapt to the intracellular environment of host plant, where only a little amount of nutrients can be obtained, with limited amino acids and vitamins. *VdThit* (thiamine transporter protein gene) participated in nutrient acquisition, the growth and conidiation, the impaired virulence of the Vd*ΔThit* mutants were partially restored by supplementing exogenous thiamine (Qi et al. 2016). Besides, *VdTHI20* was involved in the thiamine biosynthesis pathway, the deletion of *VdTHI20* resulted in several phenotypic defects including vegetative growth, conidiation, and virulence. Futhermore, *VdTHI20* increased the tolerance of *V. dahliae* to UV damage (Qin et al. 2020). Another thiamine biosynthesis member, *VdThi4* (thiazole biosynthesis protein) had putative NAD binding site, was highly conserved among *Verticillium*, and maintained mitochon-drial genome stability. Although the *ΔVdTHI4* mutant could colonize the plant roots, it did not form a systemic infection and lose pathogenicity to tomato (Hoppenau et al. 2014). In the battle aganist plants, pathogens need to obtain iron and other micronutrients from plant cells, ferric reductases are integral membrane proteins involved in the reduction of environmental ferric iron into the biologically available ferrous iron. *FreB* deletion strain exhibited significantly lower growth and spore production especially on media without iron, with highly sensitive to oxidative stress (Rehman et al. 2018). The bZip transcription factor *VdHapX* acted as a key regulator of iron homeostasis for adaptation to iron-depleted and iron-excess conditions and was required for full virulence (Wang et al. 2018b). Once adaptes to the intracellular environment, pathogenic fungus propagates quickly and invades the vascular bundles, further blocks the vascular bundle and causes a systemic infection disease.

## Protein properties related to virulence of *V. dahliae*

### Effector proteins

Successful host infection requires secretion of effector proteins to evade or suppress plant immunity, effector proteins play an important role in the host infection process of pathogens and become a research hotspot (Feng et al. 2018; Stergiopoulos and de Wit 2009; Wang et al. 2020a). Most of the effector proteins have conservative sequences at the N-terminus or C-terminus, such as RXLR, CRN, CFEM, RGD, DELD, RYWT or Y/F/WXC, etc. (Gómez-Gómez and Boller 2000; Du et al. 2017; Liu et al. 2019; Shamraĭ 2014). Effector proteins including VdNEP, PevD1, VdCP1 were secreted by *V. dahliae* into host, all of those can induce cotton cell death and trigger immunity responses (Gui et al. 2017; Wang et al. 2004, 2012). Chen et al. (2016) used isobaric tags for relative and absolute quantitation (iTRAQ) technology to systematically analyze the secreted proteins of *V. dahliae* induced by cotton, 271 secreted proteins were found to be induced to express, of which 172 proteins had signal peptide sequences. Whole-genome sequencing and

proteomics screen have been used to identify many of those proteins, including cysteine-rich proteins, necrosis-inducing proteins and enzymes (De Sain and Rep 2015). Endochitinase *VDECH* was recognized by plant to elicit defense response and also was an effective inhibitor of conidia germination (Cheng et al. 2017). Secreted small cysteine-rich proteins (SCPs) play a critical role in modulating host immunity in plant-pathogen interactions. Bioinformatic analysis was showed that the fungal pathogen *V. dahliae* encoded more than 100 *VdSCPs*, *VdSCP27*, *VdSCP113*, and *VdSCP126* vitro-expressed in tobacco leaves, resulting in cell death accompanied with reactive oxygen species (ROS) burst and callose deposition. *BAK1* and *SOBIR1* (associated with receptor-like protein) were required for host immunity triggered by those three *VdSCPs* (Wang et al. 2020a). In addition, expression of the *VdSCP7* (small cysteine containing protein) gene in *N. benthamiana* activated both salicylic acid and jasmonate signal pathway, and altered plants' susceptibility to the pathogens *Botrytis cinerea* and *Phytophthora capsici* (Zhang et al. 2017a). As a well-researched star, PevD1 with C2 domain structure and C-terminal acidic pocket activated a hypersensitive responses such as necrosis and systemic acquired resistance in many plants including cotton, tobacco and *Arabidopsis* (Liang et al. 2018; Zhang et al. 2019b; Zhou et al. 2017a). In cotton, *V. dahliae* secreted PevD1 to inhibit GhPR5 (a partner protein of PevD1) antifungal activity in order to overcome host defence system. As a PAMPs, glycoside hydrolase 12 (GH12) proteins widely present in oomycetes and filamentous fungi. Gui et al. (2017) revealed that *VdEG1* and *VdEG3* (two of GH12 proteins) acted as PAMPs to trigger host cell death. *VdEG1* and *VdEG3* associated with *BAK1* and *SOBIR1* to initiate host immunity, respectively. However, they both binded with CBM1-containing proteins to manipulate plant immunity.

#### Transcription factors

Bioinformatic analysis proved that *V. dahliae* coded approximately 530 transcription factors divided into 42 families. Jin et al. (2019) clarified that transcription factors contained fungi specific conservative domain and fungal Zn2-Cys6 binuclear cluster domain were abundance in V991w, which was an attenuated virulence strain of *V. dahliae*. Increasing evidences have been revealed that transcription factors play an important role in the interaction between pathogen and host plant (Depotter et al. 2019; Shaban et al. 2018). *Vdpf*, a fungal-specific transcription factor-encoding gene, was associated with vegetative growth and virulence. *ΔVdpf* mutants were melanin deficient, with undetectable expression of melanin biosynthesis-related genes *Brn1*, *Brn2*, and *Scd1* (Luo et al. 2016). Sarmiento-Villamil et al. (2018a, b) characterized the APSES family transcription factor *Vst1* in *V. dahliae* and *V. nonalfalfae*, the absence of *Vst1* had a great impact on sporulation, especially affected sporulation rates in liquid medium. However, *Vst1* was dispensable for virulence. Fungal transcription factors (TFs), the VdFTF1-deletion strains exhibited significantly reduced virulence in cotton, but with normal vegetative growth, mycelial pigmentation, and conidial morphology (Zhang et al. 2018). In addition, nuclear transcription factors *Som1* and *Vta3* were prerequisite for root penetration and host plant colonization (Bui et al. 2019). bZIP transcription factors are ubiquitous in animals, plants, and microorganisms, acting various biological roles in stress responses, conidiation, and pathogenicity in pathogenic fungi. Homolog of the bZIP transcription factor *Atf1*, *VdAtf1* controled pathogenesis via the regulation of nitric oxide resistance and inorganic nitrogen metabolism rather than oxidative resistance, responding to nitrosative stress and nitrogen metabolism in *V. dahliae* (Tang et al. 2020). Two bZIP transcription factors (*VDAG_08640* and *VDAG_08676*) disrupt mutants showed remarkably higher sensitivity to hydrogen peroxide stress (Fang et al. 2017). Besides, disruption of *VdHapX* (bZip transcription factor) led to decreased formation of the long-lived survival structure (Wang et al. 2018b). *V. dahliae* deployed an effector protein *VdSCP41* into plants to disrupt the functions of *SARD1* and *CBP60g*, two central transcriptional regulators of plant immunity (Ding and Redkar 2018).

## Genetic mechanism in pathogen–plant interaction

#### Horizontal gene transfer

Horizontal gene transfer (HGT) refers to the transmission of genetic material between individual organisms, internal organelles of a single cell and even distinct evolutionary lineages, which is an important source of biological innovation and a common case in the interaction between host plant and pathogen (Cai et al. 2018; Daboussi and Capy 2003). The effector gene *Ave1* and a glucosyltransferase-encoding gene *VdGT2* were identified as pathogenicity genes which were proposed to be horizontally acquired from a plant and a bacterial donor, respectively (Shi-Kunne et al. 2019). However, the direct experimental evidences of the transferred genetic material from another related or unrelated fungal species were insufficient (Depotter et al. 2019; Mehrabi et al. 2011). Klosterman et al. (2011) have sequenced the genomes of *V. dahliae* and *V. albo-atrum*, compared with the genome of *F. oxysporum*, a set of proteins were shared among all three wilt pathogens. In addition, the high level of synteny between the two *Verticillium* assemblied highlighted four flexible genomic islands in *V. dahliae*, with enriched transposable elements and contained duplicated genes

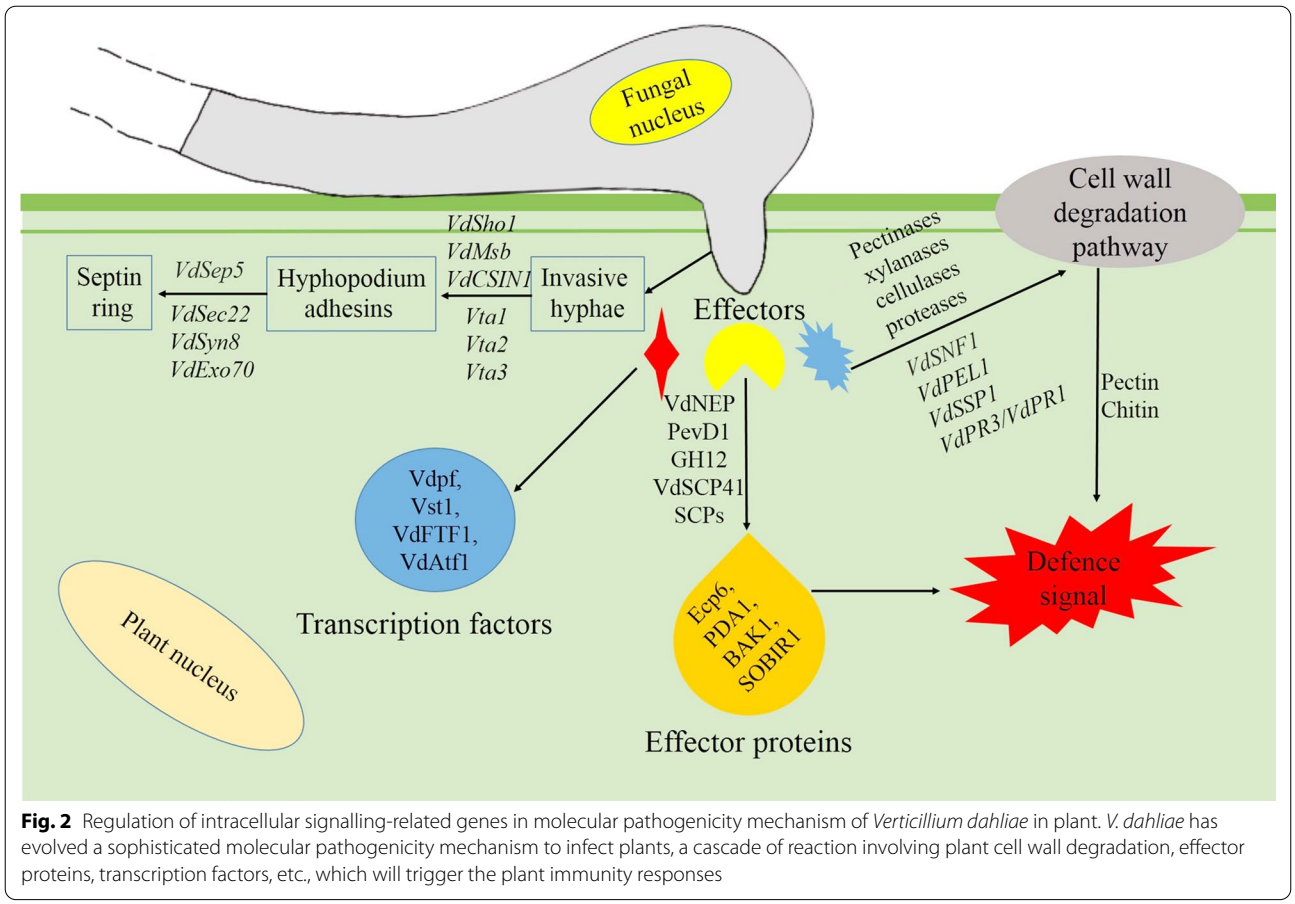


**Fig. 2** Regulation of intracellular signalling-related genes in molecular pathogenicity mechanism of *Verticillium dahliae* in plant. *V. dahliae* has evolved a sophisticated molecular pathogenicity mechanism to infect plants, a cascade of reaction involving plant cell wall degradation, effector proteins, transcription factors, etc., which will trigger the plant immunity responses

which were important in signaling/transcriptional regulation and iron/lipid metabolism (Klimes et al. 2015). Besides, LysM effector (VDAG_05180) located in an lineage-specific (LS) region contributed to virulence, which was highly up-regulated during infection (De Jonge et al. 2013). Chen et al. (2018) determined the genome of *V. dahliae* strain Vd991 and revealed that the Vd991 genome harbored several exclusive LS genes within LS regions (LSRs). Further phylogenetic analysis proved that G-LSR2 was acquired from *F. oxysporum* through HGT, whose targeted deletion mutants resulted in scarcity virulence to cotton, but did not affect virulence on lettuce and tomato. These results indicated that G-LSR2 significantly contributed to wilt pathogen infection to cotton and may represent an unique mechanism in the evolution of *V. dahliae*.

### Trans-kingdom RNA silencing

RNA interference (RNAi)-based host-induced gene silencing (HIGS) is an effective strategy against pathogenic fungi by specific silencing of fungal virulence genes with miRNAs exported from the colonized host plants. Researchers have demonstrated cotton exported miRNAs to direct specific gene silencing (*Clp-1* and *HiC-15*) in *V. dahliae* (Zhang et al. 2016b). *V. dahliae* also recruits sRNAs as effectors and transfers into *Arabidopsis* and cotton for silencing host genes, these evidences confirmed the bidirectional trans-kingdom RNAi and sRNA trafficking between pathogen and host plant (Jin and Guo 2018; Singh et al. 2010; Wang et al. 2016a, b). All these trans-kingdom transmitted molecular genetic materials contribute to the evolutionary arms race between pathogen and plant. Generally, RNAi transmission between pathogens and plant requires cell-to-cell and systemic movement of RNAi signals. As a broad host range pathogen, *V. dahliae* may have evolved to absorb and maintain RNAi signals from different hosts (Hua et al. 2018). Although the mechanism of RNA transmission is unclear, HIGS has been utilized to suppress Verticillium wilt disease by silencing virulence genes *Ave1*, *Sge1* (six gene expression 1), *NLP1, ALS* (acetolactate synthase), *VdAK* (adenylate kinase gene) and *RGS* (G protein signalling) of *V. dahliae* (Su et al. 2020; Wei et al. 2020; Xu et al. 2018). Thus, trans-kingdom sRNA transmission and RNA silencing mechanisms have been shown to be

**Table 1** Numerous genes directly/indirectly related to pathogenicity in *V. dahliae*

| Gene | Annotation | Strain | Growth characteristics | | | Pathogenicity | References |
|---|---|---|---|---|---|---|---|
| | | | Hypha growth | Sporulation | Microsclerotial formation | | |
| *VdPL3.1* and *VdPL3.3* | Pectin lyase encoding protein | Vd991 | | | | + | Chen et al. (2016) |
| *VdSSP1* | Specific secreted protein | VdG1 | + | | | + | Liu et al. (2013) |
| *VdPR1* | Pathogenesis related gene | Vd080 | + | + | + | + | Zhang et al. (2016c) |
| *VdPR3* | Pathogenesis related gene | Vd080 | + | + | + | + | Zhang et al. (2015) |
| *VdFTF1* | Transcription factor containing fungal trans domain | Vd991 | / | / | / | + | Zhang et al. (2018) |
| *Vdpf* | Transcription factor | Vd991 | + | | + | + | Luo et al. (2016) |
| *VdSCP7* | Specific secretory protein | Vd592 | | | | − | Zhang et al. (2017a) |
| *VdASP F2* | Secretory factor | Vd991 | | − | + | + | Xie et al. (2017) |
| *VdGARP1* | Glutamic acid-rich protein | Vd592 | − | | + | + | Gao et al. (2010) |
| *VdPKS1* | Polyketide synthase protein | Vd592 | | | + | + | Zhang et al. (2017b) |
| *VdNUC-2* | Neurospora crassa nuc-2 homolog protein | Vd07DF2 and VdBp2 | + | + | + | + | Deng et al. (2015) |
| *VdCYP1* | Cytochrome P450 monooxygenase encoding protein | Vd991 | | | | + | Zhang et al. (2016a) |
| *Vdisc1* | sochorismatases encoding protein | Vd991 | | | | + | Liu et al. (2014) |
| *VdNoxB/VdPls1* | Hyphopodium specific protein | Vd592 | − | | + | + | Zhao et al. (2016) |
| *VdCYC8* | Glucose repression mediator protein | Vd080 | + | + | + | + | Li et al. (2015) |
| *VDH1* | A class II hydrophobin | Dvd-T5 | / | | + | + | Klimes et al. (2008) and Klimes and Dobinson (2006) |
| *VdNLP* | NPP1 domain-containing protein | Vd592 | | − | + | + | Santhanam et al. (2013) and Zhou et al. (2012) |
| *ta2* | Transcription activator | VdJR2, Vd52, Vd73 and Va4 | + | + | − | + | Tran et al. (2014) |
| *VdEg-1* | Endoglucanase-1 | VdLs17 | + | | | + | Maruthachalam et al. (2011) |
| *VdSNF1* | Sucrose non-fermentingprotein kinase | 70wt-r1 | | | + | + | Tzima et al. (2011) |
| *CPC1* | Regulator for amino acid biosynthesis | VdJR2 | | | | + | Timpner et al. (2013) |
| *VdThi4* | Thiamine biosynthesis protein | VdJR2 and Vl43 | + | | | + | Hoppenau et al. (2014) |
| *VdThit* | Thiamine transporter protein | Vd991 | + | + | | + | Qi et al. (2016) |
| *VMK1* | MAP kinase | VdLs17 | | + | + | + | Rauyaree et al. (2005) |

**Table 1** (continued)

| Gene | Annotation | Strain | Growth characteristics | | | Pathogenicity | References |
|---|---|---|---|---|---|---|---|
| | | | Hypha growth | Sporulation | Microsclerotial formation | | |
| *VdPKAC1* | The cAMP-dependent protein kinase A | 70wt-r1 | | + | − | + | Tzima et al. (2010) |
| *VdSge1* | Transcriptional regulator | VdLs17 | + | + | | + | Santhanam and Thomma (2013) |
| *VGB* | G protein β subunit | 70wt-r1 | + | + | − | + | Tzima et al. (2012) |
| *VdSho1* | Tetraspan transmembrane protein | Vd8 and Vd991 | | | + | + | Li et al. (2019a) |
| *Vayg1* | Melanin biosynthesis | JY | | | + | + | Fan et al. (2017) |
| *VdSsk1* | Homolog to *S. cerevisiae* Ssk1 | XS11 | + | | / | + | Zheng et al. (2019) |
| *VdHog1* | High-osmolarity glycerol | XS11 | + | | + | + | Wang et al. (2016a, b) |
| *VdPbs2* | MAPK kinase | XS11 | | | + | + | Tian et al. (2016) |
| *Vta1*, *Vta2* and *Vta3* | transcription activator of adhesion 1 | VdJR2 | | | + | + | Harting et al. (2020) |
| *Som1* and *Vta3* | Nuclear transcription factor | VdJR2 | − | − | + | + | Bui et al. (2019) |
| *VdEG1* and *VdEG3* | Glycoside hydrolase 12 protein | Vd991 | | | | + | Gui et al. (2017) |
| *VdCP1* | SnodProt1-like protein | XH-8 | / | / | | + | Zhang et al. (2017c) |
| *VdICSH1* | Isochorismatase hydrolase | Vd1396-9 and Vs06-07 | / | / | / | + | Zhu et al. (2017) |
| *VdCmr1* and *VdPKS1* | Transcription factor | VdLs.17 | | | / | / | Fang et al. (2019) and Wang et al. (2018c) |
| *VdOCH1* | Initiation-specific α-1, 6-mannosyltransferase | VdGn3 | + | + | + | + | Zhang et al. (2019a) |
| *VdMsn2* | $C_2H_2$ transcription factor *Msn2* | XS11 | + | | − | + | Tian et al. (2017) |
| *VdSkn7* | Homolog to *S. cerevisiae* Skn7 | XS11 | | + | + | + | Tang et al. (2017) |
| *Vst1* | APSES family transcription factor | DvdT5 and 383-2 | | + | + | / | Sarmiento-Villamil et al. (2018a) |
| *VdPEL1* | Pectate lyase | Vd991 | | | | + | Yang et al. (2018) |
| *VdPLP* | Patatin-like phospholipase gene | Vd991 | + | + | − | + | Qi et al. (2018) |
| *VdSec22* and *VdSso1* | 22 soluble N-ethylmaleimide-sensitive factor attachment protein receptors | Vd991 | + | | | + | Wang et al. (2018a) |
| *VdFKBP12* and *VdTOR* | Rapamycin binding protein, target of rapamycin | Vd991 | | | | + | Li et al. (2019b) |
| *VdAtf1* | bZIP transcription factor | XS11 | | | | + | Tang et al. (2020) |
| *VdRGS1* | Regulators of G protein signalling | Vd8 and D-10-8F | | | + | + | Sarmiento-Villamil et al. (2020) and Xu et al. (2018) |

**Table 1** (continued)

| Gene | Annotation | Strain | Growth characteristics | | | Pathogenicity | References |
|---|---|---|---|---|---|---|---|
| | | | Hypha growth | Sporulation | Microsclerotial formation | | |
| *VdOGDH* | α-oxoglutarate dehydrogenase | Vd991 | + | | + | + | Li et al. (2020) |
| *VdNPS* | Nonribosomal peptide synthetases | Vd991 | | | + | + | Luo et al. (2020) |
| *VdQase* | Cupin domain-containing protein | Vd9 and Vd21 | | | | + | El Hadrami et al. (2015) |
| *VdDpb4* and *Vdlsw2* | Histone-fold protein, ATP-dependent chromatin-remodeling factor | V592 | + | | | + | Wang et al. (2020b) |
| *VdPDA1* | Secretory polysaccharide deacetylase | V592 | | | | + | Gao et al. (2019) |
| *VdTHI20* | Thiamine biosynthesis | Vd991 | + | + | | + | Qin et al. (2020) |
| *VdPls1/VdNoxB* | Tetraspanin, catalytic subunit of membrane-bound NADPH oxidases | V592 | | | | + | Zhao et al. (2016) |
| *VdMcm1* | MADS-box transcription factor | XS11 | | + | + | + | Xiong et al. (2016) |
| *VdFreB* | Ferric reductase | Vd991 | + | + | | + | Rehman et al. (2018) |
| *VDAG_08640* and *VDAG_08676* | bZIP transcription factor | XS11 | | | / | + | Fang et al. (2017) |
| *STT3* | Catalytic subunit of the multi-subunit oligosaccharyl transferase | Vd991 | + | + | | + | Su et al. (2018) |
| *VDECH* | Endochitinase | Vd080 | | | | + | Cheng et al. (2017) |
| *Vph1* and *Vhb1* | Transcription factor | DvdT5 | / | − | | + | Sarmiento-Villamil et al. (2018b) |
| *VdRACK1* | Gβ-like/RACK1 protein | Vd8 | + | + | + | + | Yuan et al. (2017) |
| *VdACCd* | ACC deaminase | 70V-WT | | − | + | + | Tsolakidou et al. (2019) |
| *VdMyo5* | Member of the Myosin V family | V592 | + | + | | + | Feng et al. (2018) |
| *VdILV2* and *VdILV6* | Acetolactate synthase | Vd991 | | | | + | Wei et al. (2020) |
| *VdAK* | adenylate kinase gene | Vd991 | | | | | Su et al. (2020) |
| *Clp-1* and *HiC-15* | $Ca^{2+}$-dependent cysteine protease and isotrichodermin C-15 hydroxylase | V592 | | | | + | Zhang et al. (2016b) |
| *VdSCPs* | Small cysteine-rich proteins | Vd991 | | | | + | Wang et al. (2020a) |
| *VdCSIN1* | Cellophane surface-induced gene | V592 | + | | | + | Sun et al. (2019) |
| *VdHapX* | bZip transcription factor | XS11 | + | | + | + | Wang et al. (2018b) |

"+" represents positive regulation, "−" represents negative regulation, "/" represents irrelevant, blank cell represents null value

highly efficient in controlling the pathogens with broad host ranges.

## Conclusion

In this paper, numerous genes directly/indirectly related to *V. dahliae* pathogenicity or virulence have been summarized, which covered microsclerotia development, pathogen infection, effector proteins, transcription factors, horizontal gene transfer, trans-kingdom RNA silencing, etc. (Fig. 2, Table 1), which illustrate the complexity of pathogenic mechanism of *V. dahliae*. Due to pathogens and plants have co-evolutionary relationship, therefore the pathogenic mechanism of pathogens and the mechanism of plants disease resistance, moreover, the variations between them, should be strengthened at the same time in order to achieve satisfactory results. As the ideal method to determine the particular genes' function in the living organism, *Agrobacterium tumefaciens*-mediated transformation acts an important role in constructing a library of T-DNA insertion mutants and analyzing the function of pathogenic genes in *V. dahliae*. The further application of new technologies such as host-induced gene silencing and trans-kingdom RNA interference help to improve the disease resistance of plants. In addition, multi-omics integrative analysis and in-depth genome sequencing will provide a broader perspective in the discovery of regulatory pathogenic mechanism of the destructive plant pathogens. In this review, we have comprehensively analyzed many pathogenicity genes of *V. dahliae* in cotton, providing target gene resources for effective control of cotton Verticillium wilt, which will be contributed to understand the molecular mechanism of plant resistance to *V. dahliae*.

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All data used for writing the manuscript has been cited with proper references.

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