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Proteomic Response of Cotton Leaves to Verticillium Wilt Infection

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Abstract Verticillium wilt, caused by *Verticillium dahliae*, poses a significant threat to global cotton (*Gossypium hirsutum*) production, leading to substantial yield and quality losses. In this study, we employed a proteomic approach to investigate the molecular responses of cotton leaves to *V. dahliae* infection, aiming to elucidate defense mechanisms at the protein level. Using high-resolution mass spectrometry and bioinformatics analyses, we identified and quantified differentially expressed proteins (DEPs) in infected versus healthy cotton leaves, focusing on cultivar CRI 12 as a representative case. The identified DEPs were functionally categorized into defense and stress-related proteins, metabolic reprogramming factors, and signaling regulators, reflecting a complex reorganization of cellular processes in response to infection. Comparative proteomic analysis between susceptible and resistant cultivars revealed distinct defense protein profiles and metabolic adjustments associated with disease resistance. These findings provide insights into the molecular basis of cotton defense against *V. dahliae* and highlight candidate proteins for breeding and genetic engineering. This study underscores the value of integrative omics approaches in advancing our understanding of cotton-pathogen interactions and paves the way for the development of Verticillium wilt-resistant varieties through proteomic-guided breeding strategies.

Keywords Cotton (*Gossypium hirsutum*); Verticillium wilt (*Verticillium dahliae*); Proteomics; Differentially expressed proteins; Disease resistance

1 Introduction

Among the various cotton diseases, yellow wilt is almost the most troublesome one for farmers. It is caused by the soil fungus "*Verticillium dahliae*", which can quietly invade the plant through the root system and then spread along the xylem vessels, causing systemic lesions. The most obvious symptoms often occur on the leaves. First, they turn yellow and wilt, then the vascular bundles turn brown, and finally the entire plant sheds leaves and ages prematurely. With the obstruction of photosynthesis, the vitality of cotton decreases, and both yield and fiber quality are impaired (Xiong et al., 2020).

What troubles people is not only the speed of the onset of the disease, but also the "stubbornness" of such pathogens. The Trichoderma lucidum can form black dormant structures called microsclerotia in the soil. Even without a host, it can "hibernate" in the soil for more than ten years. This endurance means that even if farmland is rotated and disease-resistant varieties are planted, it is still difficult to completely get rid of it. Although disease-resistant breeding is constantly advancing, due to the significant differences and strong adaptability among strains, complete immunity remains the ideal state (Cheng and Zhang, 2025). Previous studies have found that resistant varieties such as sea island cotton are more "alert" in defense responses, with more vigorous lignin synthesis, faster accumulation of salicylic acid (SA), and more obvious callose deposition. These characteristics were confirmed by comparing transcriptomics and histochemical analyses, indicating that the offensive and defensive battle between cotton and the Trichoderma milii is not simple, and once again reminding us that understanding the resistance mechanism requires the combination of multiple disciplines.

If transcriptomics can tell us "which genes are activated", then proteomics is more like observing "the truly moving molecular machines" (Zhu and Luo, 2024). It studies the dynamic changes of all proteins in organisms, especially capturing key signals such as post-transcriptional or post-translational modifications (Zhang et al., 2019). In the study of interactions between plants and pathogens, proteomics has revealed many defense cores,

including enzymes that alleviate oxidative stress, structural proteins that reinforce cell walls, and kinases involved in secondary metabolism and signal transduction (Huang et al., 2021). In cotton, many comparative proteomics and VIGS studies have pointed out that molecules such as gossypol, jasmonic acid, and brassinosterol are all involved in the process of resisting *V. dahliae*. Recent studies in phosphorylated proteomics have further demonstrated that signaling pathways such as the MAPK cascade, calcium-binding proteins, and hormone regulatory networks are rapidly activated upon pathogen invasion, coordinating defense responses. By integrating proteomic, transcriptomic and metabolomic data, scientists are gradually drawing a more complete "cotton immune regulation map", which provides a solid direction for molecular breeding and the development of disease-resistant varieties (Feng et al., 2023).

This study focuses on the protein changes in cotton leaves after infection with Trichoderma boldii, aiming to identify the key proteins and pathways related to host defense. By comparing the protein profiles of healthy and infected plants, we aim to capture the molecular characteristics that reflect defense activation, metabolic recombination and stress adaptation. The research content includes differential expression analysis, functional classification, and exploration of their roles in pathogen recognition, signal transduction, and defense metabolism. Ultimately, we expect these results to further reveal the molecular response pattern of cotton in the face of the Variegata boldii and provide new ideas and targets for future disease-resistant molecular breeding.

2 Pathogenesis of Verticillium Wilt in Cotton

2.1 Infection mechanism

The story of Fusarium wilt often begins deep in the soil. The long-dormant *Verticillium dahliae* hides in the root zone in the form of microsclerotia until the substances secreted by the cotton root system "awaken" it. After being stimulated, the microsclerotia germinate into hyphae. Some directly penetrate the root epidermis, while others take advantage of the natural pores to infiltrate the cortex and finally enter the vascular tissue all the way (Zhu et al., 2023). Once inside the vessels, the fungus begins to multiply in large numbers, producing conidia. These spores are carried to the above-ground parts along the transpiration flow, causing systemic infection (Figure 1).

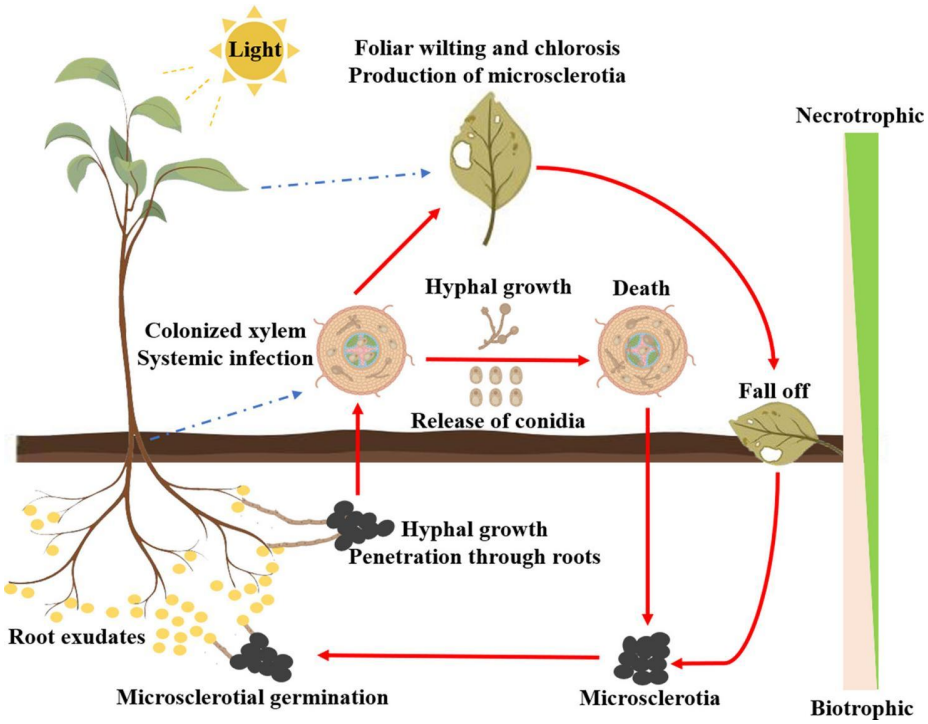

Figure 1 Infection cycle of *V. dahliae* in generic host plants (Adopted from Zhu et al., 2023)

During the process of infection, pathogenic bacteria are not merely mechanical invaders. It secretes a series of cell wall degrading enzymes (CWDEs), such as pectinase, cellulase, xylanase, etc., gradually weakening the strength of the tissue and making the vascular bundles prone to blockage and collapse. At the same time, toxins and

effector proteins like VdCP1 will also be released, quietly interfering with the host's metabolism and immune response, leaving loopholes in the plant's defense system. This is followed by yellowing of the leaves, Browning and necrosis of the vascular bundles, which often indicate the disruption of water transport. What is even more troublesome is that the microsclerotia remaining in the soil can re-enter dormancy and wait for the arrival of the next crop season, which also explains why yellow wilt is always difficult to be completely eradicated.

2.2 Physiological and cellular responses

The process of pathogen invasion is actually closely intertwined with the physiological changes of plants. The secretions from the roots of cotton first induce the germination of microsclerotia. The mycelium penetrates through the epidermis or pores, passes through the cortex, and eventually reaches the xylem vessels. All of this usually happens very quickly. In the vessels, the Trichoderma lanceolata continues to produce conidia, which move upward like an elevator along the transpiration flow, infecting the leaves and stems and causing systemic lesions.

During this period, cell wall degrading enzymes (CWDEs) released by pathogens, such as gliase, cellulase and xylanase, gradually relax the tissue structure (Umer et al., 2023). Toxins and effector proteins follow closely, and VdCP1 is one of them. It can interfere with metabolic pathways and signal transduction, weakening the host's immunity. When water cannot be transported smoothly, cotton will show typical symptoms such as wilting and leaf necrosis. And those tough microsclerotia, once they enter dormancy, can survive in the soil for many years, which also makes the prevention and control of yellow wilt a "long-term battle" in agriculture.

2.3 Host defense strategies

Cotton is not just waiting to die. It has both "innate" defenses and can be "temporarily mobilized". The former includes structural barriers such as thickening of the cell wall and secretion of antibacterial compounds; The latter, upon being stimulated by pathogen molecules (PAMPs) or effector proteins, initiates two layers of immune responses: mode-triggered immunity (PTI) and effector protein-triggered immunity (ETI).

Behind these defense responses, the synergy of three signaling channels is indispensable: salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). The SA pathway is often associated with systemic acquired resistance (SAR) and allergic reactions (HR), while JA and ET are more active in response to necrotic pathogens or mechanical injuries (Xiong et al., 2021a). Through proteomics research, many key roles have been identified: disease-related proteins (PR), phenylalanine metabolic enzymes such as PAL and POD, and secondary metabolic enzymes involved in lignin and flavonoid synthesis (Tang et al., 2019).

In addition, some transcription factors, such as WRKY, MYB and Bel1-like genes (GhBLH7-D06), are also regulating these defense networks. The MAPK cascade reaction and calcium signaling pathway are like command systems, coordinating the timing and intensity of various defense responses (Ma et al., 2020). Research generally holds that the metabolism of phenylpropyl and the synthesis of lignin are the key pathways for the differentiation of disease-resistant and susceptible varieties. Ultimately, the balance point of these complex defense systems determines the fate of a cotton plant: whether it survives infection or is invaded by the pathogen. The application of proteomics is gradually enabling us to uncover the underlying molecular logic of this resistance.

3 Proteomic Methodologies in Cotton Pathogen Research

3.1 Protein extraction and identification tools

In the research on the interaction between cotton and the Trichoderma lucidum, the first challenge often encountered is not analysis but extraction. The cotton tissue is rich in polysaccharides, phenols and various secondary metabolites, which makes protein purification extremely difficult. Researchers usually have to repeatedly explore methods. TCA/ acetone precipitation method, phenol extraction method, and buffer solution extraction method are all employed to obtain soluble protein components that are of controllable quality and suitable for subsequent analysis as much as possible.

After successful extraction, the study once relied on two-dimensional gel electrophoresis (2-DE) and two-dimensional differential gel electrophoresis (2D-DIGE) to observe the protein differences between infected and healthy tissues. However, these methods were gradually replaced by gel-free techniques later on. Nowadays, LC-MS/MS systems such as Orbitrap, Q-TOF or MALDI-TOF/TOF are more common, and they all have obvious advantages in sensitivity, coverage and reproducibility (Yang et al., 2019).

Quantitative proteomics has also undergone a similar transformation. Labeling techniques such as iTRAQ and TMT, as well as label-free quantification (LFQ) methods, have become mainstream. They enable researchers to precisely identify the key differential proteins involved in cell wall recombination, stress signaling and secondary metabolism during *V. dahliae* infection. The obtained results were then functionally annotated with the help of UniProt, NCBI or CottonGen databases, and the biological roles of these proteins were understood through GO and KEGG pathway mapping.

3.2 Advances in mass spectrometry and data analysis

The innovation of mass spectrometry technology has almost changed the research pace of plant proteomics. Low-abundance proteins that were difficult to detect in the past can now be reliably identified when cotton is under pathogen stress with the help of technologies such as DIA and SWATH-MS (Lu et al., 2022). These methods significantly broaden the dynamic range, and the results are more stable and closer to physiological reality.

In terms of data analysis tools, MaxQuant, Proteome Discoverer and Perseus remain the main players. They combine protein abundance with annotation information such as GO and KEGG, enabling researchers to sort out key biological processes such as oxidative stress, signal transduction, and secondary metabolism from complex data.

However, numbers and lists alone cannot explain all the issues. Thus, network analysis tools such as STRING or Cytoscape began to come into play. They can transform the interactions between proteins into network maps, from which the "core nodes" of defense responses can be identified. Such an integrated approach enables people to understand the defense signal mechanism of cotton against the Trichoderma lucidum from a systematic perspective and also provides a direction for the search of new disease-resistant proteins.

3.3 Limitations and opportunities

Although the application of proteomics has shown us many breakthroughs, the cotton-pathogen system remains a thorny issue. The protein types of cotton are complex and the genomic annotation is incomplete. Many low-abundance or regulated proteins are difficult to be stably detected (Bawa et al., 2022). Even if the technology is mature, minor differences in sample pretreatment, protein digestion, and mass spectrometry acquisition methods may still make it difficult to fully compare the results among studies.

However, these imperfections are precisely the breakthrough points for innovation. Nowadays, an increasing number of studies are attempting to integrate proteomics with transcriptomics, metabolomics, and even phosphorylomics to construct a systematic "defense panorama". In the future, data analysis involving single-cell proteomics, targeted validation techniques (SRM/MRM), and machine learning may make the quantification and prediction of disease-resistant proteins more accurate. In other words, the limitations still exist, but the direction is clear.

4 Case Study

4.1 Proteomic response of *Gossypium hirsutum* cultivar CRI 12 to *Verticillium dahliae*

In cotton research, CRI 12 (*Gossypium hirsutum* L. Terrestrial cotton) is often regarded as a representative of "moderate resistance". Its agronomic traits are stable. Although it is not completely immune to the Variegata officinalis, it can maintain relative tolerance. Therefore, it is widely used in the study of the resistance mechanism of Fusarium wilt. Researchers conducted a comparative proteomic analysis of leaf samples at different time points after inoculation with healthy controls. The results showed that there were significant fluctuations in the leaf protein composition of CRI 12, whether in terms of the initiation of defense responses or metabolic adjustments.

In the experiment, two-dimensional gel electrophoresis (2-DE) combined with liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to track these changes. In the early stage of infection, disease-related proteins (PR) and various antioxidant enzymes are rapidly activated, and components related to the phenylpropanin metabolic pathway are also upregulated, indicating that the basal defense is almost an instantaneous response. As the disease progresses, the number of proteins involved in signal transduction, protein folding and secondary metabolism keeps increasing, indicating that cotton cells gradually establish a coordinated defense system under continuous stress.

This proteomic remodeling over time is not a simple "enhancement", but rather a trade-off and balance: on the one hand, maintaining energy supply and cellular homeostasis, while on the other hand, it is necessary to limit the spread of pathogens. The performance of CRI 12 indicates that it achieves a dynamic integration between constitutive and inducible defense, making the defense response both timely and not excessive (Xing et al., 2024).

4.2 Key findings

From the proteomic changes of CRI 12, several key aspects of the confrontation between cotton and pathogens can be observed. The first is the activation of defense proteins. PR proteins such as chitinase, β-1, 3-glucanase and sweet proteins accumulate in large quantities. They can destroy the cell walls of fungi and at the same time strengthen the host's own barrier. Secondly, the full activation of the antioxidant system is also very obvious. The increased expression levels of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) indicate that the reactive oxygen species (ROS) levels are strictly controlled to reduce cellular oxidative damage and maintain reducing balance.

Meanwhile, the primary metabolism has shown a "stepping aside" phenomenon. The proteins related to photosynthesis and carbon metabolism decline, and energy and resources are redistributed to the defense direction to support the synthesis of lignin and flavonoids. The secondary metabolic pathways were significantly activated, and enzymes such as phenylalanine ammoniase (PAL) and chalketone synthase (CHS) were enhanced in expression, providing raw materials for structural reinforcement and the accumulation of antibacterial substances (Xiao et al., 2023). The dynamic changes of signals and folding proteins should not be ignored either. Calcium-binding proteins, heat shock proteins (HSP70, HSP90), and 14-3-3 proteins accumulate differentially at different stages. These molecules are involved in stress signal transduction and regulation of protein folding homeostasis, and are important nodes for maintaining the orderly operation of the defense network (Figure 2) (Zhou et al., 2022).

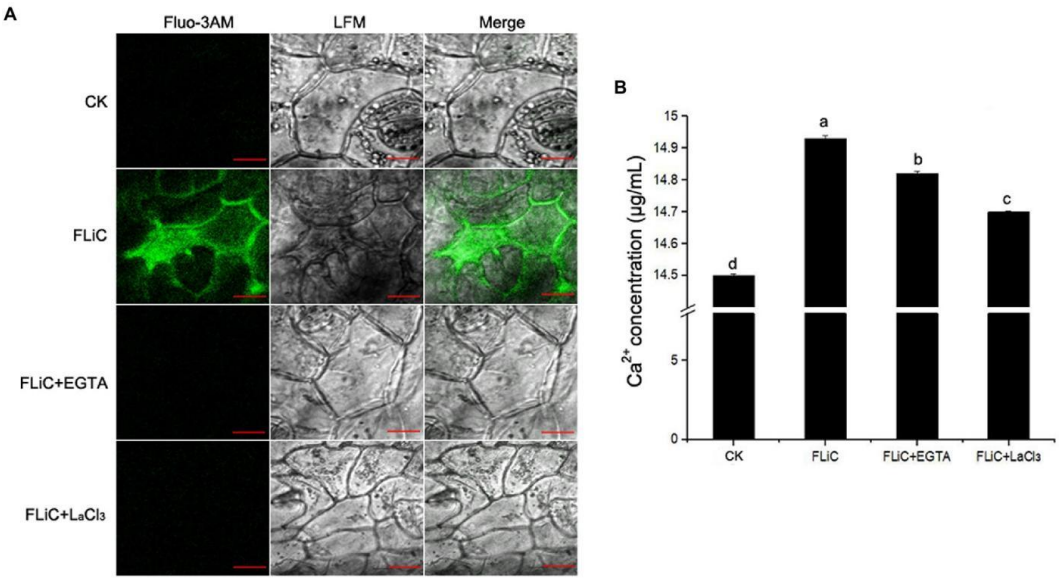

Figure 2 FLiC induces Ca^{2+} production in upland cotton epidermal cells. (A) FLiC induces increased intracellular Ca^{2+} content. (B) Intracellular Ca^{2+} quantification. The data represent the means ± SDs; $n = 3$. One-way ANOVA ($p < 0.05$) followed by Duncan's test was used for multiple comparisons. The different letters indicate significant differences at the 0.05 probability level. Bars = 50 μm (Adopted from Zhou et al., 2022)

CRI 12 exhibits a hierarchical defense model: it not only recognizes pathogens but also makes rapid adjustments at the metabolic level. This multi-level protein response enables it to maintain a relative physiological balance when infected, thereby slowing down the progression of the disease.

4.3 Implications and applications

The research on CRI 12 not only revealed the response logic of resistant cotton, but also provided molecular clues that can be directly applied to breeding. Differential proteins such as PR protein, phenylalaninase and ROS regulatory factors may serve as markers for disease resistance screening, providing support for subsequent marker-assisted breeding. Through these molecular characteristics, researchers can more specifically screen disease-resistant materials and even achieve precise editing at the genetic level.

In addition, some stress chaperone proteins and signal regulatory molecules discovered in the research are also regarded as potential genetic engineering targets. The stability of plant defense responses may be enhanced in the future through transgenic or CRISPR-mediated regulation (Li et al., 2019a).

When proteomics is integrated with transcriptomic, metabolomic and other data, a more complete immune regulatory network gradually emerges. Such system-level analysis not only explains resistance but also provides tools for precise breeding and sustainable control.

The case of CRI 12 clearly demonstrates that proteomics is no longer merely a means of observing molecular changes, but a path to understanding the panorama of plant-pathogen interactions. It has led resistance breeding from experience to mechanism, and also made the prevention and control ideas of Fusarium wilt more forward-looking.

5 Functional Categorization of Differentially Expressed Proteins

5.1 Defense and stress-related proteins

Among the cotton (*Gossypium hirsutum*) infected with *Verticillium dahliae*, defense proteins are always the first group to be "alarmed". Proteomic analysis has repeatedly shown that these proteins almost determine whether cotton can withstand the first round of pathogen attack. The most obvious changes come from disease-related proteins (PR), among which the upregulation of PR-1, chitinase, β-1, 3-glucanase and sweet-like proteins is the most significant. Their functions are simple and direct, that is, to weaken the cell wall of fungi and at the same time strengthen the host's own defense barrier.

But the reaction of cotton did not stop there. Infection is often accompanied by a sharp increase in reactive oxygen species (ROS), and thus the antioxidant system is fully activated. Superoxide dismutase (SOD), catalase (CAT), ascorbic acid peroxidase (APX), and peroxidase (POD) take turns to be used to eliminate ROS and stabilize the REDOX balance within cells (Wang et al., 2019). Without them, oxidative damage to lipids and proteins is sufficient to cause the collapse of cell structure.

Meanwhile, the expression of heat shock proteins (HSP70, HSP90) and molecular chaperones also increased accordingly. They act like "emergency workers", helping damaged proteins refold and maintaining the stability of the entire proteome. Glutathione S-transferase (GSTs) and lipoxygenase (LOXs) are involved in detoxification and lipid signaling pathways, further strengthening the resistance network.

It can be said that the combined effect of these proteins is like building a biochemical "firewall", enabling cotton to maintain cell integrity when pathogens invade and resist the chain reactions triggered by oxidative stress.

5.2 Metabolic reprogramming proteins

Once an infection occurs, the metabolic activities of cotton are almost "re-planned". After the invasion of *V. dahliae*, proteins related to photosynthesis (such as the RuBisCO subunit, oxygen-releasing enhancer protein, and chlorophyllin binding protein) are generally downregulated, and plants seem to actively slow down their growth, shifting energy to defense.

Meanwhile, the key enzymes involved in carbohydrate and amino acid metabolism experienced fluctuations. The activity changes of glyceraldehyde-3-phosphate dehydrogenase, sucrose synthase and enolase, in combination with the regulation of glutamine synthase and aspartate aminotransferase, jointly maintained the carbon flow balance and also provided raw materials for the synthesis of defensive compounds (Li et al., 2019b).

The focus of metabolism has gradually shifted to the synthesis of phenylpropyl and flavonoids. Enzymes such as phenylalanine ammonia-lyase (PAL), caffeyl-coA O-methyltransferase (CCoAOMT), and chalketone synthase (CHS) are expressed in large quantities, which enables the rapid accumulation of lignin and antibacterial compounds. These secondary metabolites can thicken the cell wall and inhibit the spread of pathogens, marking the shift of cotton's metabolic strategy from "growth first" to "defense first", which is a typical feature of adaptive resistance.

5.3 Signaling and transcription regulators

The response of cotton to the Trichoderma lucidum is not merely a structural "reinforcement", but rather a more complex dialogue at the signal level. Calcium signals are always the first to be activated. Calmodulin (CaM) and calcium-dependent protein kinase (CDPK) accumulate rapidly, triggering a series of early responses and opening the "switch" for subsequent defense genes.

Subsequently, the mitogen-activated protein kinase (MAPK) cascade makes its appearance. This system amplifies immune signals like a megaphone, promoting the activation of defense-related transcription factors. 14-3-3 proteins play the role of coordinators in this process, regulating stress responses, controlling the activity of metabolic enzymes, and preventing excessive defense responses.

At the transcriptional level, factors such as WRKY, MYB, NAC and bZIP play core roles. For instance, GhWRKY70 promotes resistance through the jasmonic acid (JA) pathway, while GhWRKY55 exerts inhibitory effects in regulating lignin and JA synthesis (Ma et al., 2024). The waxing and waning between them reflect the meticulous balance of cotton between defense and metabolism.

Finally, it is necessary to mention the interactive regulation of hormones. Salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) do not act independently. The interweaving of signals among them determines the direction and intensity of defense. Sometimes it activates allergic reactions (HR), and sometimes it leans towards systemic acquired resistance (SAR). How to switch between the two is precisely the key to maintaining a balance in disease resistance.

6 Comparative Proteomics: Susceptible vs. Resistant Cultivars

6.1 Defense protein profiles

Among different cotton genotypes, the gap between disease resistance and susceptibility can often be discerted at the protein level. The results of comparative proteomics show that the accumulation rate and extent of defense proteins almost determine the ability of cotton to resist the Trichoderma lucidum. Resistant varieties such as CRI 12, Zhongzhi Cotton No. 2, and Hai7124 Island cotton can activate disease-related proteins (PR) earlier and more strongly than disease-susceptible materials like Ape Cotton No. 3 or Jimian No. 11. Among them, chitinase, β-1, 3-glucanase, and PR-10 proteins are the most prominent. These proteins can directly degrade the cell walls of fungi. Slow down its diffusion in the vascular bundles.

Meanwhile, the antioxidant systems of resistant varieties are also more "alert". The expression levels of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbic acid peroxidase (APX) are higher, which help eliminate excess reactive oxygen species (ROS) during infection and maintain the REDOX balance of cells. In addition, the upregulation of heat shock proteins (HSP70, HSP90) and glutathione S-transferase (GSTs) makes the entire protein network more stable and detoxifying.

In susceptible varieties, the activation of the defense system is often delayed. Slow response of PR protein and low level of antioxidant enzymes result in uncontrolled oxidative stress and accelerated fungal reproduction (Zhu et al., 2021). Therefore, the key to the difference in resistance does not seem to lie merely in "whether to respond",

but in "when to respond". Only when the response is early and the amplitude is large can there be a chance to maintain the stability of the proteome and immune balance.

6.2 Key metabolic and signaling differences

The differences at the metabolic level are equally distinct. Resistant varieties often voluntarily sacrifice a portion of their photosynthesis. The down-regulation of RuBisCO subunits and oxygen-releasing enhancers indicates that they shift energy from growth to defense (He et al., 2022). Meanwhile, the activities of enzymes such as phenylalanine aminase (PAL), cinnamyl alcohol dehydrogenase (CAD), and caffeyl coA O-methyltransferase (CCoAOMT) increased significantly. These enzymes directly participate in lignin synthesis, reinforce vascular tissue, and prevent pathogen invasion (Xiong et al., 2021b). Histochemical tests also confirmed that the resistance of Hai 7124 and Zhongzhi Mian No. 2 is closely related to their stronger lignification.

At the level of signal regulation, resistant varieties have stronger "coordination". Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) related pathways are active, forming a tight regulatory network with each other, which can not only trigger systemic acquired resistance (SAR), but also maintain local defense. Calcium signaling molecules, such as calmodulin (CaM) and calcium-dependent protein kinase (CDPK), cooperate with the MAPK cascade reaction to jointly amplify defense signals (Zhang et al., 2024).

However, susceptible varieties seem to be "disconnected" at this stage. The crosstalk between the SA and JA pathways is relatively weak, and the activation of transcription factors such as WRKY, MYB, and NAC is insufficient, leading to the interruption of the signal transduction chain and a slow response of defense genes. This is also the fundamental reason why it is prone to getting out of control after infection.

6.3 Breeding implications

This difference is not merely a theoretical issue but a starting point for disease-resistant breeding. The defense proteins revealed by the comparative proteome, such as PR protein, antioxidant enzymes, and enzymes related to phenylpropanin metabolism, are all ideal molecular markers for screening resistant materials.

For instance, overexpression of the lignin-related laccase gene *GhLAC15* or the phenylalanine enzyme *Gh4CL30* can significantly enhance the resistance of cotton, enabling it to maintain a higher lignification level and antioxidant capacity under the infection of Wilt yellow. If proteomic data can be combined with transcriptomic and metabolomic results, more quantitative trait loci (QTLS) and regulatory networks related to resistance can be located, providing more precise targets for molecular breeding.

In the future, molecular marker-assisted selection (MAS) based on multi-omics fusion will be combined with CRISPR/Cas gene editing, opening up a new path for breeding broad-spectrum and long-lasting disease-resistant cotton varieties. The ultimate goal is not only to enhance resistance, but also to free cotton from its reliance on chemical control and achieve a more sustainable production method.

7 Future Directions in Cotton Proteomic Research

7.1 Integrative omics approaches

The research on cotton proteomics seems to have reached a stage where "studying alone is not sufficient". True breakthroughs often occur in its combination with other omics, such as genomics, transcriptomics, metabolomics, phosphorylomics and even epigenetics. When these data are integrated together, a more complete molecular picture can be pieced together to explain the resistance mechanism of cotton to the Variegata.

Proteomics is good at capturing changes, especially the dynamic responses of proteins under stress. However, if it is compared with the results of the transcriptome or metabolome, it is possible to distinguish which are the results of transcriptional regulation and which are the offsets caused by post-translational modifications, thereby obtaining a more comprehensive understanding. In recent years, platforms such as the "Multi-omics Database of Wheel-Plant Interactions (PPI-MD)" have provided valuable examples: when genomic, proteomic and metabolomic data are placed under the same analytical framework, key defense regulatory factors tend to be more easily identified (Shi et al., 2025).

In addition, the analysis of phosphorylation and acetylation proteomes also revealed more microscopic-level regulations, such as the activation, localization and degradation patterns of proteins during pathogen infection (Wu et al., 2021). Coupled with systems biology tools such as weighted co-expression network analysis (WGCNA) or PPI networks, researchers can now identify the "central nodes" that regulate defense systems more quickly (Zhang et al., 2025). These interwoven data networks are gradually providing actionable resistance biomarkers for cotton molecular breeding.

7.2 Functional validation and genetic engineering

Identifying defense proteins is just the beginning. What exactly they "do in the body" still needs to be verified through experiments. To establish a true causal relationship, functional research is still needed. Researchers typically validate the functions of candidate proteins in cotton or model plants (such as Arabidopsis thaliana and tobacco Bursa) using virus-induced gene silencing (VIGS), gene overexpression, or RNA interference (RNAi) (Liu et al., 2022).

Many genes have been verified to be closely related to resistance, such as those encoding PR protein, antioxidant enzymes and components of the phenylpropanin metabolic pathway. After artificial regulation, the disease resistance of cotton has been significantly enhanced. Furthermore, the precise editing of CRISPR/Cas9 makes it possible to regulate defense factors (such as WRKY, MYB, NAC and other transcription factors), thereby making the regulation of immune responses more controllable.

In addition, the ideas of synthetic biology are also entering this field. By constructing regulatory circuits that can respond to pathogen signals, dynamic expression of defense genes can be achieved. This approach, combined with proteomics results, is bridging the gap between "laboratory mechanisms" and "field performance", making the application of cotton biotechnology more practical.

7.3 High-throughput applications in breeding

Over the past few years, high-throughput technology has completely transformed the way proteomics works. Label-free quantification and the DIA/SWATH-MS platform enable researchers to detect thousands of proteins in a single experiment and compare the differences between disease-resistant and susceptible varieties. More importantly, these proteomic features can be directly associated with disease-resistant phenotypes, thus making the data "breeding".

When these results are combined with quantitative trait loci (QTL) mapping or genome-wide association studies (GWAS), the recognition speed of resistance-related proteins and molecular markers is greatly enhanced, and the efficiency of marker-assisted selection (MAS) and genomic selection (GS) also improves accordingly.

Nowadays, the construction of cotton proteome databases and the introduction of AI algorithms have opened up new channels for research. Machine learning can help identify patterns, predict resistance traits, and integrate complex omics data (Luqman et al., 2025). This data-driven approach is leading us from "seeing changes" to "predicting changes", providing a practical path for designing cotton varieties with broad-spectrum and long-lasting resistance in the future.

8 Concluding Remarks

Over the past decade, the research on cotton resistance to the Variegata has almost been completely reshaped by "omics". The introduction of proteomics has enabled us for the first time to observe the true dynamics of the defense system at the molecular level, those protein networks that are activated, rearranged and rebalance during the infection process. Researchers have found that the upregulation of disease-related proteins (PR), antioxidant enzymes, and secondary metabolism-related enzymes is the most prominent indicator of the entire resistance response. They work together to strengthen the cell wall, eliminate reactive oxygen species (ROS), and reduce oxidative damage, forming the core barrier for cotton to resist pathogens. The significance of the phenylpropanin metabolism and lignin synthesis pathways has also been repeatedly confirmed. These two pathways maintain the integrity of the vascular bundles and limit the range of pathogen spread.

Meanwhile, the integration of transcriptome and ChIP-seq studies further revealed that transcription factors such as WRKY, MYB, and NAC, especially GhWRKY41 and GHWRKY1-like proteins, play a key role in regulating lignification and phenylpropanin metabolism. Comparative proteomics results show that resistant varieties respond more rapidly and harmonically in terms of defense protein activation, metabolic reprogramming, and hormone signaling (jasmonic acid, salicylic acid, ethylene). It can be seen from this that resistance is not the product of a single gene, but rather the result of a high degree of coordination of the proteome in the spatiotemporal dimension.

However, there are still many shortcomings in the research. The proteome of cotton is extremely complex. Polyploid structure, tissue specificity, and various post-translational modifications (PTMs) all make comprehensive coverage difficult. At present, many experiments still rely on two-dimensional electrophoresis (2-DE), whose resolution and dynamic range are significantly inferior to new techniques such as LC-MS/MS or DIA. Furthermore, the lack of a unified proteome database and standardized procedures for cotton makes it difficult to compare data from different laboratories. The functional annotations of a large number of identified proteins are still incomplete. In addition, most studies only focus on a certain stage or tissue of infection, and the temporal and spatial resolution of pathogen-host interaction is limited. Although single-cell proteomics, spatial omics and other methods have emerged, they are still rare in the cotton system. More importantly, although PTMs such as phosphorylation, ubiquitination and glycosylation are crucial for the regulation of the activity of defense proteins, they have still been studied far from sufficient. Among the candidate resistance proteins that have been discovered, only a few have been verified by CRISPR, VIGS or overexpression.

Looking ahead, the direction of cotton proteomics is quietly shifting, from single determination to multi-omics integration, and from phenomenon description to functional verification and application transformation. Combining the proteome with the genome, transcriptome, metabolome and phosphorylation group is expected to reconstruct the disease resistance network of cotton at the systemic level. New technologies such as DIA/SWATH-MS and single-cell proteomics will further enhance the detection sensitivity and spatiotemporal accuracy. Meanwhile, if these findings can be combined with CRISPR/Cas9 gene editing, RNAi interference and synthetic regulatory circuits, it will not only verify the functions of defense-related proteins such as Gh4CL3, GhLac1 and GhWRKY41, but also accelerate the process of disease-resistant breeding.

From the perspective of breeding, resistance markers obtained through proteomic-genome integration are gradually becoming the core tools for marker-assisted selection (MAS) and genome selection (GS). With the integration of machine learning and artificial intelligence into the analysis process, the prediction and screening of resistance phenotypes will become more precise. Perhaps in the near future, we will be able to rely on data-driven breeding models to cultivate cotton varieties that are both high-yielding and have long-lasting resistance without the need for chemical agents, achieving true sustainable improvement.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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