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**The chitin deacetylase *TlCDA* from *Tilletia laevis* facilitates pathogen
infection by suppressing plant immunity**

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Abstract

Background: Common bunt of wheat, caused by *Tilletia laevis* Kühn, is one of the most devastating wheat diseases worldwide, prevalent in wheat-growing regions across the globe. The diseased wheat grains are replaced by galls and emit a fishy odor, seriously affects the yield and quality of flour. **Results:** In this study, TICDA was identified from *T. laevis* and shown to possess chitin deacetylase activity, which is upregulated during the early infection stage of *T. laevis* in wheat. Transient expression of *TICDA* inhibits programmed cell death, suppressed the accumulation of reactive oxygen species and reduced the expression level of defense-related genes in *Nicotiana benthamiana*, indicating that *TICDA* can promote the infection of *T. laevis* by inhibiting immune defense response in plant. Deletion of *TICDA* suppressed the growth and pathogenicity of *T. laevis*, highlighting the significance of *TICDA* in the host-pathogen interaction. Moreover, *TICDA* can protect teliospores in *T. laevis* from being damaged by chitinase in vitro. **Conclusions:** Collectively, these findings identify TICDA as a key virulence factor of *T. laevis* and a promising target for chemical control of common bunt.

Key words: *Tilletia laevis*, Chitin deacetylase, Plant immunity

1. Introduction

In the interaction between plants and pathogens, the plant immune system typically recognizes pathogen-associated molecular patterns (PAMPs) through pattern-recognition receptors (PRRs), thus initiating pattern-triggered immunity (PTI) (Wang et al., 2008; Dodds and Rathjen, 2010; Oliveira-Garcia and Valent, 2015; Buscaill and Van, 2021). PAMPs are usually conserved molecules specific to pathogenic microorganisms (Zhang and Zhou, 2010). Chitin, a β 1,4 - linked N-acetylglucosamine biopolymer present in the fungal cell wall, is a well-known PAMP (EI Gueddari et al., 2002; Liu et al., 2013; Oliveira-Garcia and Valent, 2015; Sánchez-Vallet et al., 2015; Gong et al., 2020). When attacked by fungi, plants secrete chitinases into the apoplast region to degrade chitin, release chitin oligomers, and disrupt the fungal cell wall (Gueddari et al., 2002; Shimizu et al., 2010; Lee et al.,

2014; Fiorin et al., 2018; Volk et al., 2019; Han et al., 2019). These chitin oligomers are sensed by specific cell-surface receptors containing a lysin motif (Lysm) in plants, and activate intracellular immune signaling through mechanisms such as the mitogen-activated protein kinase (MAPK) pathway, initiating PTI (Liu et al., 2012; Bigeard et al., 2015; Xu et al., 2017; Andersen et al., 2018; Figueroa et al., 2021). The PTI response triggers the activation of the MAPKs, the production of reactive oxygen species (ROS), callose deposition, and the expression of immune-response genes, ultimately preventing fungal colonization (Kämper et al., 2006; Torres et al., 2006; Kinoshita and Seki, 2014; Li et al., 2016).

In order to evade the host immune mechanisms, pathogens have evolved a variety of counter-strategies. Recent studies have shown that pathogenic fungi can secrete a chitin-remodeling enzyme called chitin deacetylase (CDA, EC 3.5.1.41) (Liu et al., 2023), which can catalyze the deacetylation of chitin in the cell walls of plant-pathogenic fungi, converting it into chitosan, which is a poor substrate for chitinases, thus masking the chitin in the cell wall (Brunner et al., 1998; Sanchez-Vallet et al., 2015), and avoiding this defense mechanism (Cord-Landwehr et al., 2016; Gao et al., 2019; Xu et al., 2020).

The CDA gene has multiple functions in the growth, development, and infection processes of pathogenic fungi. Gao et al. (2019) identified a secretory polysaccharide deacetylase (*VdPDA1*) from *Verticillium dahliae* that is specifically localized to fungus-host interfaces, which can enhance the virulence of the pathogen by directly deacetylating chitin oligomers, and inhibit the production of ROS, MAPK phosphorylation, and the expression of resistance-related genes in the immune response induced by chitooligosaccharides. Rizzi et al. (2021) analyzed seven chitin deacetylases *UmCDA1-UmCDA7* secreted by *Ustilago maydis*. The results showed that the *cda7* deletion mutant has a virulence defect, and *cda4* is involved in the formation of the chitosan layer surrounding biotrophic hyphae. By combining different *cda* gene mutations, it was found that certain CDAs have both functional redundancy and specific functions. The expression of *PoCda7* secreted by *Pyricularia oryzae* is suppressed in the early stage of infection. Subcellular localization

experiments show that PoCda7 is localized in the cell wall. As a result of decreased appressorium turgor pressure and impaired appressorium-mediated invasion, the virulence of the *ΔPocda7* mutant was significantly reduced in host plants (Dai et al., 2021). The chitin deacetylase *BgtCDA1* secreted by *Blumeria graminis* f. sp. *tritici* can deacetylate chitin to chitosan to suppress the plant immune response triggered by chitin. The aspartic protease TiAP1 that interacts with *BgtCDA1* can inhibit this function of *BgtCDA1*, enabling the wheat chitin receptor to recognize chitin and trigger an immune response (Yang et al., 2022). The chitin deacetylase *FgPDA5* secreted by *Fusarium graminearum* is an important virulence factor. Its polysaccharide deacetylase structure is crucial for the pathogenicity of *F. graminearum*. *FgPDA5* also shows high affinity for chitin substrates, and its mediated deacetylation of chitosan prevents the activation of plant defense responses (Hu et al., 2023). The chitin deacetylase *PsCDA2* secreted by *Puccinia striiformis* f. sp. *tritici* is induced to express during the initial invasion of wheat and inhibit plant programmed cell death (PCD) and the basic plant immune response triggered by chitin, including callose accumulation and the expression of defense genes. In addition, *PsCDA2* can protect the urediniospores of *P. striiformis* f. sp. *tritici* from damage by host chitinases in vitro (Xiao et al., 2023).

Tilletia laevis Kühn, the causative agent of common bunt of wheat, is one of the most devastating wheat pathogens worldwide, prevalent in wheat-growing regions across the globe (Wilcoxson and Saari, 1996; Aydoğdu, 2020). Symptoms mainly appear on the spikelets of infected wheat. The glumes open slightly outward, and the inside of the grains is filled with teliospores, emitting a fishy smell (Wilcoxson and Saari, 1996). This disease can cause a 25%-50% reduction in wheat yield or even a complete crop failure, severely affecting the yield, quality, and commercial value of wheat.

In this study, we found that *TlCDA* secreted by *T. laevis* with CDA activity. To investigate the role of *TlCDA* in the infection of wheat by *T. laevis*, we analyzed the expression pattern of *TlCDA*, CDA activity, and effects on plant programmed cell death, ROS accumulation, and the expression of pathogenesis-related genes. This is the first study on the chitin deacetylase of *T. laevis*, which helps us understand the

infection pattern of *T. laevis* and provides a theoretical basis for the comprehensive control of common bunt of wheat.

2. Material and methods

2.1 Fungal and plant material

The susceptible wheat cultivar Dongxuan 3 was used in this study. Wheat seeds were sterilized with 30% NaClO and then germinated on sterile moist muslin cloth for 1 month. The vernalized wheat seeds were then planted in pots (with a mixture of organic matter and soil at a ratio of 1:2), and cultured in growth chambers (LT-36VL; Percival Scientific, USA) under 24 hours of light and 60% relative humidity. *N. benthamiana* was grown at 25°C for 3-5 weeks in a greenhouse with 16 h of light and 8 h of dark. The teliospores of *T. laevis* were cultured in an incubator (MLR 352 H, Panasonic, USA) at 15 °C under a 24 h light cycle. After 7 days of cultivation, the germination of teliospores was observed under an automatic inverted fluorescence microscope (IX83, Olympus, Japan) and hyphae were collected with sterile water.

2.2 Bioinformatics Analysis

The conserved domain of *TlCDA* was analyzed by SMART (<https://smart.embl.de/>). SignalP 5.0 (<https://services.healthtech.dtu.dk/services/SignalP-5.0/>) was used to predict the signal peptide sequence of *TlCDA*. The alignment of homologous proteins and the construction of the phylogenetic tree were performed by ClustalW in Mega7 software.

2.3 RNA extraction and cDNA synthesis

Total RNA of wheat and *N. benthamiana* samples was extracted using the RNAsimple Total RNA Kit (Tiangen Biotech, China) following the manufacturer's protocol. Hifair® AdvanceFast 1st Strand cDNA Synthesis Kit (Yeasen Biotech, China) was used to remove the residual genomic DNA and to synthesize cDNA through the reverse transcription reaction. qPCR was performed using a SYBR green-based qPCR assay with SuperReal qPCR PreMix (Tiangen Biotech, China) on a qPCR instrument (ABI 7500, Thermo Fisher, USA). *NbAct1* (Xiao et al., 2023) and *RPS17* (Gupta et al., 2013) were used as the internal reference genes for qPCR. qPCR conditions were used

as follows: 95°C for 15 min, then 40 cycles of 95°C for 10 s, 60°C for 15 s and 72°C for 20 s. The primers used in this study are listed in Table S1.

2.4 Detection of deacetylase enzyme activity

A chitin deacetylase (CDA) ELISA kit was used to detect the deacetylase enzyme activity of *TlCDA* according to the manufacturer's instructions. CDA combined with the CDA antibody labeled with horseradish peroxidase (HRP) to form an antibody-antigen-enzyme complex. After complete washing, the substrate tetramethylbenzidine (TMB) was added, which caused the TMB substrate to turn blue due to the catalytic activity of the HRP enzyme. The intensity of the color was positively correlated with the deacetylase activity. The OD₄₅₀ was then measured using a microplate reader (Multiskan FC, Thermofisher, USA), and the activity concentration of *TlCDA* was calculated through the standard curve.

2.5 Secretory Analysis of Signal peptide

The restriction enzymes *EcoRI* and *XhoI* were used to digest the vector pSUC2. SP of *TlCDA* was predicted by SignalP 5.0 and cloned by PCR. Then, the cloned sequences were combined with pSUC2 vector and transformed into competent cells of yeast strain YTK12. Positive colonies were randomly selected and transferred to SD/Trp liquid medium and cultured at 28°C for 3 d and then moved to CMD-W and YPRAA media respectively for assay of transformant secretion. *Avr^{lb}* and *Mg87* were used as positive and negative controls.

2.6 Transient expression in *N. benthamiana*

In the Bax-induced PCD inhibition assay, recombinant vectors (pGR107:GFP, pGR107:*TlCDA*, pGR107:Bax) were constructed and transformed into *A. tumefaciens* competent cells GV3101 for transient expression. *A. tumefaciens* carrying pGR107:GFP and pGR107:*TlCDA* was injected into *N. benthamiana* leaves with injection buffer (10 mmol/L MES, 200 µmol/L AS, 10 mmol/L MgCl₂). After 24 h, a challenge inoculation of *A. tumefaciens* carrying pGR107:BAX was conducted. Cell death phenotypes in *N. benthamiana* leaves were photographed after 5 days and further validated by trypan blue staining (Li et al., 2021). The localization signal was observed under a laser confocal microscope (Zeiss, Oberkochen, Germany) 2-3 days

after agro-infiltration.

2.7 Measurement of ROS accumulation

The level of ROS in *N. benthamiana* leaves was measured using DAB and NBT. *N. benthamiana* leaves expression *TlCDA* and GFP was soaked in 2 mg/mL DAB or 0.5 mg/mL NBT solution for 5 h in the dark. The decolourization solution (ethanol: acetic acid: glycerol, 3:1:1) was used for the decolorization of *N. benthamiana* leaves at 95°C for 15 minutes.

2.8 Fungal gene deletion and complementation

The hyphae of *T. laevis* were hydrolyzed by enzymatic hydrolysis mixture (1.5% Driselase, 1.5% Snailase and 1.5% Lywallzyme in 1.2 mol/L KCl) at 28°C, 180 rpm for 2.5 h to obtain protoplasts (Shen et al., 2018). ClonExpress II One Step Cloning Kit (Vazyme, China) was used to ligate the upstream and downstream flanking sequences of *TlCDA* with hygromycin phosphotransferase (hph) fragments in pUC19 vector. The recombinant fragments were then co-introduced into protoplasts of *T. laevis* and screened on hygromycin-resistant plates. For the complementation strain, the *TlCDA* sequence was inserted into the pGNT2 vector with its native promoter and transformed into protoplasts of the $\Delta TlCDA-1$ mutant, followed by screening on G418-resistant plates. All mutants were examined by PCR. The primers used in this study are listed in Table S1.

3. Results

3.1 Bioinformatics analysis and enzyme activity assay of *TlCDA*

The sequence of *TlCDA* (KAE8185245) was analyzed using SMART (<https://smart.embl.de/>). The results showed that amino acids 1 to 19 constitute a signal peptide, and the amino acids from 109 to 231 constitute the Polysacc_deac_1 domain (PF01522) (Fig. S1A).

The sequence alignment of *TlCDA* with CDA proteins from other fungal species was performed (Fig. 1A). The results indicated that the 15 CDA proteins exhibited substantial structural similarities and possessed five conserved domains associated with CDAs, including motif 1 (TxDD), motif 2 (HTxxH), motif 3 (RxPY), motif 4

(DxxD) and motif 5 (LxH) (Aragunde et al., 2018; Grifoll-Romero et al., 2018; Xiao et al., 2023). A phylogenetic tree of 22 CDAs from different fungi was constructed, results indicated that CDAs are conserved in *Tilletia* spp. (Fig. S1B). To investigate the expression pattern of *TlCDA* during the process of *T. laevis* infecting wheat, wheat samples after inoculation at 12 h, 24 h, 36 h, 48 h, and 60 h were collected. qPCR was performed to detect the changes in the expression level of *TlCDA* (Fig. 2B). The results indicated that the expression level of *TlCDA* reached a peak at 24 h after inoculation, indicating that *TlCDA* is involved in the early infection process of *T. laevis*.

3.2 The deacetylase activity analysis of *TlCDA*

The deacetylase activity of *TlCDA* was evaluated using a chitin deacetylase (CDA) ELISA kit, with GFP as the negative control. The results showed that the substrate solution turned blue when *TlCDA* was present (Fig. 2a). A standard curve of enzyme activity was generated using serial dilutions of standard samples (Fig. 2b). These results indicate that *TlCDA* has chitin deacetylase activity and may promote the infection of *T. laevis* by evading the plant immune response triggered by chitin.

3.3 *TlCDA* is a secretory protein and located to the plasma membrane

Prediction by SignalP 5.0 revealed that a typical signal peptide (SP) is located at the N-terminus of *TlCDA* (Fig. S1A). To detect whether the SP was functional in secretion, the signal peptide of *TlCDA* was ligated to the pSUC2 vector and transformed into the yeast strain YTK12, which was then cultured on CMD-W and YPRAA screening media respectively. The SP sequence of the effector *Avr^{lb}* and the SP sequence of *Mg87* were used as positive and negative controls, respectively (Fig. 3A). The results showed that, similar to the positive control, yeast strain YTK12 transformed with *TlCDA*^{SP} could grow normally on both CMD-W medium and YPRAA medium, while YTK12 and the negative control could only grow on CMD-W medium. This indicates that *TlCDA* is a secretory protein.

To further determine the localization information of *TlCDA*, a pBin-GFP recombinant vector (*TlCDA*-GFP) was constructed, and the transient expression mediated by *A. tumefaciens* was used to observe the localization of *TlCDA*-GFP in *N. benthamiana*

under a laser confocal microscope (Zeiss LSM980), and 1300-PM-RFP was used as a marker for the plasma membrane (Fig. 3B). The results showed that the fluorescence signal of *TlCDA*-GFP was detected in the plasma membrane, which was consistent with that of 1300-PM-RFP.

3.4 *TlCDA* suppresses plant immunity in *N. benthamiana*

Bax is a mouse-derived apoptotic factor and an activator of programmed cell death, which can be used to achieve rapid high-throughput screening of effectors from various pathogens (Lacomme et al., 1999). To test whether *TlCDA* has a potential regulatory role in suppressing PCD, *A. tumefaciens* carrying pGR107:*TlCDA* was injected into *N. benthamiana*, followed 24 h later by injection of *A. tumefaciens* carrying pGR107:BAX. pGR107:GFP and injection buffer were used as negative controls (Fig. 4A). The results showed that necrosis occurred in the areas injected with pGR107:GFP and buffer but not in pGR107:*TlCDA* (Fig. 4A). Trypan blue staining of the injected *N. benthamiana* leaves indicated that *TlCDA* suppresses Bax-induced cell death (Fig. 4A).

Furthermore, peroxidases and superoxide anions (O_2^-) were stained with 3,3'-diaminobenzidine (DAB) and nitroblue tetrazolium (NBT) respectively. The results showed that, compared with the GFP control, staining intensities of peroxidases (Fig. 4B) and O_2^- (Fig. 4C) decreased in *N. benthamiana* leaves expressing *TlCDA*, indicating that *TlCDA* suppresses the accumulation of ROS.

We also evaluated the expression of plant immune marker genes in *N. benthamiana* leaves transiently expressing *TlCDA* after infiltration with chitin. It was found that the transcriptional levels of *NbPR1a*, *NbPR2*, *NbPR3* and *NbRBOHD* in *N. benthamiana* leaves were significantly decreased after transiently expressing *TlCDA* (Fig. 4D). These results suggest that *TlCDA* can inhibit chitin-induced plant immunity by suppressing the expression of immune-related genes.

3.5 The growth rate and pathogenicity of the $\Delta TlCDA$ mutant are reduced

In order to investigate the effect of *TlCDA* on the biology of *T. laevis*, *TlCDA*-deletion strains ($\Delta TlCDA$ -1, $\Delta TlCDA$ -2 and $\Delta TlCDA$ -3) and a

complementation strain (*ΔTICDA-1-C*) were generated (Fig. S2). All mutants were cultured on complete medium (CM) plates (Li et al., 2020) for 7 days. The diameters of the colonies were measured. Compared with the wild type, the colony diameters of the *ΔTICDA-1*, *ΔTICDA-2*, and *ΔTICDA-3* mutants were significantly reduced, whereas there was no significant change in the *ΔTICDA-1-C* mutant. In addition, we investigated the effect of chitinase on the mutants. The results indicated that the colony diameters of the *ΔTICDA-1*, *ΔTICDA-2*, and *ΔTICDA-3* mutants were significantly smaller than that of the wild type after the addition of 10 mM chitinase, whereas no significant difference was observed between the *ΔTICDA-1-C* mutant and the wild type. (Fig. 5).

To verify the impact of *TICDA* on the pathogenicity of *T. laevis*, wheat plants were inoculated with the *ΔTICDA* mutant or the wild type. Compared with the wild type, the number of black galls on wheat infected with *ΔTICDA* decreased (Fig. 6a). Incidence statistics indicated that the incidence of wheat infected by *ΔTICDA* was roughly 22.25%, which was significantly lower than the approximately 57.95% incidence of wheat infected by the wild type (Fig. 6b). Further detection of the biomass of *T. laevis* showed that the relative biomass of the pathogen in wheat inoculated with *ΔTICDA* was about 0.36, which was significantly lower than that of the wild type (Fig. 6c). The above results suggest that *TICDA* affects the pathogenicity of *T. laevis*.

3.6 *TICDA* mitigates the inhibition of teliospore germination caused by chitin.

To verify the protective role of *TICDA* on *T. laevis*, an experiment on the inhibition of teliospores germination by chitin was conducted. The crude extract of wheat chitinase was employed to inhibit the germination of *T. laevis* teliospores by decomposing chitin in the cell wall. The germination rate of teliospores was approximately 74.73%, dropping to roughly 5.82% after the addition of chitinase, while it rose to approximately 24.29% when *TICDA* and chitinase coexisted (Fig. 7).

4. Discussion

Chitin is the core structure of the cell wall and is widely distributed in filamentous

fungi (Bulawa, 1993; Bernard et al., 2001; Latgé, 2007; Bowman et al., 2010). Plants have developed a variety of strategies to detect fungal pathogens and initiate corresponding immune responses (Feng et al., 2019). Chitin fibril fragments derived from the fungal cell wall can be recognized by PRRs of plants and act as PAMPs, triggering PTI (Wang et al., 2008; Dodds and Rathjen, 2010; Oliveira-Garcia and Valent, 2015; Buscaill and Van, 2021).

Pathogenic fungi have evolved a new strategy: disguising chitin to evade plant recognition, thereby suppressing PTI (Volk et al., 2019; Sánchez-Vallet et al., 2020; Martínez-Cruz et al., 2022). Previous studies have shown that CDAs regulate the fungal cell wall during growth and cell division, protect chitin from degradation by chitinases, and affect pathogenicity (Christodoulidou et al., 1996; El Gueddari et al., 2002; Baker et al., 2007; Goldman et al., 2012; Gao et al., 2019; Rizzi et al., 2021; Dai et al., 2021; Yang et al., 2022; Hu et al., 2023; Xiao et al., 2023).

In this study, *TlCDA* secreted by *T. laevis* was predicted to contain a Polysacc_deac_1 domain and a signal peptide (Fig. S1A). Sequence alignment with other reported fungal CDAs revealed that CDAs from different fungi possess similar conserved domains (Fig. 1A), indicating the functions of CDAs are conserved in fungi. Phylogenetic analysis showed that CDAs are conserved in *Tilletia* spp. (Fig. S1B). Because *TlCDA* demonstrated deacetylase activity and may facilitate *T. laevis* infection, wheat samples were collected at different times after inoculation to characterize the expression pattern of *TlCDA* (Fig. 1B). The results showed that the relative expression of *TlCDA* reached a peak at 24 h after inoculation, indicating *TlCDA* is involved in the early infection process of *T. laevis*. The deacetylase activity of *TlCDA* was then measured and standard curve of deacetylase activity was conducted (Fig. 2), suggesting that *TlCDA* exert deacetylase activity and may protect the cell wall of *T. laevis* from being degraded by chitinases. The signal peptide function of *TlCDA* was verified using the yeast system, and the results indicated that *TlCDA*^{SP} exhibited secretory activity (Fig. 3A), suggesting that *TlCDA* is a secretory protein. Transient expression in *N. benthamiana* revealed that *TlCDA* is localized to the cell membrane (Fig. 3B) and can inhibit Bax-induced cell death (Fig. 4).

Moreover, *TICDA* was found to impede the accumulation of ROS and the expression of defense-associated genes in *N. benthamiana* (Fig. 4). Collectively, these results strongly suggest that *TICDA* likely promotes the infection process of *T. laevis* by inhibiting chitin-induced immunity. *TICDA* deletion and complementation strains were then generated (Fig. S2). The colony diameters of Δ *TICDA* grown for 7 d on CM medium and CM + 10 mM chitinase medium were significantly smaller than those of the WT and Δ *TICDA*-1-C (Fig. 5), indicating that *TICDA* affects both the growth and chitinase resistance of *T. laevis*. Furthermore, the incidence rate and pathogen biomass in wheat inoculated with Δ *TICDA* were significantly reduced compared with those in wheat inoculated with the complementation strain or the wild type (Fig. 6), indicating that *TICDA* affects the pathogenicity of *T. laevis*. The teliospore germination experiment indicated that *TICDA* could mitigate the inhibitory effect of chitinase on teliospore germination (Fig. 7). Based on the above results, we speculate that *TICDA* exhibits CDA activity to protect chitin from hydrolysis by chitinase, thereby suppressing chitin-induced immunity and ultimately promoting the infection and colonization of *T. laevis* (Fig. 8).

CDAs play crucial roles in the processes of fungal growth, development, and pathogenicity. Analyzing the functions of CDA in *T. laevis* can enrich and improve the physiological and biochemical understanding of fungi and deepen our understanding of the basic laws of fungal life activities. Moreover, CDAs can serve as targets for antifungal agents (Zanni et al., 2022; Liu et al., 2022; Liu et al., 2023). Future research will focus on developing novel prevention and control strategies based on CDA, aiming to ensure the safe production of wheat, safeguard global food security, reduce economic losses caused by diseases, cut down on the use of chemical pesticides, ease environmental pressure, and contribute to the realization of sustainable agricultural development. Our study has revealed a mechanism by which fungi inhibit the plant immune response. This research not only helps us understand the interaction between pathogens and hosts but also provides a new target for the control of common bunt of wheat.

350 **Abbreviations**

351 AS: Acetosyringone

352 Bax: Bcl-2-associated X protein

353 CDA: Chitin deacetylase

354 DAB: 3,3'-Diaminobenzidine

355 GFP: Green fluorescent protein

356 hph: Hygromycin phosphotransferase

357 HRP: Horseradish peroxidase

358 hpi: Hours post inoculation

359 MAPK: Mitogen-activated protein kinase

360 MES: 2-(N-Morpholino)ethanesulfonic acid

361 NBT: Nitroblue tetrazolium

362 OD: Optical density

363 PAMP: Pathogen-associated molecular pattern

364 PCD: Programmed cell death

365 PM: Plasma membrane

366 PR: Pathogenesis-related

367 PRR: Pattern-recognition receptor

368 PTI: Pattern-triggered immunity

369 RFP: Red fluorescent protein

370 ROS: Reactive oxygen species

371 SP: Signal peptide

372 TMB: Tetramethylbenzidine

373 YPRAA: Yeast peptone raffinose antimycin A medium

374 **Ethics declarations**

375 **Ethics approval and consent to participate**

376 Not applicable.

377 **Consent for publication**

378 Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ZR: Conceptualization, Data curation, Formal analysis, Investigation, Writing-original draft, Writing-review & editing. WH: Methodology, Software, Validation, Visualization, HJ: Conceptualization, Supervision, Visualization. TL: Conceptualization, Validation. WC: Methodology, Software. LG: Methodology, Funding acquisition, Supervision, Writing-review & editing.

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Data Availability Statement

All relevant data and charts of this study can be found in the article and Supporting Information.

References

- Andersen, E. J., S. Ali, E. Byamukama, Y. Yen, and M. P. Nepal. 2018. "Disease resistance mechanisms in plants." *Genes* 9: 339.
- Aragunde, H., X. Biarnés, and A. Planas. 2018. "Substrate recognition and specificity of chitin deacetylases and related family 4 Carbohydrate Esterases." *International Journal of Molecular Sciences* 19(2): 412.
- Aydoğdu, M., and Y. Kaya. 2020. "Reactions of spring wheat varieties to common bunt (*Tilletia laevis*) in Turkey." *Cereal Research Communications* 48: 333–339.
- Baker, L. G., Specht C. A., M. J. Donlin, et al. 2007. "Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in *Cryptococcus neoformans*." *Eukaryotic Cell* 6(5): 855–867.
- Bernard, M., and J. P. Latgé. 2001. "Aspergillus fumigatus cell wall: composition and biosynthesis." *Medical Mycology* 39(Suppl 1): 9–17.
- Bigeard, J., J. Colcombet, and H. Hirt. 2015. "Signaling mechanisms in pattern-triggered immunity (PTI)." *Molecular Plant* 8: 521–539.
- Bowman, S. M., and S. J. Free. 2006. "The structure and synthesis of the fungal cell wall." *Bioessays* 28(8): 799–808.
- Brunner, F., A. Stintzi, B. Fritig, et al. "Substrate specificities of tobacco chitinases." *The Plant Journal* 14: 225–234.
- Bulawa, C. E. 1993. "Genetics and molecular biology of chitin synthesis in fungi." *Annual Review of Microbiology* 47: 505–534.
- Buscaill, P., and R. A. L. van der Hoorn. 2021. "Defeated by the nines: nine

extracellular strategies to avoid microbe-associated molecular patterns recognition in plants.” *The Plant Cell* 33: 2116–2130.

Christodoulidou, A., V. Bouriotis, and G. Thireos. 1996. “Two sporulation-specific chitin deacetylase-encoding genes are required for the ascospore wall rigidity of *Saccharomyces cerevisiae*.” *Journal of Biological Chemistry* 271(49): 31420–31425.

Cord-Landwehr, S., R. L. J. Melcher, S. Kolkenbrock, and B. M. Moerschbacher. 2016. “A chitin deacetylase from the endophytic fungus *Pestalotiopsis* sp efficiently inactivates the elicitor activity of chitin oligomers in rice cells.” *Scientific Reports* 6: 1–11.

Dai, M. D., M. Wu, Y. Li, et al. 2021. “The chitin deacetylase PoCda7 is involved in the pathogenicity of *Pyricularia oryzae*.” *Microbiological Research* 248: 126749.

Dodds, P. N., and J. P. Rathjen. 2010. “Plant immunity: towards an integrated view of plant-pathogen interactions.” *Nature Reviews Genetics* 11(8): 539–548.

El Gueddari, N. E., U. Rauchhaus, B. M. Moerschbacher, and H. B. Deising. 2002. “Developmentally regulated conversion of surface-exposed chitin to chitosan in cell walls of plant pathogenic fungi.” *New Phytologist* 156(1): 103–112.

Feng, F., J. Sun, G. V. Radhakrishnan, et al. 2019. “A combination of chitooligosaccharide and lipochitooligosaccharide recognition promotes arbuscular mycorrhizal associations in *Medicago truncatula*.” *Nature Communications* 10(1): 5047.

Figueroa, M., D. Ortiz, and E. C. Henningsen. 2021. “Tactics of host manipulation by intracellular effectors from plant pathogenic fungi.” *Current Opinion in Plant Biology*

450 62: 102054.

451 Fiorin, G. L., A. Sánchez-Vallet, D. P. T. Thomazella, et al. 2018. “Suppression of
 452 Plant Immunity by Fungal Chitinase-like Effectors.” *Current Biology* 28(18): 3023–
 453 3030.e5.

454 Gao, F., et al. 2019. “Deacetylation of chitin oligomers increases virulence in
 455 soil-borne fungal pathogens.” *Nature Plants* 5: 1167–1176.

456 Goldman, D. L., and A. G. Vicencio. 2012. “The chitin connection.” *mBio* 3(2):
 457 e00056-12.

458 Gong, B. Q., F. Z. Wang, and J. F. Li. 2020. “Hide-and-seek: chitin-triggered plant
 459 immunity and fungal counter strategies.” *Trends in Plant Science* 25: 805–816.

460 Grifoll-Romero, L., S. Pascual, H. Aragunde, et al. 2018. “Chitin Deacetylases:
 461 Structures, Specificities, and Biotech Applications.” *Polymers (Basel)* 10(4): 352.

462 Han, L. B., Y. B., Li, F. X., Wang, et al. 2019. “The cotton apoplastic protein CRR1
 463 stabilizes chitinase 28 to facilitate defense against the fungal pathogen *Verticillium*
 464 *dahliae*.” *The Plant Cell* 31: 520–536.

465 Hu, S., M. Jin, Y. Xu, et al. 2023. “Deacetylation of chitin oligomers by *Fusarium*
 466 *graminearum* polysaccharide deacetylase suppresses plant immunity.” *Molecular*
 467 *Plant Pathology* 24(12): 1495–1509.

468 Kämper, J., R. Kahmann, M. Bölker, et al. 2006. “Insights from the genome of the
 469 biotrophic fungal plant pathogen *Ustilago maydis*.” *Nature* 444(7115): 97–101.

470 Kinoshita, T., and M. Seki. 2014. “Epigenetic memory for stress response and
 471 adaptation in plants.” *Plant and Cell Physiology* 55(11): 1859–1863.

472 Lacomme, C., and S. Santa Cruz. 1999. "Bax-induced cell death in tobacco is similar
473 to the hypersensitive response." *Proceedings of the National Academy of Sciences of*
474 *the United States of America* 96(14): 7956–7961.

475 Latgé, J. P. 2007. "The cell wall: a carbohydrate armour for the fungal cell."
476 *Molecular Microbiology* 66(2): 279–290.

477 Lee, W. S., J. J. Rudd, K. E. Hammond-Kosack, and K. Kanyuka. 2014.
478 "*Mycosphaerella graminicola* LysM effector-mediated stealth pathogenesis subverts
479 recognition through both CERK1 and CEBiP homologues in wheat." *Molecular*
480 *Plant-Microbe Interactions* 27(3): 236–243.

481 Li, B., X. Meng, L. Shan, and P. He. 2016. "Transcriptional Regulation of
482 Pattern-Triggered Immunity in Plants." *Cell Host and Microbe* 19(5): 641–650.

483 Li, D., X. Wei, T. Liu, et al. 2020. "Establishment of an *Agrobacterium*
484 *tumefaciens*-mediated transformation system for *Tilletia foetida*." *Journal of*
485 *Microbiological Methods* 169: 105810.

486 Li, X., C. Jin, H. Yuan, W. Huang, F. Liu, R. Fan, J. Xie, and Q. Shen. 2021. "The
487 barley powdery mildew effectors CSEP0139 and CSEP0182 suppress cell death and
488 promote *B. graminis* fungal virulence in plants." *Phytopathology Research* 3(1): 7.

489 Liu, L., Y. Xia, Y. Li, et al. 2023. "Inhibition of chitin deacetylases to attenuate plant
490 fungal diseases." *Nature Communications* 14(1): 3857.

491 Liu, T. T., et al. 2012. "Chitin-induced dimerization activates a plant immune receptor."
492 *Science* 336: 1160–1164.

493 Liu, W., J. Liu, Y. Ning, et al. 2013. "Recent progress in understanding PAMP- and

494 effector-triggered immunity against the rice blast fungus *Magnaporthe oryzae*.”
495 *Molecular Plant* 6(3): 605–620.

496 Liu, Y., S. Ahmed, Y. Fang, et al. 2022. “Discovery of Chitin Deacetylase Inhibitors
497 through Structure-Based Virtual Screening and Biological Assays.” *Journal of*
498 *Microbiology and Biotechnology* 32(4): 504–513.

499 Martínez-Cruz, J. M., Á. Polonio, L. Ruiz-Jiménez, et al. 2022. “Suppression of
500 Chitin-Triggered Immunity by a New Fungal Chitin-Binding Effector Resulting from
501 Alternative Splicing of a Chitin Deacetylase Gene.” *Journal of Fungi (Basel)* 8(10):
502 1022.

503 Oliveira-Garcia, E., and B. Valent. 2015. “How eukaryotic filamentous pathogens
504 evade plant recognition.” *Current Opinion in Microbiology* 26: 92–101.

505 Rizzi, Y. S., P. Happel, S. Lenz, et al. 2021. “Chitosan and Chitin Deacetylase Activity
506 Are Necessary for Development and Virulence of *Ustilago maydis*.” *mBio* 12(2):
507 e03419-20.

508 Sánchez-Vallet, A., J. R. Mesters, and B. P. Thomma. 2015. “The battle for chitin
509 recognition in plant-microbe interactions.” *FEMS Microbiology Reviews* 39(2): 171–
510 183.

511 Sánchez-Vallet, A., H. Tian, L. Rodriguez-Moreno, et al. 2020. “A secreted LysM
512 effector protects fungal hyphae through chitin-dependent homodimer polymerization.”
513 *PLoS Pathogens* 16(6): e1008652.

514 Shen, H. M., C. Li, L. Gao, T. G. Liu, B. Liu, and W. Q. Chen. 2018. “Protoplast
515 preparation and regeneration of *Tilletia controversa*.” *Plant Protection* 44(2): 140–

516 144.

517 Shimizu, T., T. Nakano, D. Takamizawa, et al. 2010. “Two LysM receptor molecules,
518 CEBiP and OsCERK1, cooperatively regulate chitin elicitor signaling in rice.” The
519 Plant Journal 64(2): 204–214.

520 Torres, M. A., J. D. G. Jones, and J. L. Dangl. 2006. “Reactive oxygen species
521 signaling in response to pathogens.” Plant Physiology 141(2): 373–378.

522 Volk, H., K., Marton, M., Flajšman, et al. 2019. “Chitin-binding protein of
523 *Verticillium nonalfalfae* disguises fungus from plant chitinases and suppresses
524 chitin-triggered host immunity.” Molecular Plant-Microbe Interactions 32: 1378–
525 1390.

526 Wan, J., X. C. Zhang, D. Neece, et al. 2008. “A LysM receptor-like kinase plays a
527 critical role in chitin signaling and fungal resistance in *Arabidopsis*.” The Plant Cell
528 20(2): 471–481.

529 Wilcoxson, R. D., and E. E. Saari (eds.). 1996. Bunt and Smut Diseases of Wheat:
530 Concepts and Methods of Disease Management. Mexico, D. F.: CIMMYT.

531 Xiao, M., D. Chen, S. Liu, et al. 2023. “A chitin deacetylase PsCDA2 from *Puccinia*
532 *striiformis* f. sp. *tritici* confers disease pathogenicity by suppressing chitin-triggered
533 immunity in wheat.” Molecular Plant Pathology 24(12): 1467–1479.

534 Xu, J., G., Wang, J., Wang, et al. 2017. “The Lysin motif-containing proteins, Lyp1,
535 Lyk7 and LysMe3, play important roles in chitin perception and defense against
536 *Verticillium dahliae* in cotton.” BMC Plant Biology 17: 1–18.

537 Xu, Q., J., Wang, J., Zhao, et al. 2020. “A polysaccharide deacetylase from *Puccinia*

striiformis f. sp. *tritici* is an important pathogenicity gene that suppresses plant immunity.” Plant Biotechnology Journal 18: 1830–1842.

Yang, Y., P. Fan, J. Liu, et al. 2022. “*Thinopyrum intermedium* TiAP1 interacts with a chitin deacetylase from *Blumeria graminis* f. sp. *tritici* and increases the resistance to Bgt in wheat.” Plant Biotechnology Journal 20(3): 454–467.

Zanni, R., J. Martínez-Cruz, M. Gálvez-Llompарт, et al. 2022. “Rational Design of Chitin Deacetylase Inhibitors for Sustainable Agricultural Use Based on Molecular Topology.” Journal of Agricultural and Food Chemistry 70(41): 13118–13131.

Zhang, J., and J. M. Zhou. 2010. “Plant immunity triggered by microbial molecular signatures.” Molecular Plant 3(5): 783–793.

Figure Legends

Figure 1. Multiple protein sequences alignment and expression pattern of *TlCDA*. (A) Multiple protein sequences alignment of *TlCDA*. *TlCDA* (KAE8185245), *UmCDA1-7* (XP_011386452, XP_011387091, XP_011389242, XP_011387743, XP_011387928, XP_011391520, XP_011388681), *SsCDA6* (CDW97409), *BgtCDA1* (KE374986), *FgPDA5* (FGSG_03544), *PsCDA2* (KNE92947), *ClCDA* (AY633657), *VdPDA1* (NW_009276934), *PoCDA7* (MGG_05023). The “His-His-Asp” metal binding triad (★), catalytic base (▲), and catalytic acid (※) are labelled (Aragunde et al., 2018). (B) Expression pattern of *TlCDA*. Data shown are the means ± standard deviation calculated from three independent biological replications. ns: $P > 0.05$, ***: $P < 0.001$, ****: $P < 0.0001$. hpi: hours post inoculation.

Figure 2. The deacetylase activity of *TlCDA*. a: the deacetylase activity of *TlCDA* (100 µg/mL) was measured using the chitin deacetylase ELISA kit. b: the standard curve of deacetylase activity according to the dilution of the standard samples. The standard samples were 32, 64, 96, 128, 160 IU/L. Green circles indicate *TlCDA* and black circles indicates standard samples.

Figure 3. *TlCDA* is a secretory protein. (A) Function detection of *TlCDA*^{SP}. *Mg87* and *YTK12* were used as negative control, *Avr^{lb}* was used as positive control. (B) Subcellular localization of *TlCDA* in *N. benthamiana*. 1300-PM-RFP was used as positive control. 1 bar=20 μ m.

Figure 4. *TlCDA* suppress plant immunity in *N. benthamiana*. (A) *TlCDA* suppresses Bax induced programmed cell death. (B) *TlCDA* suppresses chitin-induced plant immunity. a: *N. benthamiana* leaves stained by DAB (a) and NBT (b) after expressing *TlCDA* (left) and GFP (right). (C) The relative expression of defense-associated genes in *N. benthamiana*. Data was analysed by one-way ANOVA, **** indicating $P < 0.0001$.

Figure 5. *TlCDA* affects the growth of *T. laevis*. (A) Colony morphology of *TlCDA* deletion and complementary mutants after 7 days of growth. (B) Statistics of colony diameters. Different letters indicate significant differences (one-way ANOVA, $P < 0.01$).

Figure 6. *TlCDA* affects the pathogenicity of *T. laevis*. (A) Disease symptoms of wheat infected with WT, Δ *TlCDA* and Δ *TlCDA*-1-C. (B) Statistics of the incidence of wheat inoculated with the wild type and Δ *TlCDA* respectively. (C) Statistics of the pathogen biomass of wheat inoculated with the wild type and Δ *TlCDA* respectively. Different letters indicate significant differences (one-way ANOVA, $P < 0.01$).

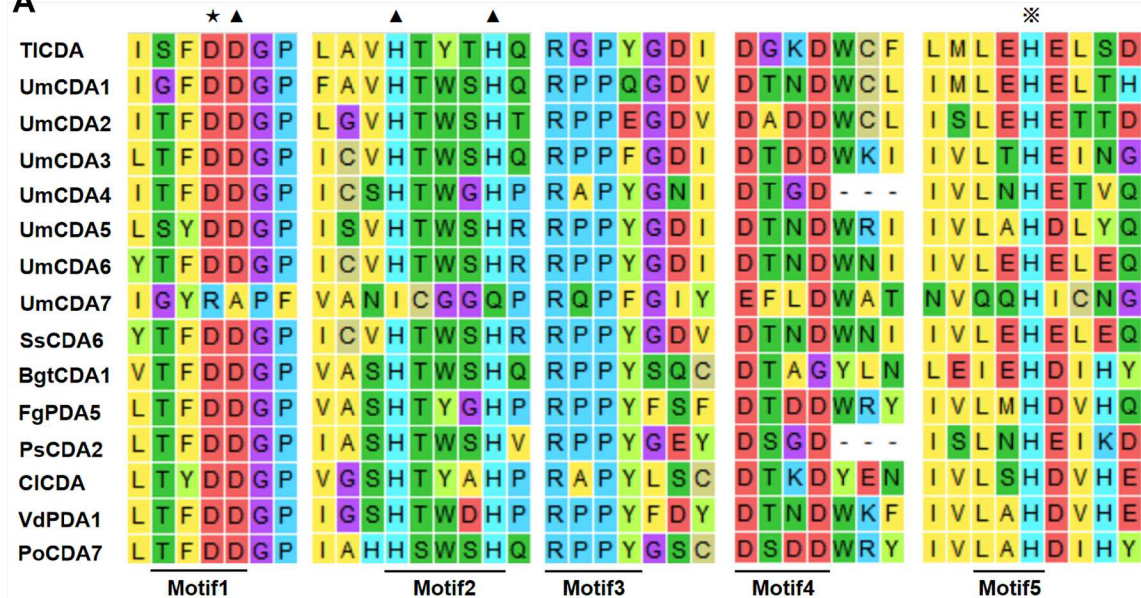
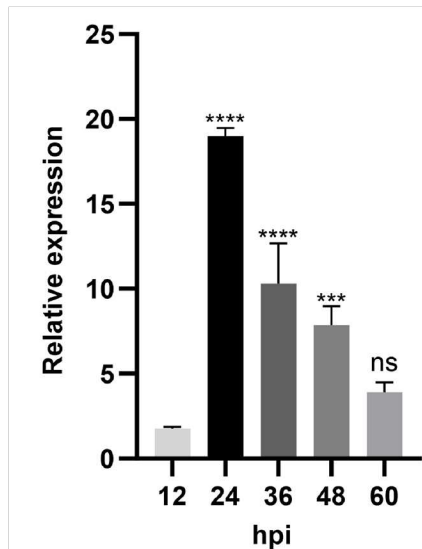
Figure 7. *TlCDA* mitigates the inhibition of teliospores germination caused by chitinase. (A) Germination of teliospores was observed under optical microscope, 1 bar=50 μ m. (B) The germination rate of teliospores was significantly differences under different treatments. Data was analysed by one-way ANOVA, ** indicating $P < 0.01$, **** indicating $P < 0.0001$.

Figure 8. The mechanism by which chitin deacetylase inhibits plant immunity.

Supplementary Materials

Fig. S1. Bioinformatics analysis of *TlCDA*. (A) Three-dimensional structure of *TlCDA*. Blue color indicates signal peptide, yellow color indicates polysacc_deac_1 domain. (B) Phylogenetic analysis of 22 CDAs from different fungi.

593 **Fig. S2.** Examination of *T/CDA* deletion and complementation stains. M: DL 2000
594 DNA ladder. Lane 1, 5, 9, 13, 17: Amplification of upstream fragments. Lane 2, 6, 10,
595 14, 18: Amplification of downstream fragments. Lane 3, 7, 11, 15, 19: Amplification
596 of *T/CDA* fragments. Lane 4, 8, 12, 16, 20: Amplification of *hph* fragments.
597 **Table S1.** List of primers used in this study.

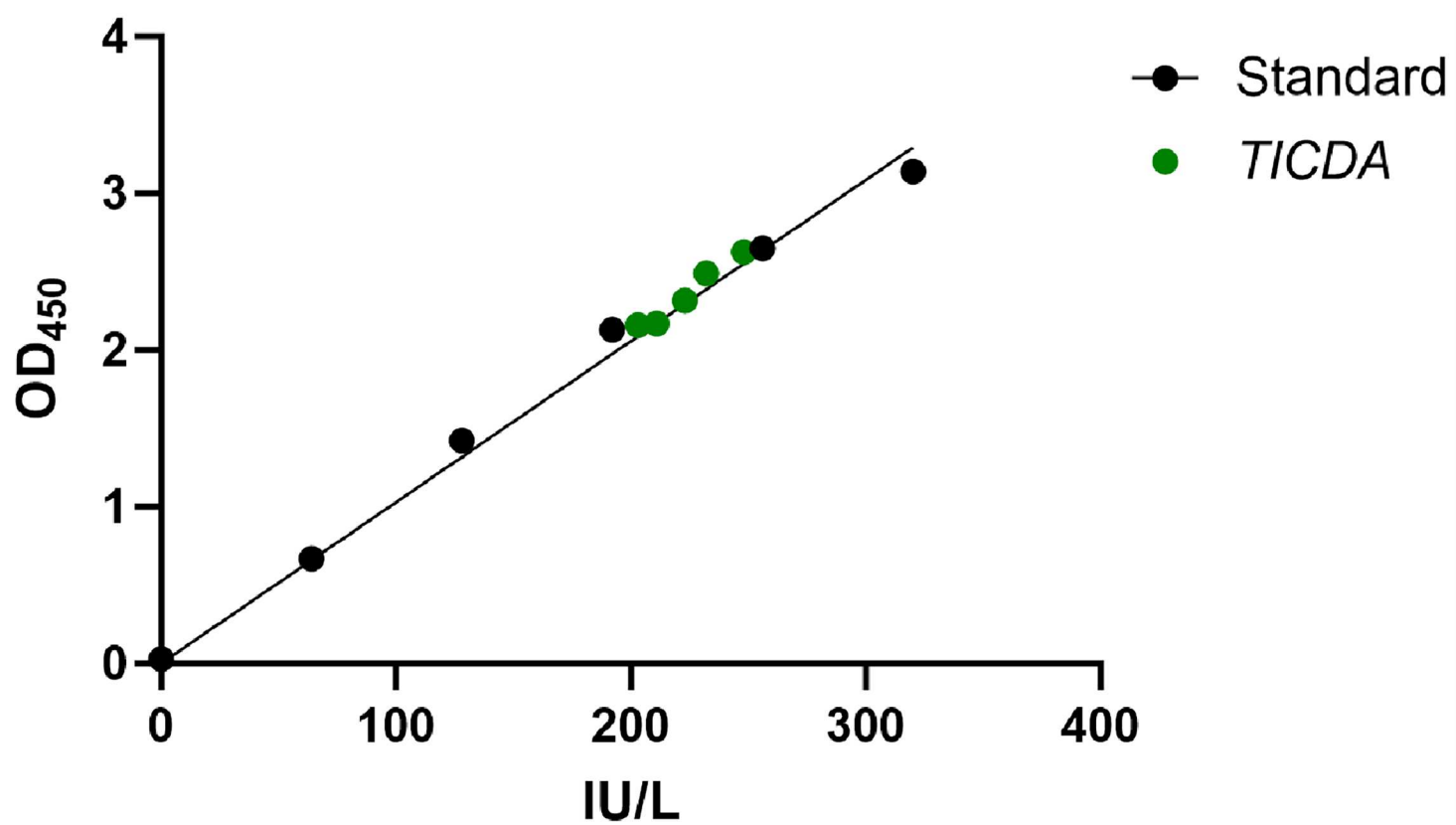
A**B**

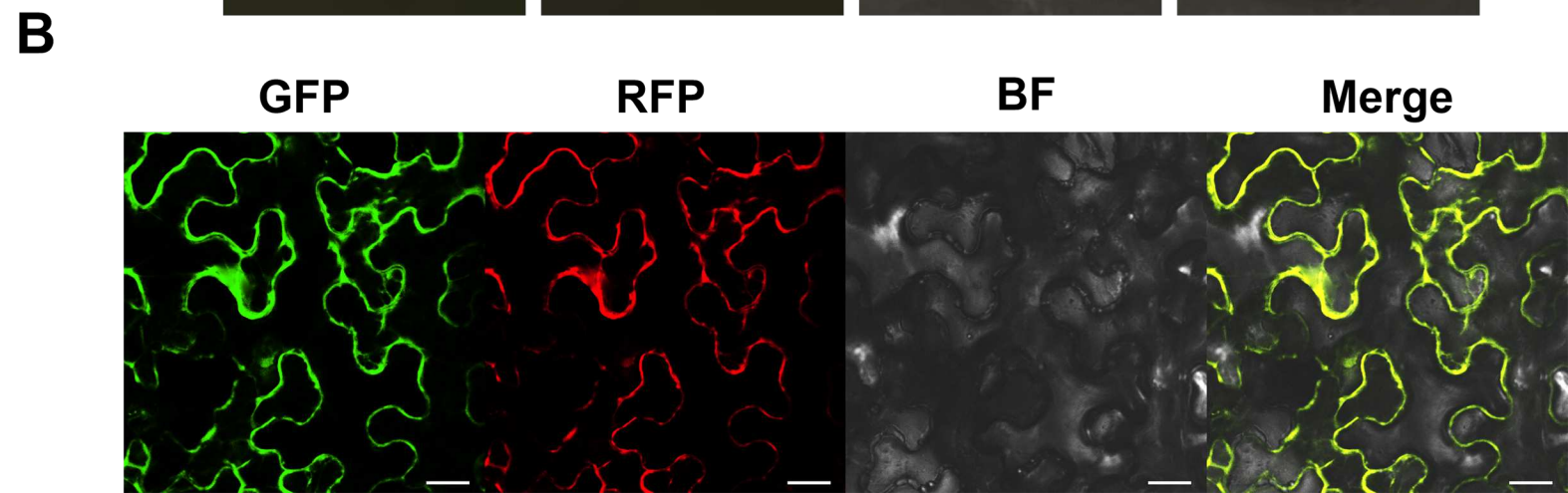
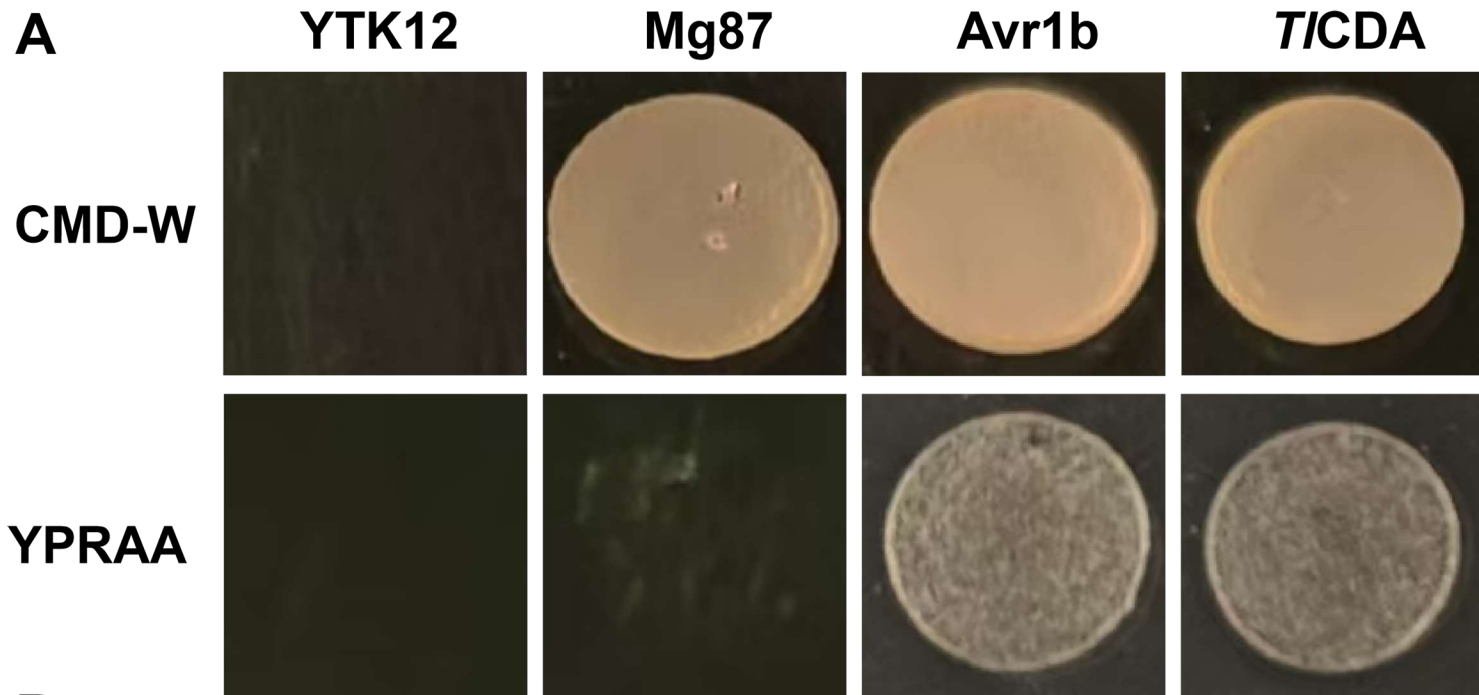
A

Standard
samples

TICDA

Negative
control

**B**

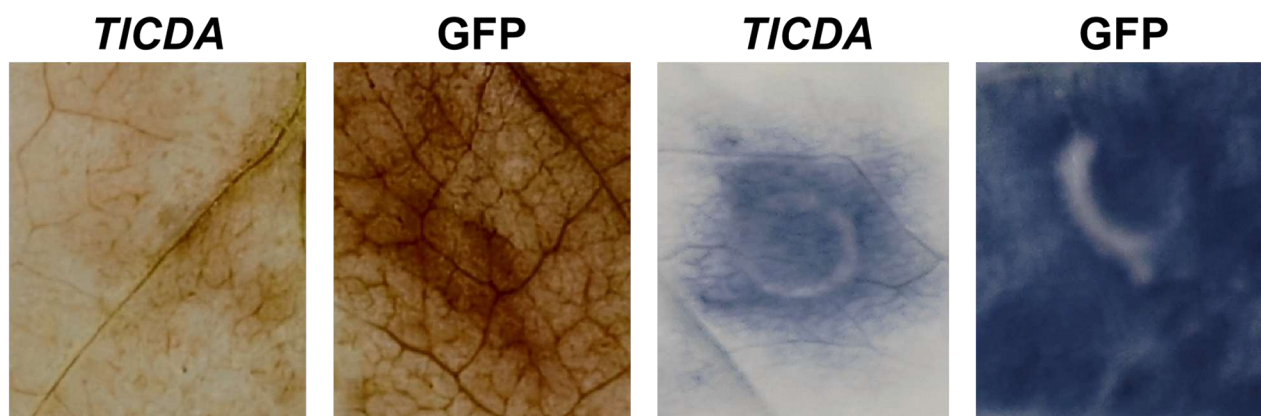


A

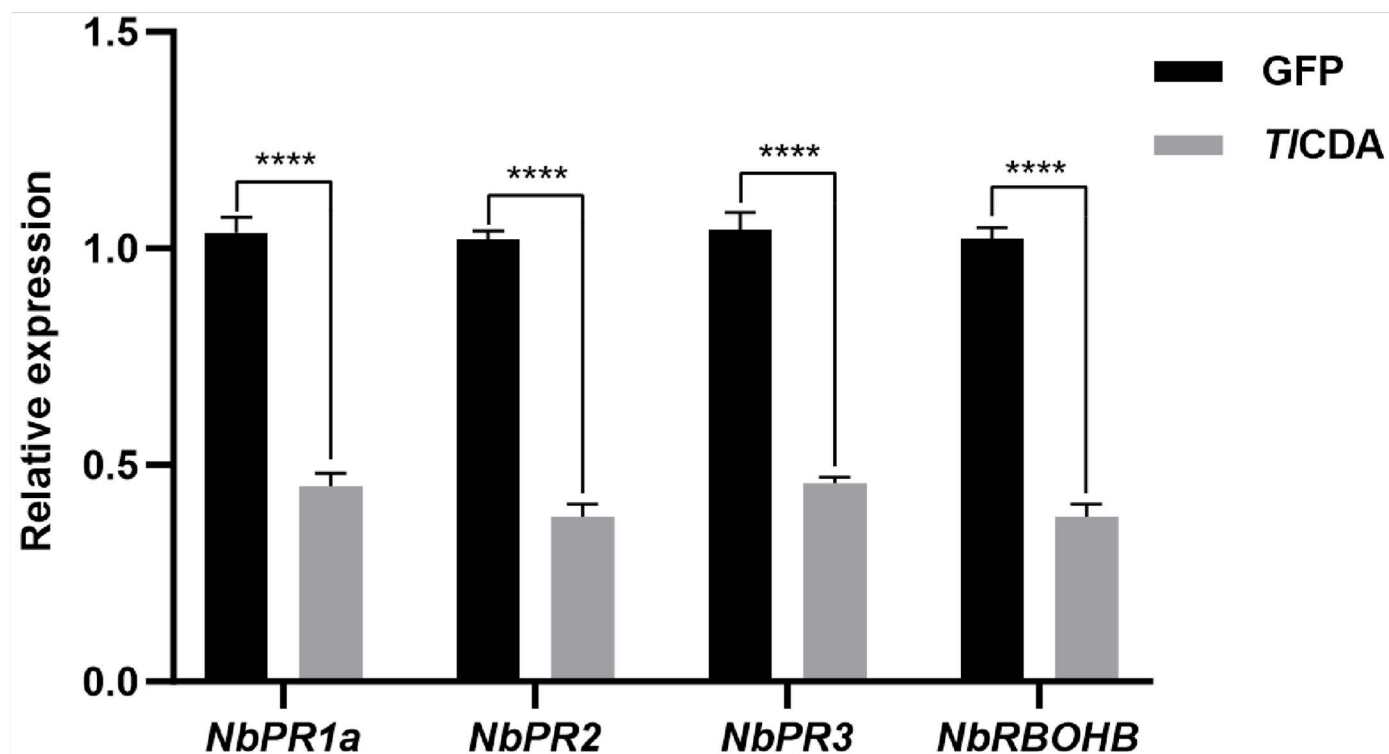
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2-GFP	5-GFP + <u>Bax</u>
3-Buffer	6-Buffer +Bax



B



C



A

WT

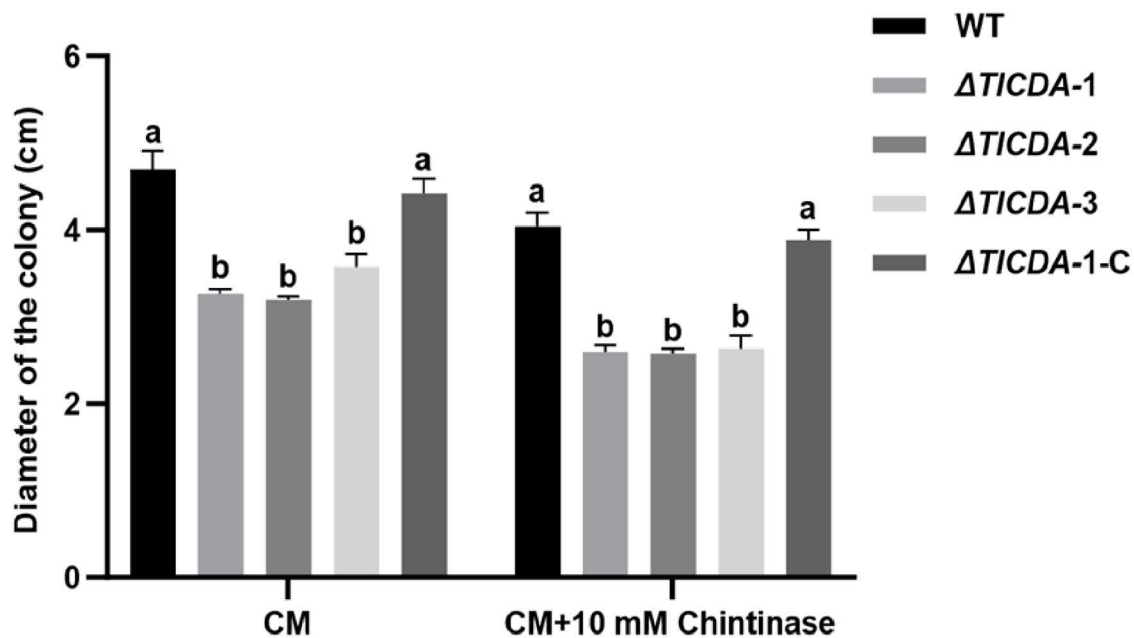
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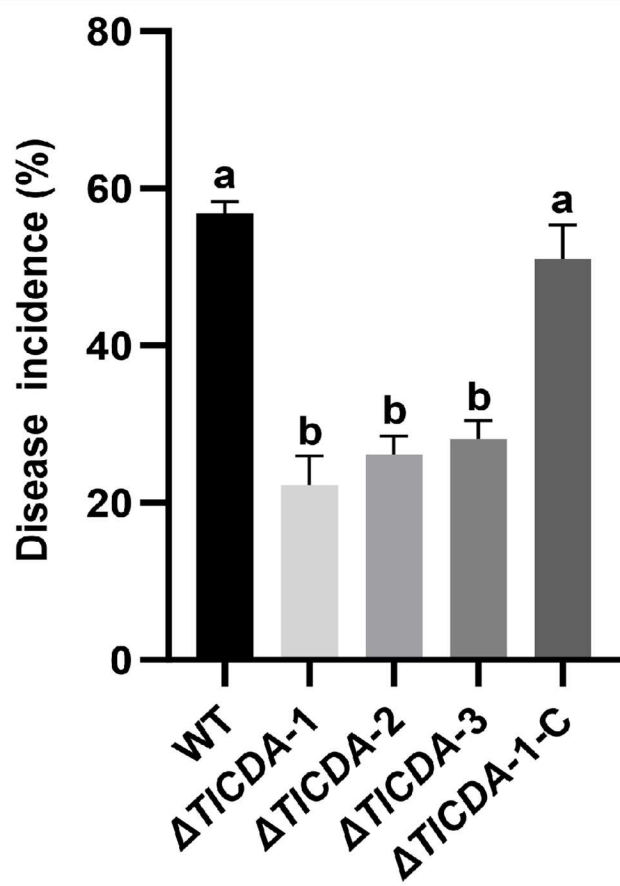
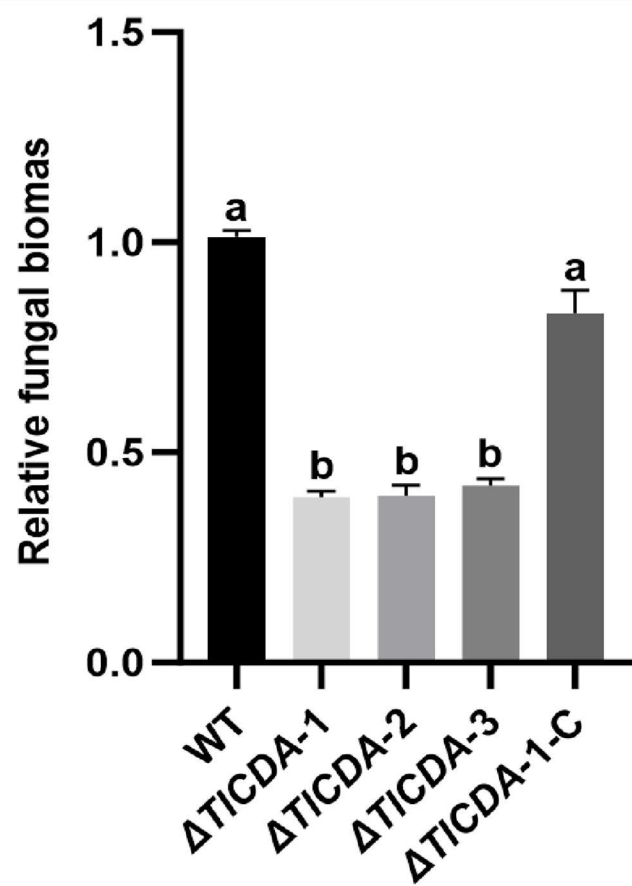
CM

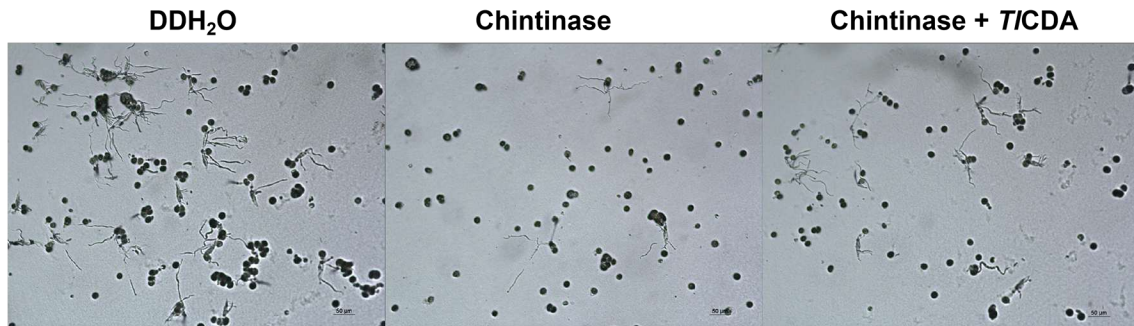
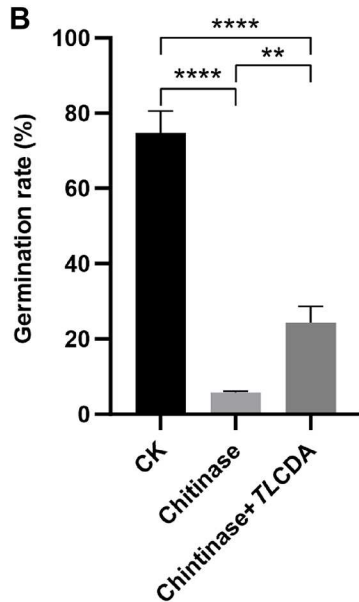


CM+

10 mM Chitinase

**B**

A**B****C**

A**B**



Plant chitinase



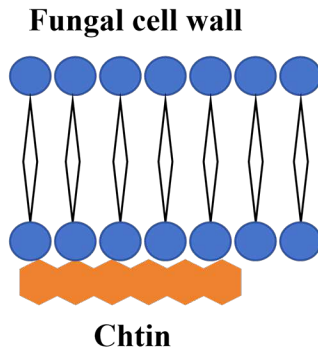
chitin oligomers



Chitin-triggered immunity ↑



PR gene ↑, Cell death ↑, ROS ↑



Chitin deacetylase protection

Plant chitinase



chitin oligomers



Chitin-triggered immunity ↓



PR gene ↓, Cell death ↓, ROS ↓

Supplementary Files

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