

Physiological and Chitinase Gene Expression Responses of Male *Zizania latifolia* to *Ustilago esculenta* Infection

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ABSTRACT. This study explored the effects of *Ustilago esculenta* inoculation on physiological responses (activities of defense and antioxidant enzymes) and chitinase gene expression in male *Zizania latifolia* “jiaobai” (without *U. esculenta* infection, with no galls, but normal flowering). Male jiaobai seedlings were injected at the five-leaf stage with *U. esculenta* suspension, and the impact on transcription of several genes encoding enzymes was examined. Compared with controls, expression of most enzymes was significantly different at 3 or 12 hours postinjection, and most *ZlChi* genes were involved in the response to *U. esculenta* inoculation. Fluorescence quantitative polymerase chain reaction results showed that *U. esculenta* was present in the roots of male jiaobai inoculated with *U. esculenta* at the shoot tips. Paraffin sections revealed many fungal hyphae in the roots at 15 d after inoculation, but few in controls. The results provide a basis for further study of the responses of male *Z. latifolia* to *U. esculenta* infection.

Zizania latifolia, also known as Manchurian wild rice, is an asexual perennial herb and the second most widely cultivated aquatic vegetable in China (Yu et al. 2020). The endophytic smut fungus *Ustilago esculenta* induces the formation of culm galls (jiaobai) in *Z. latifolia*. Normal jiaobai (edible swollen galls in stem bases filled with hyphae), gray jiaobai (inedible galls filled with dark teliospores, with an unacceptable taste), and male jiaobai (without *U. esculenta* infection, with no galls but normal flowering) are the three phenotypes of *Z. latifolia* found in their habitats (Wang et al. 2020). At present, the method of isolation and selection of *Z. latifolia* used in cultivation and conservation breeding requires considerable labor and material resources. Moreover, population diversity and physiological race differentiation of *U. esculenta* may exist among *Z. latifolia* populations (Tu et al. 2019). The molecular mechanisms of *Z. latifolia* responses to *U. esculenta* infection are not completely

understood, although the mechanism of swelling and stem enlargement in infected *Z. latifolia* has received attention (Li et al. 2022; Zhang et al. 2021).

Research on the physiological and biochemical aspects of *Ustilago*-induced stem expansion of *Z. latifolia* has focused mainly on changes in antioxidant enzymes, including peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), and polyphenol oxidase (PPO). The activities of SOD, CAT, and PPO decrease during the process of stem expansion, whereas POD activity increases during the middle stage of stem expansion, suggesting that POD might participate in the differentiation and expansion of *Zizania* cells and tissues (Jiang et al. 2007). Meanwhile, proteomic studies have explored the stem expansion process of *Z. latifolia*. The number and expression level of unique proteins in stems of *Z. latifolia* at different developmental stages varies significantly, and the formation of fleshy stem bases of *Z. latifolia* result from interactions between plants and endophytic fungi (Liu et al. 2011). During the process of fleshy stem expansion, 4389 upregulated and 3700 downregulated genes were detected in normal *Z. latifolia*. By comparison, in gray *Z. latifolia*, 3121 genes were upregulated and 2130 genes were downregulated (Wang et al. 2017). Jose et al. (2019) detected qualitatively and quantitatively differentially expressed proteins in response to local *U. esculenta* formation during the interaction between *Z. latifolia* and *U. esculenta* and found that *U. esculenta*

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produced proteases and energy acquisition proteins that enhanced its defense and survival in the host.

Plants can be invaded by various pathogens. To cope with pathogen infection, a series of physiological and biochemical changes take place in challenged plants. Among these, changes in key enzyme activities related to plant disease resistance can effectively enable plants to withstand further infection by pathogens (Espinosa-Vellarino et al. 2020; Waszczak et al. 2018). These defense enzymes can improve the tolerance and resistance of plants to external abiotic and biotic stresses (Zhang 2020). Pathogenesis-related proteins (PRs), such as chitinases and β -1,3-glucanase, are a class of proteins induced by biotic or other external factors that play an important role in plant disease resistance (Tuan et al. 2019; Zhou et al. 2020). In the absence of pathogens, chitinase and β -1,3-glucanase activities in plants are very low, but when plants are infected by fungal phytopathogens, the activities of β -1,3-glucanase and chitinase increase within a short time, rapidly degrading chitin in the cell walls of phytopathogenic fungi and inhibiting the growth and reproduction of pathogenic fungi in resistant plants, thereby increasing the resistance of plants to pathogens (Bai et al. 2018; Hu et al. 2017; Khan et al. 2017).

In previous studies on interactions between *Z. latifolia* and *U. esculenta*, we established an in vitro *Z. latifolia* regeneration system, explored the *U. esculenta* inoculation system (Yang 2018), and studied the expression of *Z. latifolia* chitinase in response to abiotic stress (Zhou et al. 2020). However, the mechanism underpinning the interaction between *U. esculenta* and *Z. latifolia* that leads to enlargement of swollen stem galls remains unclear. To explore the physiological basis and molecular mechanisms of the *Z. latifolia* responses to *U. esculenta* infection, male (uninfected) *Z. latifolia* plants at the five-leaf stage were used as the experimental material to explore the effects of artificial inoculation on the responses of disease resistance-associated enzymes, phenylpropanoid metabolism-related enzymes and antioxidant enzymes, as well as the responses of membrane lipid peroxidation. In addition, paraffin sections of roots and stems of *Z. latifolia* plants at 15 d after *U. esculenta* inoculation were collected to probe changes in *U. esculenta* fungus development. This work provides an important basis for future studies on the mechanism of culm gall formation in *Z. latifolia*.

Materials and Methods

U. ESCULENTA PREPARATION AND INOCULATION TECHNIQUES. Single colonies (mycelium) of *U. esculenta* were selected and cultured in potato dextrose liquid medium at 28 °C with shaking at 220 rpm on an orbital shaker. When the optical density at 600 nm (OD_{600}) reached 0.6 to 0.8, cultures were expanded at a dilution of 1:50 overnight. The suspension was then centrifuged at 2632 g_n for 20 min to collect cells, and the pellet was resuspended in potato dextrose liquid medium and diluted to $OD_{600} = 2.5$ (Geng 2018; Yang 2018).

Male jiaobai plants at the five-leaf stage (Fig. 1A) were injected at the stem tip growth point with the resulting *U. esculenta* cell suspension, 0.5 cm from the base of the bolting tube (Fig. 1B, red arrow). Male jiaobai plants injected with potato dextrose liquid medium served as controls (CK). Experiments were repeated three times with six plants per replicate.

MEASURING THE PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF *Z. LATIFOLIA* TO *U. ESCULENTA* INJECTION. Crude extracts of leaves of *Z. latifolia* plants were prepared at 3, 6, 12, 18, and 24 h

after inoculation, and the activities of defense enzymes and antioxidant enzymes were assayed. The activities of ascorbate peroxidase (APX) and phenylalanine ammonia lyase (PAL) were determined according to the methods of Gao and Cai (2018); the activity of POD was assayed according to the method of Wu (2018); the activities of SOD and CAT were determined according to the methods of Gao and Cai (2018); the activity of PPO was determined using the methodology described by Dalmadi et al. (2006). Extraction of chitinase and β -1,3-glucanase was performed according to the methods of Liu (2013) and Boller et al. (1983), respectively. The activity of β -1,3-glucanase was determined using the method of Jiang et al. (2015), and a glucose standard curve was prepared according to the method of Li (1998). Determination of chitinase activity and preparation of an *N*-acetylglucosamine standard curve were conducted according to the methods of Liu (2013) and Zhang et al. (2013), respectively. Three biological replicates were performed for each treatment, with six plants per replicate.

RNA EXTRACTION AND QUANTITATIVE REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION. Leaves at 3, 6, 12, 18, and 24 h, and 3 and 5 d after inoculation, were ground with liquid nitrogen, total RNA was extracted using TRIzol reagent (RNAiso Plus; Takara, Dalian City, China), and treated using a PrimeScript RT Reagent Kit and gDNA Eraser (Takara) to remove genomic DNA contamination. The quality and purity of samples were monitored using a spectrophotometer (NanoDrop 2000C; Thermo Fisher Scientific, Waltham, MA, USA). Total RNA (1 μ g) was reverse-transcribed to first-strand cDNA using a PrimeScript RT Reagent Kit with gDNA Eraser (Takara) following the manufacturer's instructions. The resulting cDNA was diluted 10-fold with sterile water. Gene-specific primers (Supplemental Table 1) used for quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis of the *Z. latifolia* chitinase gene were designed using Primer Premier 5.0 software (Kakhki et al. 2019). The expression level of the *Z. latifolia Actin2* gene (primer sequences listed in Supplemental Table 1) (Wang et al. 2017), which is consistent in different organs and under different treatments, was used as an internal control (Supplemental Fig. 1). qRT-PCR was carried out according to the instructions of the PrimeScript RT Reagent Kit with gDNA Eraser (Takara). Three biological replicates were performed for each treatment, and each cDNA sample was tested using four technical replicates. Relative expression levels were calculated as $2^{-\Delta\Delta Cq}$ where $\Delta Cq = Cq$ (gene of interest) – Cq (*Actin2*), $\Delta\Delta Cq = \Delta Cq$ (treatment, inoculated with *U. esculenta*) – ΔCq (CK, inoculated with potato dextrose broth).

DETECTION OF *U. ESCULENTA* IN INOCULATED PLANTS. Genomic DNA was extracted from roots of plants at 15 d after inoculation, and expression of the *actin* gene of *U. esculenta* was measured by qRT-PCR (Ge et al. 2021). Male jiaobai plants inoculated with potato dextrose liquid medium served as a negative control, and *U. esculenta* DNA served as a positive control. Primers P1 (CAATGGTTCGGGAATGTGC) and P2 (GGGATACTTGAGCGTGAGGA) were used to amplify the *actin* gene sequence (KU302684.1). qRT-PCR was performed according to the preceding instructions, and products were detected by electrophoresis on a 2.0% agarose gel and purified. The obtained target fragments were ligated into the pMD19-T vector, transformed, and sequenced (Sangon Biotech Shanghai Co. Ltd., Shanghai, China).

At 15 d after inoculation, roots of male *Z. latifolia* were quickly fixed in formaldehyde-acetic acid-ethanol fixative comprising 5% formalin (37% to 40% formaldehyde) + 5% glacial

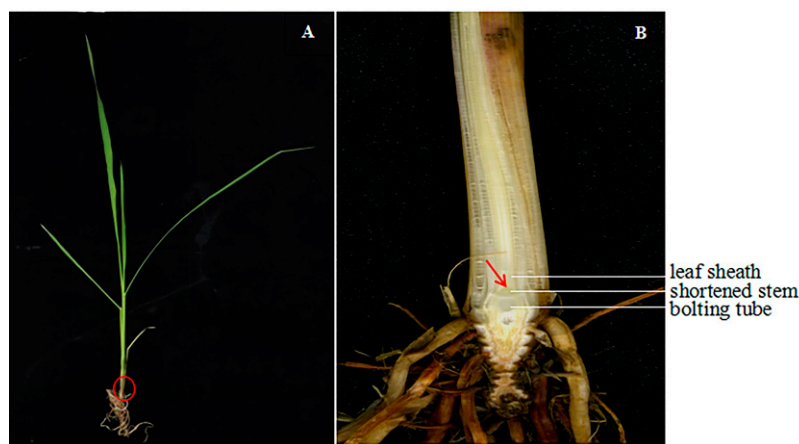


Fig. 1. Images of the five-leaf stage of male *Zizania latifolia* seedlings: (A) seedlings and (B) enlarged view of partial longitudinal cutting.

acetic acid + 90% alcohol (70%). Using qRT-PCR detection, roots that only amplified the *Ue-actin* gene of *U. esculenta* and roots without any amplified band were analyzed by paraffin section, including tissue cleaning, fluorescent staining of fungal cell walls with fluorescein isothiocyanate-conjugated wheat germ agglutinin (FITC-WGA), and counterstaining of plant cell walls with propidium iodide (Redkar et al. 2018), performed by Wuhan Seville Biotechnology Co., Ltd. (Wuhan, China).

DATA PROCESSING. Spreadsheet software (Microsoft Excel 2016; Microsoft Corp., Redmond, WA, USA) was used for basic processing of test data, statistical analysis software (IBM SPSS statistics ver. 25; IBM Corp., Armonk, NY, USA) was used for analysis of variance ($P \leq 0.05$ indicates statistical significance), and data analysis/scientific graphing software (SigmaPlot 14.0, Systat International Software, San Jose, CA, USA); Origin 2019b 32-bit (OriginLab, Northampton, MA, USA) were used for mapping.

Results

ACTIVITIES OF ANTIOXIDANT AND DISEASE-RELATED ENZYMES. APX activity in leaves of male *Z. latifolia* inoculated with *U. esculenta* was significantly higher than in control plants at 12 h after inoculation, indicating that 12 h was a suitable timepoint for assessing APX involvement in the response to *U. esculenta* injection (Fig. 2). CAT activity in the treated group was also higher than that in the control group, with the highest level of 73.67 U/g fresh weight per minute occurring at 3 h after inoculation, and the difference was significant ($P \leq 0.05$; Fig. 2). The same trend was observed for POD activity, indicating that the response of POD activity to *U. esculenta* mainly occurred shortly after inoculation (Fig. 2). There was no significant difference in SOD activity and malondialdehyde (MDA) content between treated and control plants within 24 h after inoculation (Fig. 2).

PPO activity of the treated group was significantly lower than that of the control at 6 h after inoculation ($P \leq 0.05$), whereas PAL activity in the treated group was insignificant compared with that of the control group ($P \leq 0.05$; Fig. 3). Chitinase in leaves of male *Z. latifolia* showed a pattern of decreasing activity over time, and there was a significant difference between treated and control groups only at 12 h after inoculation ($P \leq 0.05$; Fig. 4). The activity of β -1,3-glucanase in the treated group was higher than that of the control group at 3, 6, and 18 h after

inoculation, with a significant difference at 3 h after inoculation ($P \leq 0.05$; Fig. 4).

EXPRESSION PROFILING OF *ZlChi* GENES IN RESPONSE TO *U. ESCULENTA* INFECTION. Expression of individual *ZlChi* genes was detected at 3, 6, 12, 18, and 24 h, and 3 and 5 d after treatment. The expression patterns of *ZlChi* genes were different, among which expression of three *ZlChi* genes (*ZlChi2*, *ZlChi5*, and *ZlChi9*) could not be detected, whereas that of the other eight responded to *U. esculenta* inoculation (Fig. 5). *ZlChi1* expression was upregulated, and the difference was significant ($P \leq 0.05$) at 18 h and 3 d after inoculation. Expression of *ZlChi6* was upregulated at 3, 6, and 12 h after inoculation, and the difference was significant ($P \leq 0.05$). The relative expression level of *ZlChi8* was highest at 5 d after inoculation, and the difference was significant ($P \leq 0.05$), whereas the relative expression level of *ZlChi10* was also upregulated at 3, 6, and 18 h after inoculation. The relative expression level of *ZlChi11* reached a peak (10.50) at 3 h after injection, but expression of the other *ZlChi* genes was low or downregulated in response to *U. esculenta* inoculation (Fig. 5).

DETECTION OF *U. ESCULENTA* IN INOCULATED PLANTS. The root DNA templates at 15 d after inoculation were amplified using primers targeting *Ue-Actin*. Agarose gel electrophoresis showed that only one band was detected in some roots of male jiaobai plants inoculated with *U. esculenta*, and sequencing results showed that the fragment was 243 bp in size, with 100% homology to *Ue-Actin* (KU302684.1). In addition, two bands of 243 bp (*Ue-Actin*) and 139 bp (86% identical to the *NIH2624 actin* gene XM_001209659.1 of *Aspergillus terreus*) were detected in other inoculated roots. Meanwhile, some roots were not infected at all by any fungi, and some were only infected with one fungal species (Fig. 6). This indicated that some *Z. latifolia* plants had been successfully infected by *U. esculenta* 15 d after inoculation.

Roots that only amplified bands (243 bp) of *U. esculenta* at 15 d after inoculation and control roots were selected for paraffin sectioning. The root tissue of male *Z. latifolia* plants was quickly fixed in formaldehyde-acetic acid-ethanol (FAA) fixative, and paraffin sections were stained with FITC-WGA and counter-stained with propidium iodide. In the same field of vision (10 $\times$ field of view), numerous fungal hyphae could be observed in the roots of male *Z. latifolia* plants inoculated with *U. esculenta*, whereas few fungal hyphae of *A. terreus* could be seen in the roots of control

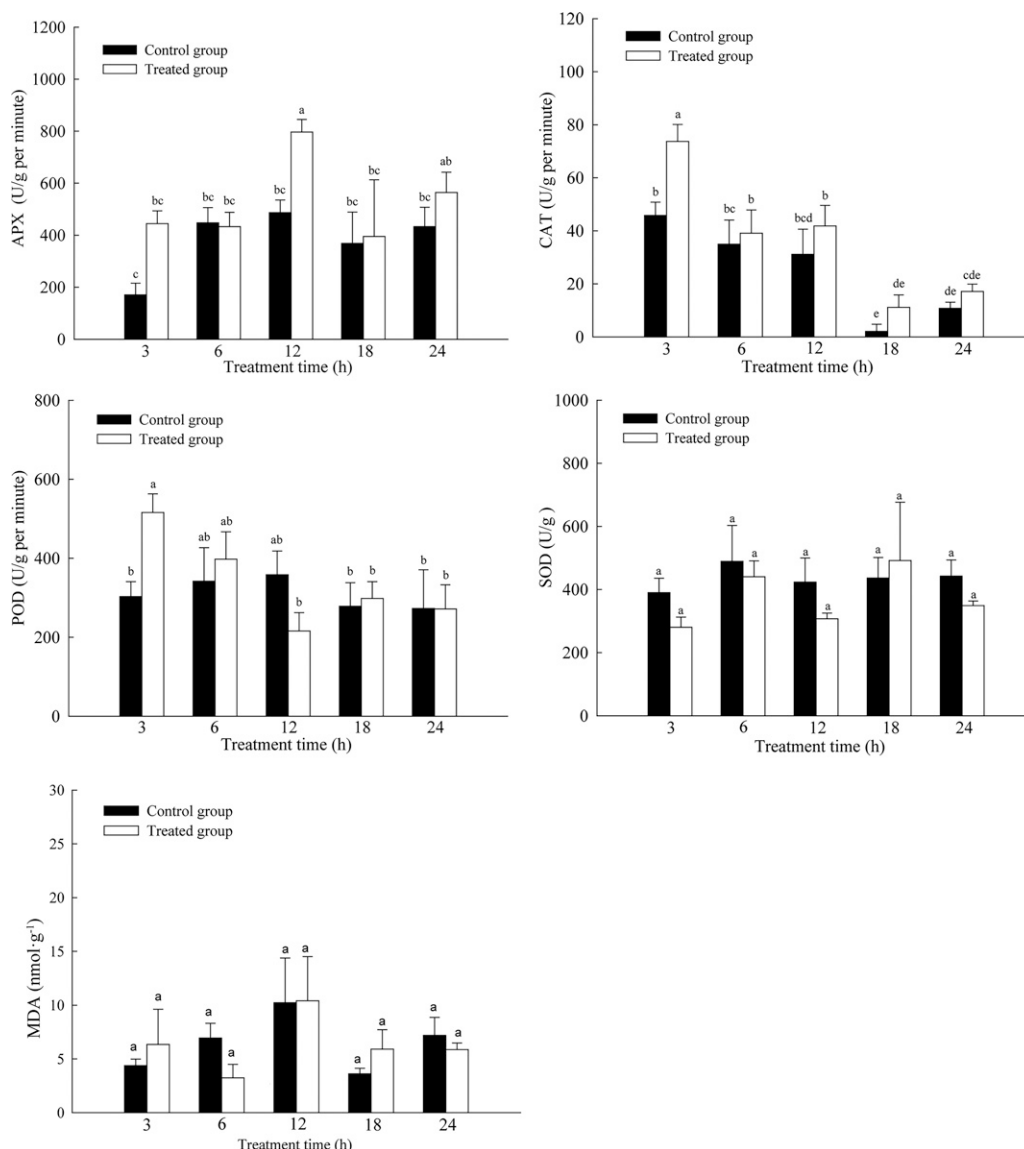


Fig. 2. Changes in the activities of antioxidant-related enzymes ascorbate peroxidase (APX), catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and malondialdehyde (MDA) content following infection of male *Zizania latifolia* by *Ustilago esculenta*. Error bars represent standard deviations of mean values from three biological replicates. Shared lowercase letters indicate no significant differences in activities of a particular enzyme between different treatments via analysis of variance ($P \leq 0.05$).

male *Z. latifolia* plants (Fig. 7). This indicated that *U. esculenta* had successfully infected male *Z. latifolia* plants.

Discussion

PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF *Z. LATIFOLIA* TO *U. ESCULENTA* INOCULATION. When plants encounter stress, it can regulate various antioxidant enzymes (e.g., APX, POD, SOD, and CAT) and disease resistance-related enzymes (chitinase and β -1,3-glucanase) to reduce damage to cells and tissues (Tyagi et al. 2020; Wang et al. 2021a). Key enzymes of the phenylpropane metabolic pathway (e.g., PAL and PPO) can also respond quickly to invasion of plant pathogens (Sakhuja et al. 2021; Syed et al. 2021). Yan et al. (2013) reported that *U. esculenta* infection led to an increase in APX, CAT, POD, glutathione reductase, and SOD activities in *Z. latifolia* leaves, and a decrease in the concentrations of superoxide free radicals and MDA. Our results showed that inoculation of *Z. latifolia*

with *U. esculenta* significantly increased the activities of APX and CAT enzymes in leaves. The activities of CAT and POD were highest at 3 h after inoculation, whereas SOD activity was highest at 18 h after inoculation. Changes in antioxidant enzyme activity were consistent with previous reports by Yan et al. (2013).

Chitinase (EC 3.2.1.14) is a type of glycosidase that degrades chitin from fungi and responds to fungal infection (Zhou et al. 2020). β -1,3-glucanase (EC 3.2.1.39) inhibits the growth of fungi by catalyzing the hydrolysis of β -1,3-glucan in fungal cell walls, participates in the defense of plants against pathogenic fungi (Ali et al. 2021). When crops are infected by phytopathogenic fungi, chitinase and β -1,3-glucanase can inhibit the growth of pathogenic fungi, thus mediating resistance to fungal pathogens (Toufiq et al. 2018). Our results showed that chitinase activity in male *Z. latifolia* leaves was higher than in controls, and the gradual decrease in activities of chitinase and β -1,3-glucanase was similar to that reported by Wang et al. (2021b).

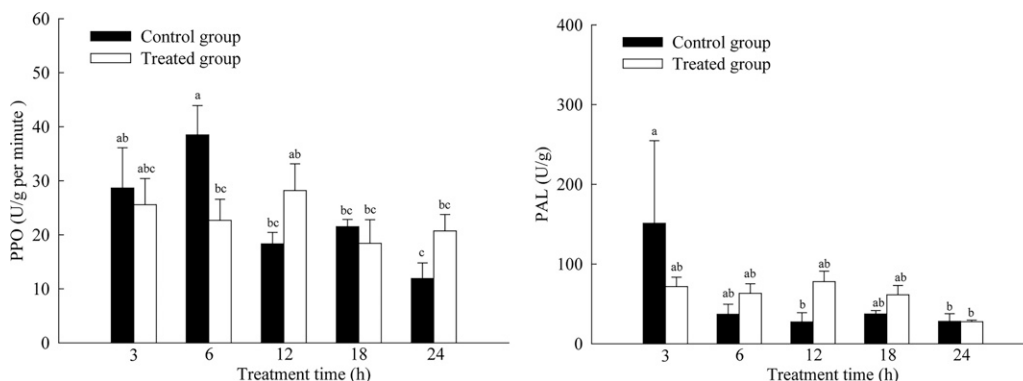


Fig. 3. Changes in the activities of enzymes related to the phenylpropanoid metabolism pathway, namely polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL), after infection of *Zizania latifolia* by *Ustilago esculenta*. Error bars represent standard deviations of the mean values of three biological replicates. Shared lowercase letters indicate no significant differences in activities of a particular enzyme between different treatments via analysis of variance ($P \leq 0.05$).

PPO (EC 1.10.3.1) is associated with disease resistance, pest resistance, and stress tolerance (Kanjapongkul and Baibua 2021; Zhao et al. 2021), and PAL (EC 4.3.1.24) regulates the biosynthesis of secondary metabolites associated with plant disease resistance (Dong and Lin 2021). In the current study, PPO activity in treated plants was significantly lower than that in controls at 6 h post-injection. PAL activity in treated plants was also higher than that in controls. These results revealed that the increases in PPO and PAL activities in treated plants mainly occurred in the early stages of the colonization process. Previous research also showed that PAL activity was higher during the early stages of *U. esculenta* colonization in *Z. latifolia* (Cheng 2002). However, in the present study, PAL activity reached the highest level at 12 h after inoculation. Thus, infection by *U. esculenta* altered antioxidant enzyme activity in *Z. latifolia* plants. Endophytic fungi and their hosts need to adapt and evolve together over a long period of time to form a mutually beneficial symbiotic relationship (Delavaux et al. 2019; Saikkonen et al. 2004). The observed changes in the activities of enzymes in *Z. latifolia* plants may be related to the formation of a mutually beneficial symbiosis between *Z. latifolia* and *U. esculenta*. However, infection of male jiaobai plants by *U. esculenta* and the resulting symbiotic relationship require further investigation.

EXPRESSION PATTERNS OF *ZlChi* GENES IN RESPONSE TO INOCULATION WITH *U. ESCULENTA*. Plant chitinases are pathogenesis-related proteins that degrade chitin, damage the fungal cytoskeleton,

inhibit the growth of pathogenic fungi, and elicit antifungal effects (Bartholomew et al. 2019; Chen et al. 2018). Previous research showed that chitinase genes regulate the responses of *Morus laevigata* to *Sclerotinia sclerotiorum* infection (Jiang et al. 2020). Similarly, upregulated expression of *GH19* (TEA028279 and TEA019397) indicated that chitinase genes play defensive roles in responses to fungal infection (Bordoloi et al. 2021). The current study showed that, apart from *ZlChi2*, *ZlChi5*, and *ZlChi9* genes, expression of the other eight *ZlChi* genes responded to *U. esculenta* infection. Thus, we speculated that these genes may be involved in the infection process of male *Z. latifolia* by *U. esculenta*. Previous research revealed time differences in the expression patterns of different *CmCHT* genes in *Cucumis melo* induced by pathogen infection (Wang et al. 2021b). Similarly, in the present study, expression of most *ZlChis* also showed temporal differences. Expression of most chitinase genes peaked during the early stages after inoculation, indicating an involvement in the colonization of *U. esculenta*. However, expression of *ZlChi1*, *ZlChi4*, and *ZlChi8* peaked at 3 or 5 d after inoculation, indicating that these genes might play a key role in the later stages of *U. esculenta* infection.

DETECTION OF *U. ESCULENTA* IN MALE *Z. LATIFOLIA* 15 D AFTER INOCULATION. In the current study, fluorescence quantitative PCR was used to detect fungal genomic DNA in male *Z. latifolia* roots at 15 d after inoculation, and the results showed that only the *Ue-Actin* gene, or two fragments (the *Ue-Actin* gene fragment and the

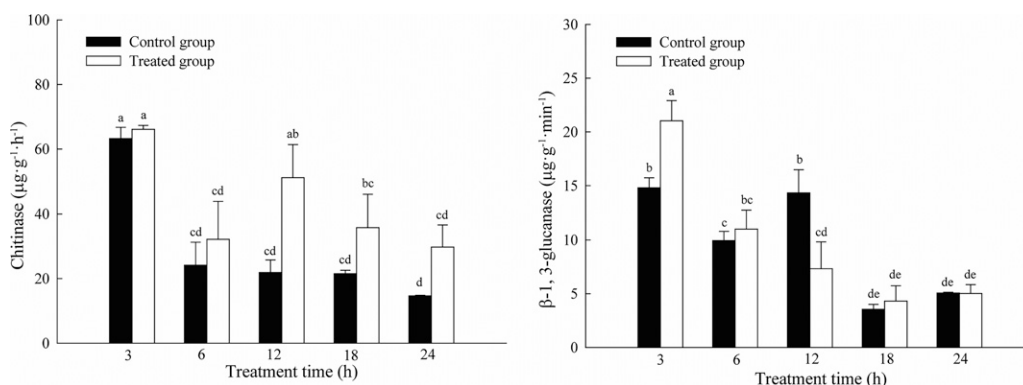


Fig. 4. Changes in chitinase and β-1,3-glucanase activities in *Zizania latifolia* following infection by *Ustilago esculenta*. Error bars represent standard deviations of the mean values of three biological replicates. Shared lowercase letters indicate no significant differences in activities of a particular enzyme between different treatments via analysis of variance ($P \leq 0.05$).

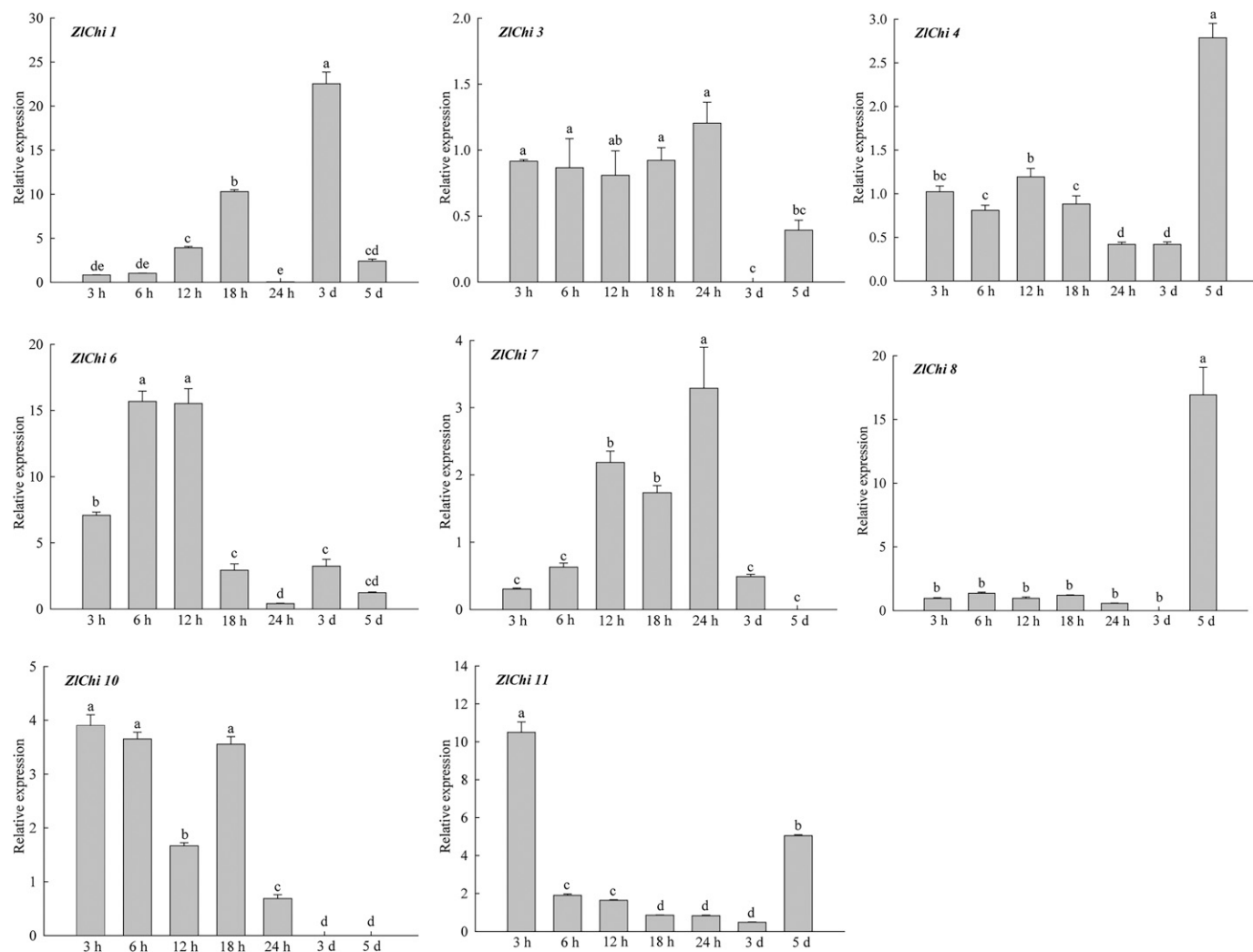


Fig. 5. Patterns of relative gene expression analysis of ZlChi genes in *Zizania latifolia* leaves revealed by real-time fluorescence quantitative polymerase chain reaction in response to *Ustilago esculenta* infection. ZlActin2 served as an internal control to normalize data. The y-axes, indicating relative expression levels, are drawn to different scales depending on the magnitude and direction of change. Upregulated expression is represented by whole-number y-axis values, whereas downregulated gene expression is indicated by decimal fractions on the y-axis scale. Error bars represent standard deviations of the mean values of three biological replicates. Shared lowercase letters indicate no significant differences in activities of a particular enzyme between different treatments via analysis of variance ($P \leq 0.05$).

NIH2624 actin gene fragment), were amplified from the roots of some inoculated plants, indicating that *U. esculenta* successfully infected and reached the roots of male jiaobai plants at 15 d after inoculation. Meanwhile, the NIH2624 actin gene fragment was

amplified from other treated plants, which indicates that there were other fungi (*A. terreus*) within male *Z. latifolia* plants.

Li (2016) found that *U. esculenta* in normal *Z. latifolia* (with swollen stem bases) was mainly distributed in the root and stem

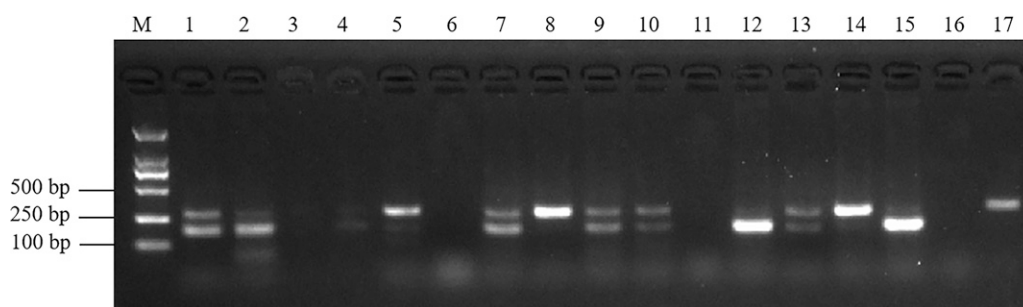
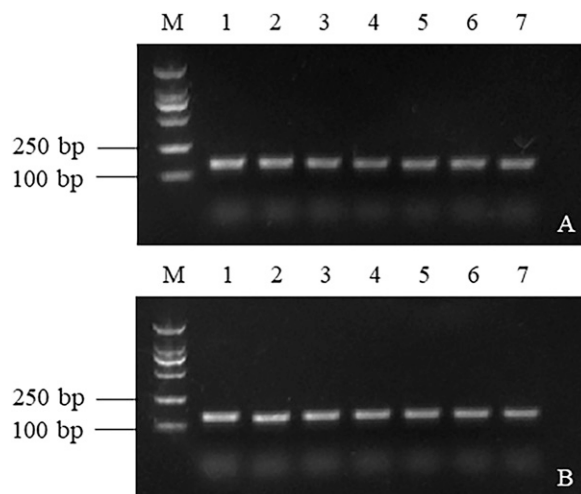


Fig. 6. Electrophoretic image (2.0% agarose gel) of real-time quantitative polymerase chain reaction products of Ue-Actin from roots of male *Zizania latifolia* 15 d after inoculation with *Ustilago esculenta*; M = DNA markers; lanes 1–15 = extracts of male *Z. latifolia* plants inoculated with *U. esculenta*; lane 16 = negative control; lane 17 = positive control.

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Supplemental Fig. 1. Electrophoretic image (2.0% agarose gel) of polymerase chain reaction amplification of *ZlActin* (primer sequences listed in Supplemental Table 1) from treated and control samples of *Zizania latifolia*. M = DNA markers. (A) Male *Z. latifolia* inoculated with potato dextrose liquid medium; (B) male *Z. latifolia* plants inoculated with *U. esculenta*; lanes 1 – 7 = 3, 6, 12, 18, and 24 h, and 3 and 5 d after treatment.

Supplemental Table 1. Basic information on *ZlChis* in *Zizania latifolia*, and primer sequences used for real-time fluorescence quantitative polymerase chain reaction.

Gene name	Sequence ID	Primers	
		Forward primer	Reverse primer
ZlChi1	Zlat_10003182	GGGCATATCCAACAATGGCGAACT	CCGGAGATGAGGAGCACTAA
ZlChi2	Zlat_10047807	GGAAAGCTCAGCCATCGAGA	GAGGAGTGGGAGAAAGCGAAGT
ZlChi3	Zlat_10012767	GGTTCTGCTGCAACGAGAC	GGTGATGAAGGCCTGGTAGT
ZlChi4	Zlat_10016842	CTCCGAAGACGACTCCAAG	CGATCTCCTCGATGTAGCAG
ZlChi5	Zlat_10017446	GCTGCACCGATGTACAGATTG	CTTGTGCAGGAACAGCGAC
ZlChi6	Zlat_10048858	CGCTCTGGTTCTGGATGA	ATCTGTTGCCGTTGCACT
ZlChi7	Zlat_10029194	GACCGCATCGGCTACTACAAG	CTGGTTGTAGCAGTCGAGGTT
ZlChi8	Zlat_10031220	TTACTGCGACGCCACTGACA	CCCGTAGTTGAAGTTCCATGAG
ZlChi9	Zlat_10048857	ATGATGTCGTTCTGGCTC	CTTGAAGAACGCCTCAGTG
ZlChi10	Zlat_10004398	CCCTGTGCAACAAACCACTGGAAAC	CCCCTGCGATCATCATGCCAAAT
ZlChi11	Zlat_10041954	ATGGTTTCATCGCACTTCC	GGTGAGACACGTTTCGTGAACAT
ZlActin 2	LOC102711684	TAACCGGCCACGTGTATTTA	AGAGCAGAGGCATTCCAAGT
Ue-actin	KU302684.1	CAATGGTTTCGGGAATGTGC	GGGATACTTGAGCGTGAGGA