

A Novel Fungal Metabolite 1-Methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic Acid Enhances Apple Resistance to *Glomerella cingulata* through Activation of the Phenylpropanoid Pathway and Lignin Biosynthesis

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ABSTRACT: Fungal metabolites with diverse structures serve as valuable natural resources for green biopreparations and immune elicitors in sustainable agricultural biocontrol. As a small-molecule metabolite derived from the edible fungus *Pholiota adiposa*, 1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid (MCA) has been reported to enhance stress tolerance and plant growth, yet its potential and mechanism against fruit tree fungal diseases remain unclear. This study investigated MCA-mediated resistance to *Glomerella* leaf spot (GLS) in apples. MCA pretreatment significantly alleviated lesion formation in apple leaves and fruits, upregulated immunity associated genes and increased defense-related enzyme activities. Transcriptomic analysis revealed that MCA activated the phenylpropanoid pathway, elevated lignin biosynthetic gene expression, and promoted lignin accumulation and cell wall reinforcement, while also boosting apple seedling growth. As the first application of MCA as a novel immune elicitor in fruit trees, these findings confirm its dual functions of disease resistance and growth promotion, expanding MCA's application scope and providing theoretical support for green sustainable apple production.

KEYWORDS: 1-methyl-1,2,3,4-tetrahydro- β -carboline-3-carboxylic acid, plant immune elicitors, plant disease resistance, *Glomerella cingulata*, lignin

1. INTRODUCTION

Fungal metabolites are widely derived from various fungal species including edible and biocontrol fungi, serving as a rich reservoir of natural bioactive substances for plant health regulation.^{1,2} They promote plant growth and enhance stress adaptability under normal and stressful conditions by directly inhibiting mycelial growth and spore germination of pathogenic microorganisms and disrupting pathogen cell structure and physiological metabolism, and indirectly improve host defense capacity by activating plant immune signaling pathways, regulating growth- and defense-related phytohormone homeostasis, and boosting host antioxidant system activity.^{3–5} These fungal metabolites cover diverse structural categories such as polyketides, terpenoids, alkaloids and polypeptides, and exert pleiotropic biological functions that modulate plant growth and development, enhance biotic and abiotic stress resistance, and elevate cellular antioxidant capacity.^{6–8} Recent efforts devoted to the isolation, structural identification, bioactivity screening and mechanistic investigation of these metabolites provide valuable material resources and theoretical support for developing green, efficient and eco-friendly biogenic preparations, plant immune elicitors and functional bioactive agents, with promising application prospects in sustainable agricultural production and biological control.^{9,10} Notably, bioactive fungal metabolites with immune induction activity are a class of potential

plant immune elicitors that have attracted increasing attention in agricultural biocontrol research.

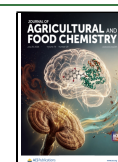
Plant immune elicitors function primarily by modulating endogenous signaling pathways to activate the plant's innate immune system.¹¹ Although they lack direct biocidal activity, these agents effectively induce robust defense responses against both biotic stresses and abiotic stresses. Besides, these compounds can boost plant growth, improve crop yield and quality, reduce dependence on synthetic pesticides, and lower chemical residues, making them valuable for sustainable and organic agricultural production.^{12,13} Their mechanisms of action have been well-elucidated: they activate surface receptors and signaling cascades to regulate the production of pathogenesis-related (PR) proteins and the accumulation of secondary metabolites, thereby blocking pathogen infection and movement. Simultaneously, they stimulate cellular growth processes to enhance the tolerance of infected plants.¹⁴ Polysaccharides, including chitosan,^{15,16} oligosaccharins,¹⁷ algal polysaccharides,¹⁸ and β -glucans,¹⁹ represent the most

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Quercetin 2,3-dioxygenase genes *VmQDO1* and *VmQDO2* of *Valsa mali* utilizes flavonoid to produce two key toxins for pathogenesis

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ABSTRACT

Apple *Valsa* canker, caused by the necrotrophic fungus *Valsa mali*, is a devastating disease in East Asia. *V. mali* employs cell wall-degrading enzymes and toxins to induce cortical necrosis, while also degrading host flavonoids, key plant defense metabolites, to facilitate infection and toxin production. Here, we identified two quercetin 2,3-dioxygenase genes, *VmQDO1* and *VmQDO2*, in *V. mali*, which are significantly upregulated during infection and in response to quercetin and rutin. *VmQDO1* preferentially degrades quercetin, achieving 96.10 % degradation within 3 h, while *VmQDO2* preferentially targets rutin, with a degradation rate 3.25-fold higher than *VmQDO1*. Although *VmQDOs* (refers to *VmQDO1* and *VmQDO2*) do not affect fungal growth, their knockout significantly reduces pycnidia production, lesion lengths on apple branches by 43.13 % and 56.86 % and leaves by 20.00 % and 28.00 %, and levels of the toxin 3-hydroxybenzoic acid by 45.38 % and 30.18 %. Double knockout mutants exhibit even greater reductions in virulence and toxin production compared to single mutants. *VmQDOs* represent the first reported genes in plant pathogenic fungi specifically degrading quercetin. *VmQDOs* disrupt host defense responses by degrading flavonoids, furthermore, the small-molecule metabolites like toxins generated from this degradation process destroying host cells, offering critical insights into *V. mali*'s pathogenicity and potential targets for disease control. This study advances our understanding of fungal virulence mechanisms and provides a foundation for developing novel antifungal strategies.

1. Introduction

Apple (*Malus domestica*), one of the most widely cultivated fruits globally. As a common fruit, apples are rich in various bioactive components [1,2]. Flavonoid compounds in apples include quercetin, myricetin, kaempferol, rutin, and proanthocyanidins [3,4]. Quercetin as a natural flavonoid with strong antioxidant properties, capable of scavenging free radicals. Rutin is the glycoside form of quercetin. The glucose in rutin is β -D-glucopyranose. As a constituent unit of the disaccharide rutinose, it is linked to the 3-position hydroxyl group of quercetin via a β -O-1-3 glycosidic bond at one end, to rhamnose via an α -O-1-6 glycosidic bond at the other end, and has no additional chemical modifications itself, formed by the combination of quercetin with a glucose molecule, which has better water solubility and bioavailability [5,6].

Flavonoids are a class of natural compounds widely found in plants,

belonging to the polyphenol group [7]. They are commonly found in fruits, vegetables, tea, red wine, and some grains [8]. Flavonoids not only give plants their color but also play important roles in plant growth, pest resistance, and defense against pathogenic infections [9,10]. The role of flavonoids in plants' resistance to pests and diseases, stress tolerance, and growth promotion makes them an important focus in agricultural research. By breeding plant varieties rich in flavonoids or applying flavonoid compounds, it is possible to enhance crop resilience and yield [10].

Currently, there are many reports on the antibacterial activity of flavonoids [11]. In *Arabidopsis*, increased levels of anthocyanins and flavonoids enhance resistance to *Pieris brassicae*. *Sclerotinia sclerotiorum* evade flavonoid defenses by decomposing flavonoids glycosides and aglycones [12]. Hesperidin inhibits the growth of the rice pathogen *Xanthomonas oryzae* [13]. Additionally, pathogenic infections often induce local accumulation of flavonoids, such as the increase of

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RESEARCH

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β -Glucosidase VmGlu1 is required for toxin production and pathogenicity of *Valsa mali*

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Abstract

Flavonoids, serving as crucial secondary metabolites, are widely distributed in plants and play a key role in inhibiting microbial growth, protecting plants from pathogen invasion, and conserving energy. Our previous study revealed that the β -glucosidase VmGlu2 effectively catalyzes the degradation of phloridzin, a typical flavonoid in apples, and regulates the pathogenic process of *Valsa mali*. However, VmGlu2 exhibits a lower efficiency in degrading rutin, another characteristic flavonoid in apples. In this study, we found that the expression level of the β -glucosidase gene *VmGlu1* was induced by 9.42-fold following rutin treatment, and VmGlu1 possessed the capability to degrade both rutin and phloridzin, but under the same conditions, VmGlu1 showed a distinct preference for rutin degradation, with an initial degradation rate 8.66 times higher than that of VmGlu2. Targeted deletion of *VmGlu1* resulted in a 99.32% reduction in pycnidia production, a nearly 33% decrease in toxin production, and a significant decrease in pathogenicity on apple twigs and leaves. Furthermore, we verified that VmGlu1 and VmGlu2 exhibit functional redundancy in pycnidia production, toxin-related β -glucosidase activity, and pathogenicity by generating double-deletion mutants. The results of this study provide valuable insights into the roles of VmGlus in the interaction of *V. mali* and apple during the infection process.

Keywords *Valsa mali*, β -glucosidase, Flavonoids, Pathogenicity

Background

Apple tree canker caused by *Valsa mali* leads to severe damage to the tree trunk, which can extend to the entire tree, potentially resulting in orchard destruction and significant reductions in apple yield, posing a substantial

threat to the apple industry (Wang et al. 2014). The pathogen infects the woody parts of apple branches and rapidly proliferates in the xylem vessels, making complete removal of the pathogen from the wood during the treatment challenging, which contributes to the recurrence of old lesions (Wang et al. 2012; Li et al. 2017; Wang et al. 2018).

Necrotrophic fungi, including *V. mali*, are capable of infecting living cells and tissues, as well as thriving within host tissues. A key pathogenic strategy involves secreting enzymes and synthesizing toxins that weaken host tissue by degrading the cell wall, facilitating fungal invasion (McCombe et al. 2022; Fei and Liu 2023). Therefore, the pathogenic mechanism of *V. mali* is considered complex. Previous studies have shown that the cell wall-degrading enzymes (CWDEs), such as xylanase, pectinase and β -glucosidase, along with toxins like protocatechuic acid

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Xylanase VmXyl2 is involved in the pathogenicity of *Valsa mali* by regulating xylanase activity and inducing cell necrosis

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Xylanase plays a key role in degrading plant cell wall during pathogenic fungi infection. Here, we identified a xylanase gene, *VmXyl2* from the transcriptome of *Valsa mali* and examined its function. *VmXyl2* has highly elevated transcript levels during the infection process of *V. mali*, with 15.02-fold increase. Deletion mutants of the gene were generated to investigate the necessity of *VmXyl2* in the development and pathogenicity of *V. mali*. The *VmXyl2* deletion mutant considerably reduced the virulence of *V. mali* in apple leaves and in twigs, accompanied by 41.22% decrease in xylanase activity. In addition, we found that *VmXyl2* induces plant cell necrosis regardless of its xylanase activity, whereas promoting the infection of *V. mali* in apple tissues. The cell death-inducing activity of *VmXyl2* dependent on BRI1-associated kinase-1 (BAK1) but not Suppressor of BIR1-1 (SOBIR1). Furthermore, *VmXyl2* interacts with Mp2 *in vivo*, a receptor-like kinase with leucine-rich repeat. The results offer valuable insights into the roles of *VmXyl2* in the pathogenicity of *V. mali* during its infection of apple trees.

KEYWORDS

Valsa mali, xylanase VmXyl2, inducing cell necrosis, protein interaction, apple tree

Introduction

Apple is a globally significant crop; however, the branch disease severely hampers the progress of the apple industry and leads to substantial economic losses for fruit farmers. *Valsa mali*, the pathogen responsible for apple tree canker, is a harmful fungus that primarily infects the branches and trunks of apple trees (Li et al., 2013). This pathogen mainly infects through the dead epidermal tissues and different types of wounds, particularly pruning wounds

ORIGINAL ARTICLE

A novel MAP kinase-interacting protein MoSmi1 regulates development and pathogenicity in *Magnaporthe oryzae*

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Abstract

The cell wall is the first barrier against external adversity and plays roles in maintaining normal physiological functions of fungi. Previously, we reported a nucleosome assembly protein, MoNap1, in *Magnaporthe oryzae* that plays a role in cell wall integrity (CWI), stress response, and pathogenicity. Moreover, MoNap1 negatively regulates the expression of MoSMI1 encoded by MGG_03970. Here, we demonstrated that deletion of MoSMI1 resulted in a significant defect in appressorium function, CWI, cell morphology, and pathogenicity. Further investigation revealed that MoSmi1 interacted with MoOsm1 and MoMps1 and affected the phosphorylation levels of MoOsm1, MoMps1, and MoPmk1, suggesting that MoSmi1 regulates biological functions by mediating mitogen-activated protein kinase (MAPK) signalling pathway in *M. oryzae*. In addition, transcriptome data revealed that MoSmi1 regulates many infection-related processes in *M. oryzae*, such as membrane-related pathway and oxidation reduction process. In conclusion, our study demonstrated that MoSmi1 regulates CWI by mediating the MAPK pathway to affect development and pathogenicity of *M. oryzae*.

KEYWORDS

appressorium formation, cell wall integrity, pathogenicity, regulation, rice blast fungus

1 | INTRODUCTION

Rice blast, caused by *Magnaporthe oryzae*, is one of the most destructive fungal diseases that decrease rice yield and seriously threaten food security (Dean et al., 2012). *M. oryzae* infection begins with a three-celled conidium. The conidium attaches to the host leaf surface and develops into a germ tube within 2 h. Subsequently, a domed-shaped structure called the appressorium differentiates

from the tip of the germ tube (Hamer et al., 1988; Hamer & Talbot, 1998; Howard & Valent, 1996). With the accumulation of high concentrations of glycerol, the appressorium generates sufficient turgor pressure to form an infection peg and penetrates the host cell (Howard et al., 1991). Subsequently, typical necrotic spots of rice blast appear on plant surfaces after about 5 days. Finally, the invasive hyphae spread within plant cells, resulting in typical lesions, and the secondary conidia spread the disease to adjacent plants (Gupta et al., 2021). In the process by which the rice blast fungus infects the

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RESEARCH ARTICLE

Protein disulfide isomerase MoPdi1 regulates fungal development, virulence, and endoplasmic reticulum homeostasis in *Magnaporthe oryzae*

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Highlights

- MoPdi1 is involved in regulation of ER homeostasis of *M. oryzae*.
- MoPdi1 contributes to regulating the formation of intermolecular disulfide bonds in substrate proteins of *M. oryzae*.
- MoPdi1 affects the secretion of cytoplasmic effectors in *M. oryzae*.

Abstract

Rice blast, caused by *Magnaporthe oryzae*, is a fungal disease that causes devastating damage to rice production worldwide. During infection, pathogens secrete effector proteins that modulate plant immunity. Disulfide bond formation catalyzed by protein disulfide isomerases (PDI) is essential for protein folding and maturation. However, the biological function of Pdi1 in *M. oryzae* has not yet been characterized. In this study, we identified the endoplasmic reticulum (ER)-located protein, MoPdi1, in *M. oryzae*. MoPdi1 regulates conidiation, cell wall stress, and pathogenicity of *M. oryzae*. Furthermore, the CGHC active sites in the a and a' redox domain of MoPdi1 were essential for the biological function of MoPDI1. Further tests demonstrated that MoPdi1 was involved in the regulation of ER stress and positively regulated ER phagy. We also found that MoPdi1 interacted with MoHut1. Deletion of *MoPDI1* led to the bereft of MoHut1 dimerization, which depends on the formation of disulfide bonds. In addition, MoPdi1 affected the normal secretion of the cytoplasmic effector AVR-Pia. We provided evidence that MoHut1 is important for the vegetative growth, conidiation, and pathogenicity in *M. oryzae*. Therefore, our findings could provide a suitable target point for designing antifungal agrochemicals against rice blast fungus.

Keywords: *Magnaporthe oryzae*, protein disulfide isomerase, MoPdi1, fungal development, pathogenesis

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