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ORIGINAL PAPER

Induction of DIMBOA accumulation and systemic defense responses as a mechanism of enhanced resistance of mycorrhizal corn (*Zea mays* L.) to sheath blight

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Abstract Arbuscular mycorrhizas are the most important symbioses in terrestrial ecosystems and they enhance the plant defense against numerous soil-borne pathogenic fungi and nematodes. Two corn (*Zea mays*) varieties, Gaoyou-115 that is susceptible to sheath blight disease caused by *Rhizoctonia solani* and Yuenong-9 that is resistant, were used for mycorrhizal inoculation in this study. Pre-inoculation of susceptible Gaoyou-115 with arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* significantly reduced the disease incidence and disease severity of sheath blight of corn. HPLC analysis showed that AMF inoculation led to significant increase in 2,4-dihydroxy-7-methoxy-2 H-1,4-benzoxazin-3(4 H)-one (DIMBOA) accumulation in the roots of both corn varieties and in leaves of resistant Yuenong-9. *R. solani* inoculation alone did not result in accumulation of DIMBOA in both roots and leaves of the two corn varieties. Our previous study showed that DIMBOA strongly inhibited mycelial growth of *R. solani* in vitro. Real-time PCR analysis showed that mycorrhizal inoculation itself did not affect the transcripts of most genes tested. However, pre-inoculation with *G. mosseae* induced strong responses of three defense-related genes *PR2a*, *PAL*, and *AOS*, as well as *BX9*, one of the key genes in DIMBOA biosynthesis pathway, in the leaves of corn plants of both Yuenong-9 and Gaoyou-115 after the pathogen attack. Induction of defense responses in pre-inoculated plants was much higher and quicker than that in non-inoculated plants upon *R. solani* infection. These results indicate that induction of accumulation of DIMBOA, an important phytoalexin in corn, and systemic defense responses by AMF, plays a vital role in enhanced disease resistance of mycorrhizal plants of corn against sheath blight. This study also suggests that priming is an important mechanism in mycorrhiza-induced resistance.

Keywords Arbuscular mycorrhizal fungus · *Glomus mosseae* · Induced disease resistance · Systemic defense responses · *Rhizoctonia solani* · *Zea mays* · DIMBOA · BX_9 gene

Abbreviations

AMF	Arbuscular mycorrhizal fungus
DIMBOA	2,4-dihydroxy-7-methoxy-2 H-1, 4- benzoxazin-3(4 H)-one
PAL	Phenylalanine ammonia-lyase
AOS	Allene oxide synthase

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Introduction

In addition to improving plant nutrient supply and overcoming abiotic stress, mycorrhizal colonization has been demonstrated to protect host plants against many pathogens (Cordier et al. 1998; Whipps 2004). The pre-inoculation of onion (*Allium cepa*) with *Glomus mosseae* significantly reduces the pink root disease caused by *Pyrenochaeta terrestris* (Safir 1968; Azcón-Aguilar and Barea 1996). The incidence and disease indices of verticillium wilt of cotton were significantly reduced in all growth stages of cotton after colonization with *Glomus mosseae, Glomus versiforme,* and *Sclerocystis sinuosa* (Liu 1995). Mycorrhizal colonization enhances tomato resistance to diseases caused by *Erwinia carotovora* (García-Garrido and Ocampo 1988), *Fusarium oxysporum* f. sp. *lycopersici* (Akköprü and Demir 2005), *Phytophthora nicotianae* var. parasitica (Cordier et al. 1996), *Phytophthora parasitica* (Cordier et al. 1998), and *Pseudomonas syringae* (García-Garrido and Ocampom 1989). Mycorrhiza also reduces susceptibility of tomato to foliar disease of early blight caused by *Alternaria solani* (Fritz et al. 2006). Mycorrhizal colonization increases corn resistance to sheath blight disease (Huang et al. 2006). Over the last 30 years, there has been an increasing interest in using arbuscular mycorrhizal fungi (AMF) for bioprotection of plant diseases (St-Arnaud and Vujanovic 2007; Khaosaad et al. 2007; Elsen et al. 2008).

Plants elaborate a vast array of secondary metabolites, many of which can protect plants against a wide variety of microorganisms (viruses, bacteria, fungi) (Dixon 2001; Bednarek and Osbourn 2009). Mycorrhizal colonization leads to an array of changes in secondary metabolism both quantitatively and qualitatively in host plants, which may be one of the important mechanisms of induced resistance in mycorrhizal plants (Pozo and Azcon-Aguilar 2007; Zubek et al. 2010). Many studies show that mycorrhizal colonization induces accumulation of phenolic compounds, alkaloids, terpenoids and essential oil (Araim et al. 2009; Bi et al. 2007; Toussaint et al. 2007; Zhu and Yao 2004).

Cyclic hydroxamic acids (Hx), a novel class of alkaloids, are naturally occurring benzoxazinones which possess the 2-hydroxy-2 H-1,4-benzoxazin-3-(4 H)-one skeleton. Hx have been shown as main natural defense compounds against bacteria, fungi, and insects in Gramineae (Bohidar et al. 1986; Niemeyer 1988; Figueroa et al. 1999; Wilkes et al. 1999; Rostás 2007). They have been found in a wide range of Gramineae, including wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and rye (*Secale cereale* L.) (Niemeyer 1988; Niemeyer and Perez 1995). The most abundant of Hx in corn and wheat is 2,4-dihydroxy-7-methoxy-1,4- benzoxazin-3-one (DIMBOA) (Bohidar et al. 1986; Sicker et al. 2000). DIMBOA possesses strong biological activity towards various organisms, whereas its glucoside is almost biologically inactive. DIMBOA levels significantly increase in corn and wheat after plant infection by pathogenic fungi, herbivore insects and mechanical damage (Gutierrez et al. 1988; Niemeyer et al. 1989; Morse et al. 1991; Weibull and Niemeyer 1995). Correlations between the susceptibility to Fusarium head blight and the concentrations of the benzoxazinoids DIMBOA-glc, HMBOA-glc, HMBOA, DIBOA-glc, HBOA-glc, and DIM2BOA-glc were found by Søltoft et al. (2008).

Our previous study has shown that mycorrhizal colonization increased disease resistance of corn (cv. Gaoyou-115) to sheath blight disease caused by *Rhizoctonia solani* and improved the plant growth (Huang et al. 2006). The aim of the present study was to investigate effects of mycorrhizal colonization by *Glomus mosseae* on local and systemic DIMBOA production, and thereby to determine the role of DIMBOA in plant-microbe interactions. Systemic induction of defense-related genes by mycorrhizal colonization was also examined.

Materials and methods

Plant and fungal materials

Corn seeds (Gaoyou-115 and Yuenong-9) were provided by Professor Xiulan Liang in the College of Agriculture of South China Agricultural University. Gaoyou-115 is susceptible to sheath blight disease caused by *R. solani* (Huang et al. 2006) and Yuenong-9 is resistant (Liu et al. 1999). Seeds were surface sterilized with 10% H_2O_2 and rinsed five times with sterile distilled water before sowing in autoclaved sand–soil mixture.

The starting inocula of mycorrhizal fungus *G. mosseae* (Nicol. & Gerd) Gerdemann & Trappe used in this experiment were provided by Professor Runjin Liu at Qingdao Agricultural University. The mycorrhizal inoculum was produced in pot culture using corn plants and autoclaved sand media (Chellappan et al. 2002). A mixture of rhizospheric sand from these pot cultures containing 35 infective propagules per gram was used for inoculation.

The pathogen (*R. solani AG1-IA*) was provided by Plant Pathology Laboratory of College of Agriculture at Guangxi University. This pathogen was maintained in Potato Dextrose Agar (PDA) and the inoculum was cultured in PDA at 25°C in the darkness. After 10 days, the microconidia were obtained by flooding with sterilized water and filtering through cheesecloth.

Chemicals

TRIzol reagent, AMV reverse transcriptase, Taq polymerase, dNTPs, primer inhibitor, were purchased from TaKaRa

(Shuzo Co. Ltd., Shiga, Japan), while 4-morpholine-propanesulfonic acid and diethylpyrocarbonate were purchased from AMRESCO (Solon, OH, USA). All solvents used were high-performance liquid chromatography (HPLC) grades.

Bioassay

A bioassay experiment was conducted to compare the disease incidence between mycorrhizal and non-mycorrhizal plants of susceptible corn Gaoyou-115 in a brown loam soil. The soil collected from the university campus in Guangzhou (China) contained 1.59% organic matter, 0.767 g/kg total N, 0.40 g/kg total P, 1.83 g/kg total K, 36.16 mg/kg available N, 1.25 mg/kg available P, and 36.93 mg/kg available K with a pH of 4.70. Each plastic pot was filled with 1.5 kg of the mixture of autoclaved (121°C, 2 h) soil and sand (2:1, *v*/*v*). *G. mosseae* inoculum was added to pots for mycorrhizal treatments (225 g per port), whilst equal amount of sterilized soil mixture was added into pots designated as non-mycorrhizal control. Four pre-germinated corn seeds were transplanted into each pot with the growth substrate. Seedlings were thinned to two per pot 10 days after transplanting. The plants were grown in a growth chamber at 25±1°C with a 16-h photoperiod, 150 Md/m^2/s PAR, and 60% relative humidity. Plants were watered daily and fertilized every 5 days with 50 mL of Hoagland nutrient solution. Twenty-nine days after transplanting the sheath of the corn, plants was wounded with a sterile dissecting needle and inoculated with one piece of inoculum substrate (5 mm in diameter) of *R. solani* cut from the edge of fungal colonies cultured in Petri dishes (9 mm in diameter) with PDA. The inoculated portion of the plants were covered with absorbent cotton, tied with parafilm and moistened with sterile water. There were three replicate pots per treatment.

The disease incidence and severity of corn plants were recorded at 5, 7, 9, and 10 days after the inoculation of pathogen. Disease incidence was defined as percentage of diseased sheaths. Disease severity was estimated using disease index (DI) calculated from disease grades 0–5 (Sriram et al. 1997) using the formula:

$$\mathrm{DI} = \frac{\textit{Sum of individual sheath ratings}}{\textit{Maximum disease score} \times \textit{Number of sheaths sampled}} \times 100$$

Individual sheath ratings in the formula refer to disease grade of each leaf of corn. The maximum disease score refers to the maximum disease grade observed during the entire period of the experiment. Fifty one-centimeter-long root segments were taken from each corn plant, cleaned and stained to measure AM colonization (Mukerji et al. 2002).

Chemical analysis

Four treatments were designed to determine the effects of pathogen and mycorrhizal inoculation on DIMBOA accumulation in corn leaves and roots, i.e., (1) CK, control plants without pathogen and mycorrhizal inoculation; (2) R, plants inoculated with *R. solani*; (3) G, plants inoculated with *G. mosseae*; (4) GR, plants inoculated with both *G. mosseae* and *R. solani*. Pathogen and mycorrhizal inoculation was the same for bioassay experiment described above. One gram of fresh corn leaves and roots was collected from each treatment 1, 5, and 10 days after pathogen inoculation, respectively, and ground in 5 ml distilled water with pH 3.0 adjusted with 0.1 M H_3PO_4. The water extracts were centrifuged at 12,000 g for 20 min at 4°C. The supernatant was partitioned against equal volume of diethyl ether for three repeats. The diethyl ether extracts were combined and concentrated to dryness at 40°C under reduced pressure, then dissolved with 1.0 ml methanol. Methanol extracts were passed through sterilized filter paper in a syringe. DIMBOA in the filtrate was determined by Agilent Technologies HP1100 series HPLC system equipped with an ODS reverse phase C18 column (250×4 mm, 5 μm) and diode array detector (G1315 B) monitoring the absorbance of the elution at 280 nm. The solvent system was a methanol/gradient water 20:80, 0–6 min; linear gradient from 20:80 to 30:70, 6–13 min; 30:70, 13–25 min; linear gradient from 30:70 to 20:80, 25–30 min. The water pH was adjusted to 2.6 with acetic acid. Ten microliters of extracted sample were injected with a flow rate of 1.0 ml/min at 20°C. Standard DIMBOA identified in our laboratory (Huang et al. 2007) was used to determine DIMBOA in the samples by comparing retention times and UV spectrum with the standards. Each treatment had three pots, and three biological replicates per treatment were analyzed.

Real-time PCR analysis

Differential expression of selected genes was verified by RT-PCR using the RNA samples isolated from corn leaves obtained from the four treatments. The total RNA was extracted and isolated according to the method described by Kiefer et al. (2000) with slight modification. Ground fresh leaves (0.2 g) using a mortar and pestle in liquid nitrogen was transferred to a 2-ml Eppendorf tube, and added with 1,000 μl of TRIzol reagent (Invitrogen). The mixture was incubated on ice for 8–10 min and mixed with 200 μl of chloroform. After 5 min incubation at room temperature, the mixture was centrifuged at 12,000 g for 15 min at 4°C. The supernatant was transferred to a 1.5-ml Eppendorf tube added with 500 μl of isoamylalcohol, followed by vortexing at room temperature for 10 min and centrifuging at 13,000 g for another 10 min at 4°C. The supernatant was discarded and

the pellet was washed with 1 ml of 75% ethanol (*v*/*v*), dissolved in 30 μl RNA free water and kept at −80°C until used.

The expression patterns of DIMBOA biosynthetic gene (*BX9*) and defense-related genes (*PAL*, *PR2a*, and *AOS*) in corn leaves of each treatment were analyzed by using real-time polymerase chain reaction (RT-PCR). The primers for target's genes *PR2a*, *PAL*, *AOS*, and *BX9* were designed by Primer 3.0 software (Applied Biosystems, http://fokker.wi.mit.edu/primer3/input.htm) based on corn mRNA sequences deposited in GenBank. Gene-specific forward/reverse primer sets used in these reactions were (F: 5′- TCATCTCGCTGG TACTGCTG -3′/R: 5′- TAGAGCGCAACAGGTTGAT -3′) for *PR2a*, (F: 5′- AAGAAGGTGAACGAGCTGGA -3′/R: 5′- GTTGTCGTTCACGGAGTTGA -3′) for *PAL*, (F: 5′- ACCGGTGTCACGAAAGCTAC -3′/R: 5′- AGCGA CAAACACCTCCAATC -3′) for *AOS*, (F: 5′- GCAACAT-GAGGTACGTGTGC -3′/R: 5′- GCAGCGATCTT GAATTCCTT -3′) for BX_9, and (F: 5′- CCTGCTTCTCATG-GATGGTT -3′/R: 5′- CTCACCACAAGCAGCAAAAA -3′) for *GAPc* which was used as a reference. PCR were carried out with 0.2 μl (0.15 μM) of each specific primer, 1 μl of cDNA, 12.5 μl of the SYBR green master mix (Quanti Tech SYBR Green kit, Qiagen, Gmbh Hilden, Germany) and the final volume made up to 25 μl with RNase-free water. In the negative control, cDNA was replaced by RNase-free water. The reactions were performed on a DNA Engine Opticon 2 Continuous Fluorescence Detection System (MJ Research Inc., Waltham, MA, USA). The program used for PCR was 3 min initial denaturation at 95°C, followed by 35 cycles of denaturation for 20 s at 95°C, annealing for 20 s *PAL*: 51.5°C; *AOS*: 51.5°C; *PR2a*: 55°C; *BX9*: 55°C; *GAPc*: 55°C, and extension for 20 s at 72°C. The fluorescence signal was measured immediately after incubation for 2 s at 75°C following the extension step, which eliminates possible primer dimer detection. At the end of the cycles, melting temperatures of the PCR products was determined between 65°C and 95°C. The specificity of amplicons was verified by melting curve analysis and agarose gel electrophoresis. Each treatment had three pots. The leaves collected from two plants in the same pot were used for RNA extraction. Separate analyses were performed on plants from each of the three pots.

Statistical analysis

For each treatment, three replicates were maintained in a completely randomized design. SAS 8.0 (SAS Institute, Cary, NC, USA) package for windows was used for statistical analysis. The data were analyzed with a one-way analysis of variance with the significant differences among means identified by Duncan's multiple range test ($P<0.05$).

Results

Induction of disease resistance

Inoculation with AMF *G. mosseae* led to significant decrease in disease incidence and disease severity of sheath blight of susceptible corn Gaoyou-115 compared with the control plants that had no mycorrhizal inoculation (Fig. 1). Disease incidences were reduced by 50% and 67.7% at 5 and 10 days after inoculation with pathogen *R. solani*, respectively, in mycorrhizal plants (Fig. 1a). Mycorrhizal plants had significantly less sheath blight symptoms than non-mycorrhizal plants. Disease indices were reduced by 25% and 57% at 5 and 10 days after pathogen inoculation, respectively (Fig. 1b). Furthermore, disease development in plants colonized by *G. mosseae* was significantly slower compared with that in control plants. Mycorrhizal colonization rates were 33% and 29.3% in inoculated plants of Gaoyou-115 and Yuenong-9, respectively.

Induction of DIMBOA accumulation

DIMBOA concentrations in corn leaves and roots were determined by HPLC. DIMBOA concentrations in the roots

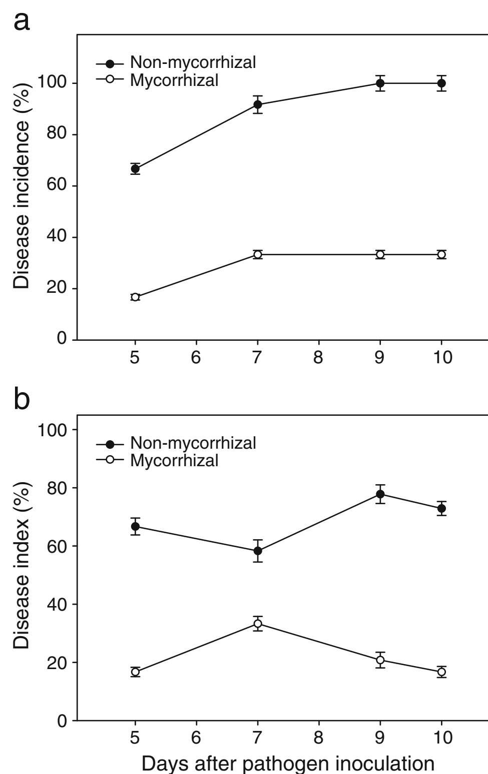

Fig. 1 Effects of mycorrhizal colonization by *Glomus mosseae* on disease incidence (**a**) and severity (**b**) of *Zea mays* cv Gaoyou-115 infected by *Rhizoctonia solani.* Values are means±standard errors from three replicates

were much higher than those in the leaves in both cultivars (Figs. 2 and 3). In susceptible cultivar Gaoyou-115, pathogen inoculation alone did not result in accumulation of DIMBOA in both leaves and roots (Fig. 2). Mycorrhizal colonization, however, led to substantial accumulation of DIMBOA in the roots at three time points of sampling (Fig. 2a). DIMBOA concentration in mycorrhizal roots was 164.5 μg/g FW, while it was only 67.3 μg/g FW in non-mycorrhizal control at 5 days after pathogen inoculation. Pathogen infection further increased DIMBOA accumulation in the mycorrhizal roots, and DIMBOA concentration reached 244.1 μg/g FW 5 days after pathogen inoculation (Fig. 2a). Ten days after pathogen inoculation, DIMBOA concentrations in two mycorrhizal treatments, were increased by 60.6% and 55.8%, respectively than those in roots with no inoculation of both mycorrhizal fungus and pathogen. In the leaves, the induction of DIMBOA was found at 5 days after pathogen inoculation only (Fig. 2b). Five days after pathogen inoculation, the amount of DIMBOA in the leaves of mycorrhizal colonization treatment (G) increased by 92.2%

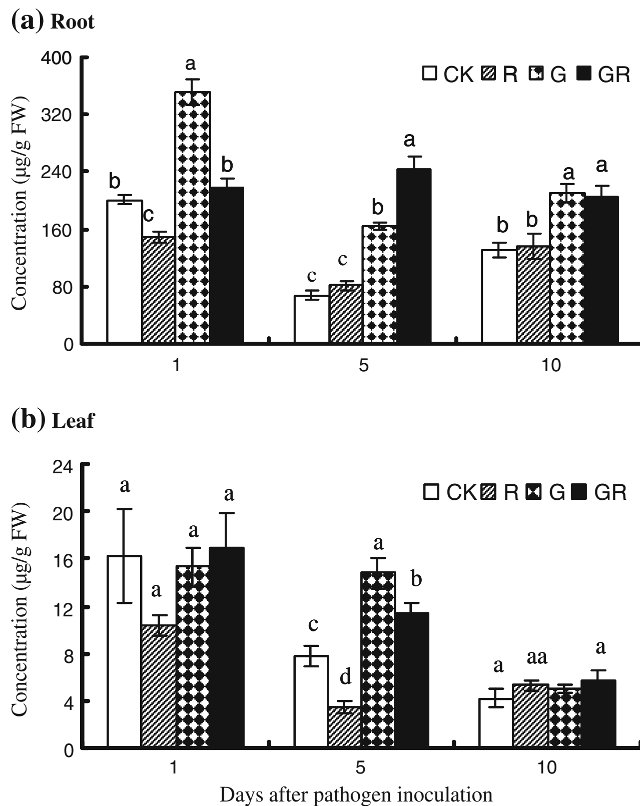

Fig. 2 Concentrations of DIMBOA in the roots (**a**) and leaves (**b**) of *Zea mays* cv Gaoyou-115. *CK* control plants without pathogen and mycorrhizal inoculation; *R* plants inoculated with *Rhizoctonia solani; G* plants inoculated with *Glomus mosseae; GR* plants inoculated with both *G. mosseae* and *R. solani.* Values are means+standard errors from three replicates. Significant differences ($P<0.05$ using Duncan's multiple range test) among treatments in a group are indicated by *different letters above the bars*

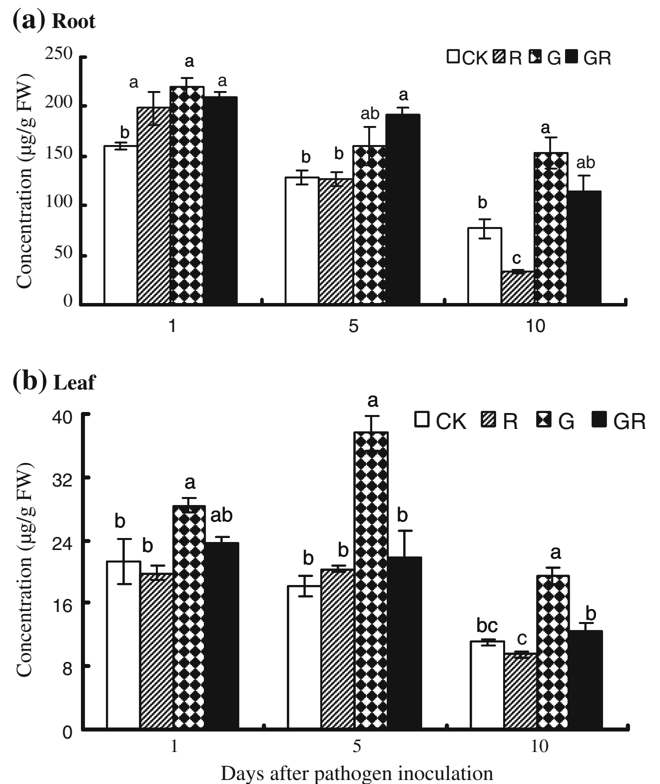

Fig. 3 Concentrations of DIMBOA in the roots (**a**) and leaves (**b**) of *Zea mays* cv Yuenong-9. *CK* control plants without pathogen and mycorrhizal inoculation; *R*: plants inoculated with *Rhizoctonia solani; G*: plants inoculated with *Glomus mosseae; GR*: plants inoculated with both *G. mosseae* and *R. solani.* Values are means+standard errors from three replicates. Significant differences ($P<0.05$ using Duncan's multiple range test) among treatments in a group are indicated by *different letters above the bars*

compared with that in non-mycorrhizal control (CK); the amount of DIMBOA in the leaves of combined inoculation (GR) increased by 235% than that of inoculation with pathogen alone (R) (Fig. 2b).

In resistant cultivar Yuenong-9, pathogen inoculation induced temporary accumulation of DIMBOA in the roots at 1 day after pathogen inoculation, but did not induce DIMBOA production in the roots at later two observations and in the leaves at all stages (Fig. 3a). However, mycorrhizal colonization increased DIMBOA accumulation both in the roots and in the leaves at three time points of sampling (Fig. 3a, b). DIMBOA concentrations in mycorrhizal roots were increased by 36.4% and 98.7% than those in non-mycorrhizal control roots 1 and 10 days after pathogen inoculation, respectively. Mycorrhizal colonization and later pathogen inoculation (GR) increased DIMBOA in the roots by 48.6% and 51.4% compared with non-inoculated control (CK), and sole pathogen inoculation (R), respectively 5 days after pathogen inoculation (Fig. 3a). Dual inoculation (GR) increased DIMBOA in the roots by 241.7% compared with sole pathogen inoculation (R)

10 days after pathogen inoculation. Pathogen inoculation (R) increased DIMBOA in Yuenong-9 roots after 1 day, but it reduced DIMBOA after 10 days. Mycorrhizal colonization in corn roots also led to significant increase in DIMBOA accumulation in the leaves (Fig. 3b). DIMBOA concentrations in the leaves of mycorrhizal plants were increased by 107.7% and 77.3% than those in non-mycorrhizal control leaves 5 and 10 days after pathogen inoculation, respectively. However, dual inoculation with both mycorrhizal fungus and pathogen (GR) resulted in a decrease in DIMBOA accumulation in the leaves compared to sole mycorrhizal inoculation (G) (Fig. 3b).

Transcript induction of defense-related genes

To determine whether mycorrhizal colonization enhances the disease resistance and DIMBOA accumulation by inducing transcription of defense-related genes (*PR2a*, *PAL*, and *AOS*) (van Loon et al. 2006) and a gene encoding DIMBOA biosynthesis *(BX9*) (Jonczyk et al. 2008), the expression patterns of the four genes were analyzed by using RT-PCR from the leaves of *Z. mays* cv Gaoyou-115 and Yuenong-9 at 1, 5, and 10 days after pathogen inoculation. Pre-inoculation of susceptible cultivar (Gaoyou-115) with the AMF (G) as well as dual inoculation with the AMF and pathogen (GR) induced transcripts of *PAL* 2.5 and 3.7-fold, respectively compared with non-mycorrhizal control (CK) 5 days after pathogen inoculation (Fig. 4a). Dual inoculation induced transcripts of *PR2a* 3.4 and 14.4-fold 1 and 5 days after pathogen inoculation, respectively (Fig. 4b), and induced transcripts of *AOS* 2.6 and 3.0-fold 5 and 10 days after pathogen inoculation, respectively (Fig. 4c). *BX9* transcripts in the leaves of corn were induced approximately 7.7 and 23.7-fold by dual inoculation (GR) 1 and 10 days after pathogen inoculation, respectively (Fig. 4d). Pathogen infection alone did not induce transcripts of all four genes tested in the leaves of corn Gaoyou-115.

Pre-inoculation of resistant cultivar (Yuenong-9) with AMF *G. mosseae* and later inoculation with pathogen *R. solani* (GR) induced accumulation of *PAL* and *AOS* tran-

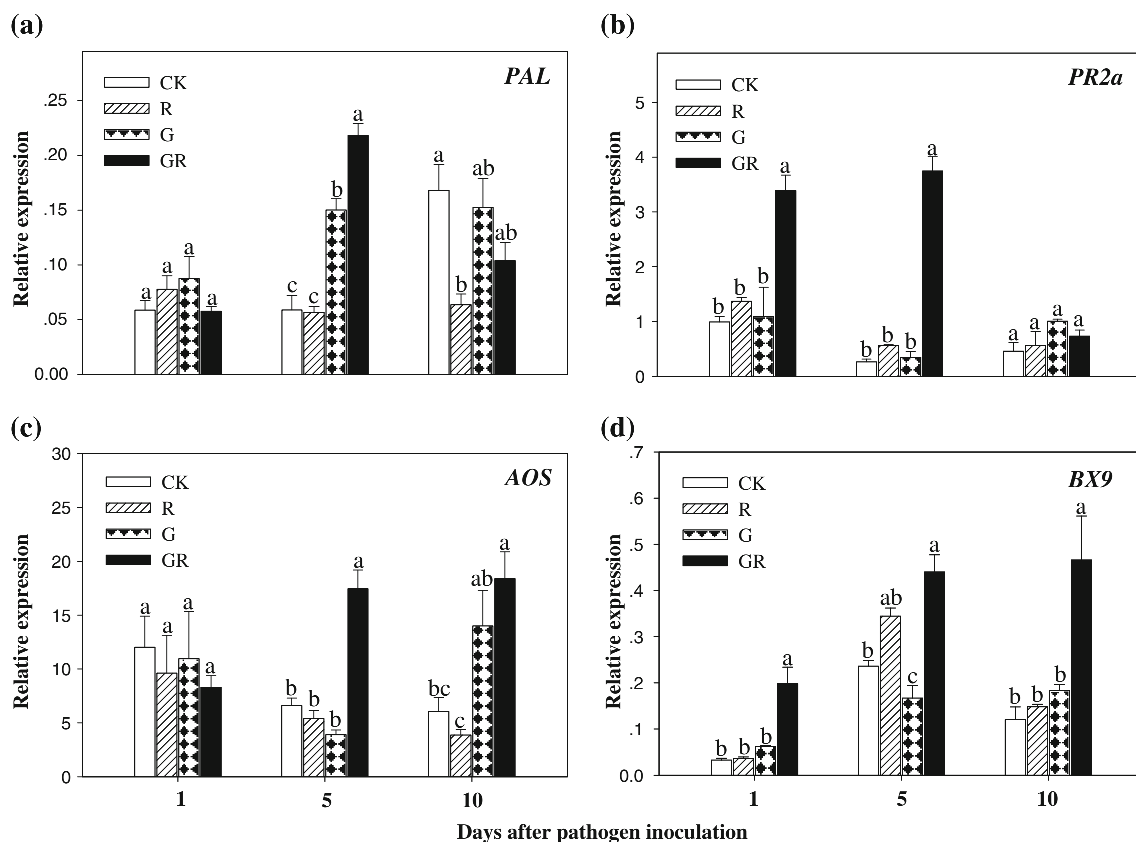

Fig. 4 Gene expression of phenylalanine ammonia-lyase (*PAL*) (**a**), basic type pathogen-related protein *PR-2a* (β-1,3-glucanase) (**b**) allene oxide synthase (*AOS*) (**c**), and benzoxazinoid UDP-glucosyltransferase (*BX9*) (**d**) in the leaves of *Zea mays* cv Gaoyou-115. Quantitative RT-PCR was used to detect the transcript levels. Glyceraldehyde-3-phosphate dehydrogenase (*GAPc*) was used as the reference internal control for RT-PCR, and the expression levels of target genes were normalized to its abundance. *CK* control plants without pathogen and mycorrhizal inoculation; *R*: plants inoculated with *Rhizoctonia solani; G:* plants inoculated with *Glomus mosseae; GR:* plants inoculated with both *G. mosseae* and *R. solani.* Values are means+standard error from three biological replicates. Significant differences ($P<0.05$ using Duncan's multiple range test) among treatments in a group are indicated by *different letters above bars*

scripts over basal levels present in the leaves of non-mycorrhizal control (CK) and sole pathogen inoculation (R) at 1 day after pathogen inoculation (Fig. 5a, c). Particularly, *PAL* transcripts accumulated approximately to 7.7, 2.0 and 23.7-fold higher levels in response to dual inoculation (GR) compared with those in non-mycorrhizal control (CK) (Fig. 5a). However, mycorrhizal inoculation (G) alone did not induce *PAL* and *AOS* transcripts (Fig. 5a, c). Dual inoculation also induced transcripts of *BX9* (4.3-fold) 5 days after pathogen inoculation (Fig. 5d) and transcripts of *PR2a* (9.4-fold) 10 days after pathogen inoculation (Fig. 5b). Ten days after pathogen inoculation, the transcripts of *BX9* were induced 3.4-, 3.1- and 2.7-fold of those of inoculation with pathogen (R), AMF (G), and both (GR), respectively in the leaves of Yuenong-9 (Fig. 5d).

Discussion

Corn is one of the most important cereal crops in the world. In recent years, sheath blight, caused by *R. solani*, has become a major disease of this crop in many parts of China (Li et al. 1998). This study showed that corn sheath blight could be prevented through mycorrhizal inoculation. Pre-inoculation of susceptible Gaoyou-115 with AMF *G. mosseae* significantly reduced disease incidence and disease severity of sheath blight of corn. *G. mosseae* inoculation also significantly improves root and shoot growth of corn (Huang et al. 2006). The growth improvement was particularly obvious in corn plants co-inoculated with *G. mosseae* and *R. solani* compared with the plants only inoculated with the pathogen. Mycorrhizal fungi appeared to be good bioprotection agents since they are natural components of soil biota and can establish stable and long-lasting mutualistic symbiosis with the majority of vascular plant species including most crops (Smith and Read 1997). In terms of ubiquity and partnerships throughout the plant kingdom, mycorrhizas are the most important plant–microbe symbiosis. They not only improve the plant nutrient absorption (Varma and Hock 1995) and plant resistance to various abiotic stresses (Ruiz-Lozano et al. 1996), but also enhance the host plant defense against many

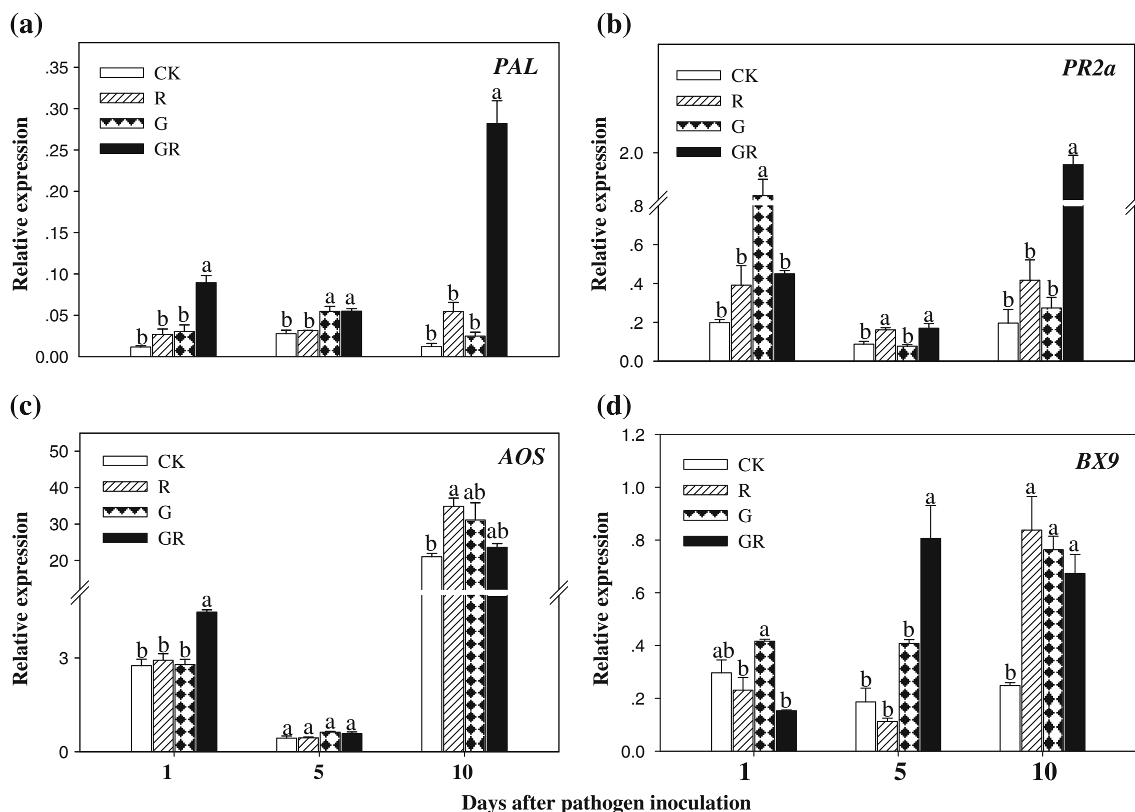

Fig. 5 Gene expression of phenylalanine ammonia-lyase (*PAL*) (**a**), basic type pathogen-related protein *PR-2a* (β-1,3-glucanase) (**b**) allene oxide synthase (*AOS*) (**c**), and benzoxazinoid UDP-glucosyltransferase (*BX9*) (**d**) in the leaves of *Zea mays* cv Yuenong-9. Quantitative RT-PCR was used to detect the transcript levels. Glyceraldehyde-3-phosphate dehydrogenase (*GAPc*) was used as the reference internal control for RT-PCR, and the expression levels of target genes were normalized to its abundance. CK: control plants without pathogen and mycorrhizal inoculation; *R*: plants inoculated with *Rhizoctonia solani; G:* plants inoculated with *Glomus mosseae; GR:* plants inoculated with both *G. mosseae* and *R. solani.* Values are means+standard error from three biological replicates. Significant differences ($P<0.05$ using Duncan's multiple range test) among treatments in a group are indicated by *different letters above bars*

soil-borne fungal pathogens (Bi et al. 2007; Azcón-Aguilar and Barea 1996). Maintenance of inoculum potential of AMF in agricultural soils will be particularly important for both crop nutrient acquisition and disease control. This may be achieved through bioaugmentation, the addition of microorganisms to enhance a specific biological activity, by inoculating soils with AMF or by using transplanted seedlings that already have the appropriate AMF in their roots (Douds and Millner 1999). However, many agricultural practices, including the use of fertilizers and biocides, tillage, monocultures, and the cultivation of non-mycorrhizal crops, may affect functioning of mycorrhizal fungi in the soils (Gosling et al. 2006). As a result, agroecosystems are impoverished in AMF and may not provide bioprotection to crops.

Plants defense microbial attack by using a combination of constitutive defenses as well as induced defenses that develop after infection of plants with various biological agents or after pretreatment with various chemical or physical agents (Tollrian and Harvell 1999). The induced defenses involve an array of defense mechanisms, including synthesis of phytoalexins and activation of large number of defense-related genes encoding pathogenesis-related (PR) proteins or enzymes involved in biosynthesis of antimicrobial compounds (Maldonado-Bonilla et al. 2008). Phytoalexins are antimicrobial low-molecular-weight compounds that are synthesized by, and accumulated in, plants in response to microbial infection. Production of antimicrobial phytoalexins is one of the most important defensive mechanisms in plants against pathogen attack (Dixon 2001; Grayer and Kokubun 2001; Bednarek and Osbourn 2009). DIMBOA and 2,4-dihydroxy-1,4- benzoxazin-3-one (DIBOA) are major phytoalexins produced by corn, wheat, rye, and related monocotyledoneae plants (Hashimoto and Shudo 1996; Frey et al. 1997). DIMBOA confers corn resistance to first-brood European corn borer (*Ostrinia nubilalis*), *Setosphaeria turcica* (Rostás 2007), corn plant louse (*Rhophalosiphum maydis*), northern corn leaf blight (*Helminthosporium turcicum*), stalk rot (*Diplodia maydis*) (Niemeyer 1988), and other plant pathogens (Corcuera et al. 1978; Bravo and Lazo 1993). DIMBOA and other hydroxamic acids in cereal roots inhibit the pathogen growth of *Gaeumannomyces graminis* var. tritici, the causal agent of take-all disease of wheat and barley (Wilkes et al. 1999). This study showed that DIMBOA accumulated in both leaves and roots of mycorrhizal corn plants, suggesting that mycorrhizal colonization induces systemic production of DIMBOA. DIMBOA accumulated in mycorrhizal roots and pathogen infection further increased DIMBOA accumulation. Our previous study showed that DIMBOA inhibited mycelial growth of *R. solani* (Huang et al. 2007). This indicates that DIMBOA accumulation induced by the AM fungus may play a practical role in reducing disease infection of corn by *R. solani*. Corn plants also produce several DIMBOA derivatives in the form of glucosides (Glc), which are important especially in roots (Cambier et al. 1999). These glucosylated hydroxamic acids are enzymatically converted into aglycone forms by the action of β-glucosidases after the plant infection by pathogens and insects (Niemeyer 1988; Weibull and Niemeyer 1995). The aglycone forms may further enhance fungitoxicity of corn. Nevertheless, some corn-associated fungi in the genus *Fusarium* and endophytes can metabolize MBOA and BOA (Saunders and Kohn 2008). The pathogen *R. solani* used in this study may also adapt these hydroxamic acids in corn. The complex interaction between Hx production in corn and pathogenic adaptation should be further investigated.

Mycorrhizal colonization also induced transcripts of *BX9*, one of the key genes in DIMBOA biosynthesis pathway (Jonczyk et al. 2008; Morant et al. 2008), in the leaves of corn. BX9 catalyzes the conversion of DIBOA to DIBOA-glucoside, which is further transformed into DIMBOA-glucoside by BX6 and BX7 (Jonczyk et al. 2008). Upon insect damage and pathogen infection DIBOA-Glc and DIMBOA-Glc are hydrolyzed by β-glucosidases (BX9 and BX8) in corn to yield glucose and the bio-active benzoxazinoids (Cicek and Esen 1999; Morant et al. 2008). Transcript induction of *BX9* in mycorrhizal corn suggests increased biosynthesis of DIMBOA, consistent with increased accumulation of DIMBOA in the roots and leaves. In susceptible cultivar (Gaoyou-115) *BX9* was only induced in mycorrhizal plants after pathogen inoculation (dual inoculation treatment GR), whereas in resistant cultivar (Yuenong-9) mycorrhizal colonization alone induced transcripts of *BX9*, and pathogen infection also induced the gene transcripts 10 days after pathogen inoculation.

Furthermore, mycorrhizal colonization induced transcripts of other three defense-related genes *PR2a*, *PAL*, and *AOS* in leaves of corn. Induction of pathogenesis-related proteins is thought to play a role in pathogen-induced plant defense responses. Accumulation of hydrolytic enzymes such as β-1,3-glucanase (PR2) was associated with induced resistance in corn to the fungal infection (Cordero et al. 1994). Phenylalanine-ammonia-lyase (PAL) is the key enzyme involved in the biosynthesis of the signal molecule salicylic acid (Mauch-Mani and Slusarenko 1996). Induction of *PAL* and allene oxide synthase gene (*AOS*), encoding one of the key enzymes of jasmonic acid biosynthesis (Schaller et al. 2005), indicates that mycorrhizal colonization initiates jasmonate and salicylate signaling pathways. Hause et al. (2002) found that mycorrhizal colonization of barley roots by AMF *G. intraradices* led to elevated levels of endogenous jasmonic acid (JA) and increased expression of *AOS*.

The results of the current study indicate that mycorrhizal fungi induce not only local defense responses, but also systemic defense responses in corn. Pozo et al. (2002)

found that *G. mosseae* colonization in tomato plants reduced both local and systemic disease symptoms caused by *Phytophthora parasitica* infection, as well as led to local and systemic induction of defense-related enzymes including hydrolytic enzymes chitinase, chitosanase and β-1,3-glucanase, and superoxide dismutase. *G. mosseae* also induced systemic resistance of tomato to *P. parasitica* in a split-root experimental system (Cordier et al. 1998). Take-all disease caused by *Gaeumannomyces graminis* var. tritici is systemically reduced in roots of mycorrhizal barley (*Hordeum vulgare*) plants (Khaosaad et al. 2007). Root colonization in sweet basil (*Ocimum basilicum*) by *G. caledonium* and *G. mosseae* enhances the production of phenolic compounds (rosmarinic and caffeic acids) in the shoots irrespective of phosphorus nutrition (Toussaint et al. 2007). Mycorrhizal inoculation in *Medicago truncatula* leads to local and systemic induction of many genes that are involved in defense responses, which further results in enhanced resistance to a virulent bacterial pathogen, *Xanthomonas campestris* (Liu et al. 2007).

Pathogen inoculation did not induce accumulation of DIMBOA and defense responses in susceptible plants of Gaoyou-115 at any time point of sampling (Figs. 2 and 4). However, it induces accumulation of DIMBOA and defense responses in resistant corn Yuenong-9 at some time points (Figs. 3 and 5). Pathogen inoculation increased DIMBOA accumulation in the roots after 1 day. It induced gene expression of *AOS* and *BX9* after 10 days, although the response was much slower than those in dual inoculated plants. These indicate that different mechanisms exist in the two genotypes and resistant variety can develop defense responses to pathogen infection. It is concluded that infection by *R. solani* induced systemic defense responses in the resistant cultivar, which did not happen in the susceptible cultivar. Furthermore, with the very slow and mild disease development occurring in the resistant cultivar, the defense responses in the resistant cultivar became weaker with the time and this attenuated the effect of mycorrhization on DIMBOA accumulation in the leaves 5 and 10 days after pathogen inoculation.

Infection by necrotizing pathogens or beneficial microbes may provoke some plants to develop a unique physiological state called “priming” (Conrath et al. 2006; Engelberth et al. 2004; van Hulten et al. 2006). Primed plants display faster and/or stronger activation of various cellular defense responses after pathogen and insect attack (Conrath et al. 2002; Conrath et al. 2006). Exposure to volatile organic compounds from caterpillar-infested plants does not activate defense responses directly, but primes defense responses for earlier and/or stronger induction upon subsequent defense elicitation (Ton et al. 2006). Our study showed that mycorrhizal inoculation itself did not affect the transcripts of most genes tested (Figs. 4 and 5) or only had marginal effects on transcripts of *BX9*. However, pre-inoculation with *G. mosseae* primed defense responses of all four tested genes in corn plants after pathogen attack. Induction of defense responses in pre-inoculated plants was much higher and quicker than that in non-inoculated plants upon *R. solani* infection. Our study confirms that priming is an important mechanism in mycorrhiza-induced resistance (Pozo and Azcon-Aguilar 2007).

In conclusion, mycorrhizal inoculation with *G. mosseae* enhanced disease resistance of susceptible corn to sheath blight caused by *R. solani* and resulted in significantly higher concentrations of DIMBOA in the roots of both susceptible and resistant cultivars of corn. Mycorrhizal colonization systematically induced DIMBOA biosynthesis gene *BX9* and other two defense-related genes *PR2a* and *AOS* in the leaves of corn. These results suggest that accumulation of DIMBOA, an important phytoalexin in corn, and systemic defense responses induced by mycorrhizal colonization, play a vital role in enhanced disease resistance in mycorrhizal plants of corn.

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References

Akköprü A, Demir S (2005) Biological control of Fusarium wilt in tomato caused by *Fusarium oxysporum* f. sp. lycopersici by AMF *Glomus intraradices* and some *Rhizobacter*. J Phytopathol 153:544–550

Araim G, Saleem A, Arnason JT, Charest C (2009) Root colonization by an arbuscular mycorrhizal (AM) fungus increases growth and secondary metabolism of purple coneflower, *Echinacea purpurea* (L.) Moench. J Agr Food Chem 57:2255–2258

Azcón-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens—an overview of the mechanisms involved. Mycorrhiza 6:457–464

Bednarek P, Osbourn A (2009) Plant-microbe interactions: chemical diversity in plant defense. Science 324:746–748

Bi HH, Song YY, Zeng RS (2007) Biochemical and molecular responses of host plants to mycorrhizal infection and their roles in plant defence. Allelopathy J 20:15–28

Bohidar K, Wratten SD, Niemeyer HM (1986) Effects of hydroxamic acids on the resistance of wheat to the aphid *Sitobion avenae*. Ann Appl Biol 109:193–198

Bravo HR, Lazo W (1993) Antimicrobial activity of cereal hydroxamic acids and related compounds. Phytochemistry 33:569–571

Cambier V, Hance T, de Hoffmann E (1999) Non-injured maize contains several 1,4-benzoxazin-3-one related compounds but only as glucoconjugates. Phytochem Anal 10:119–126

Chellappan P, Christy SAA, Mahadevan A (2002) Multiplication of arbuscular mycorrhizal fungi on roots. In: Mukerji KG,

Manoharachary C, Chamola BP (eds) Techniques in mycorrhizal studies. Kluwer Academic Publishers, The Netherlands, pp 285–297

Cicek M, Esen A (1999) Expression of soluble and catalytically active plant (monocot) β -glucosidases in *E. coli*. Biotechnol Bioeng 63:392–400

Conrath U, Pieterse CMJ, Mauch-Mani B (2002) Priming in plant–pathogen interactions. Trends Plant Sci 7:210–216

Conrath U, Beckers GJM, Flors V, Garcia-Agustin P, Jakab G, Mauch F, Newman MA, Pieterse CMJ, Poinssot B, Pozo MJ, Pugin A, Schaffrath U, Ton J, Wendehenne D, Zimmerli L, Mauch-Mani B (2006) Priming: getting ready for battle. Mol Plant Microbe Interact 19:1062–1071

Corcuera LJ, Woodward MD, Helgeson JP, Kelman A, Upper C (1978) 2,4-Dihydroxy-7-methoxy-2 H-1,4-benzoxazin-3(4 H)-one, an inhibitor from *Zea mays* with differential activity against soft rotting *Erwinia* species. Plant Physiol 61:791–795

Cordero MJ, Raventos D, San Segundo B (1994) Differential expression and induction of chitinases and *β*-1,3-glucanases in response to fungal infection during germination of maize seeds. Mol Plant Microbe Interact 7:23–31

Cordier C, Gianinazzi S, Gianinazzi Pearson V (1996) Colonisation patterns of root tissues by *Phytophthora nicotianae* var. *parasitica* related to reduced disease in mycorrhizal tomato. Plant Soil 185:223–232

Cordier C, Pozo M, Barea J, Gianinazzi S, Gianinazzi-Pearson V (1998) Cell defence responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. Mol Plant Microbe Interact 11:1017–1028

Dixon RA (2001) Natural products and plant disease resistance. Nature 411:843–847

Douds DD, Millner P (1999) Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. Agric Ecosyst Environ 74:77–93

Elsen A, Gervacio D, Swennen R, De Waele D (2008) AMF-induced bioprotection against plant parasitic nematodes in *Musa* sp.: a systemic effect. Mycorrhiza 18:251–256

Engelberth J, Alborn HT, Schmelz EA, Tumlinson JH (2004) Airborne signals prime plants against insect herbivore attack. Proc Natl Acad Sci U S A 101:1781–1785

Figueroa CC, Koenig C, Araya C, Santos MJ, Niemeyer HM (1999) Effect of DIMBOA, a hydroxamic acid from cereals, on peroxisomal and mitochondrial enzymes from aphids: evidence for the presence of peroxisomes in aphids. J Chem Ecol 25:2465–2475

Frey M, Chomet P, Glawischnig E, Stettner C, Grun S, Winkl-mair A, Eisenreich W, Bacher A, Meeley RB, Briggs SP, Simcox K, Gierl A (1997) Analysis of a chemical plant defense mechanism in grasses. Science 277:696–699

Fritz M, Jakobsen I, Lyngkjær MF, Thordal-Christensen H, Pons-Kühnemann J (2006) Arbuscular mycorrhiza reduces susceptibility of tomato to *Alternaria solani*. Mycorrhiza 16:413–419

García-Garrido JM, Ocampo JA (1988) Interaction between *Glomus mosseae* and *Erwinia carotovora* and its effects on the growth of tomato plants. New Phytol 110:551–555

García-Garrido JM, Ocampom JA (1989) Effect of VA mycorrhizal infection of tomato on damage caused by *Pseudomonas syringae*. Soil Biol Biochem 21:165–167

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35

Grayer RJ, Kokubun T (2001) Plant-fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants. Phytochemistry 56:253–263

Gutierrez C, Castanera P, Torres V (1988) Wound-induced changes in DIMBOA (2,4 dihydroxy-7-methoxy-2 H-1,4 benzoxzinone-3 (4 H)-one) concentration in maize plants caused by *Sesamia nonagrioides* (Lepidoptera: Noctuidae). Ann Appl Biol 113:447–454

Hashimoto Y, Shudo K (1996) Chemistry of biologically active benzoxazinoids. Phytochemistry 43:551–559

Hause B, Maier W, Miersch O, Kramell R, Strack D (2002) Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. Plant Physiol 130:1213–1220

Huang JH, Zeng RS, Luo SM (2006) Studies on disease resistance of maize toward sheath blight induced by arbuscular mycorrhizal fungi. Chin J Appl Ecol Agric 14(3):167–169 (in Chinese)

Huang JH, Zeng RS, Luo SM, Gu WX, Nie CR, Cao M, Li XH (2007) Antifungus activity of DIMBOA from maize seedlings compared with several phenolic acids. Nat Prod Res Dev 19:572–577

Jonczyk R, Schmidt H, Osterrieder A, Fiesselmann A, Schullehner K, Haslbeck M, Sicker D, Hofmann D, Yalpani N, Simmons C, Frey M, Gierl A (2008) Elucidation of the final reactions of DIMBOA-glucoside biosynthesis in maize: characterization of Bx6 and Bx7. Plant Physiol 146:1053–1063

Khaosaad T, Garcia-Garrido JM, Steinkellner S, Vierheilig H (2007) Take-all disease is systemically reduced in roots of mycorrhizal barley plants. Soil Biol Biochem 39:727–734

Kiefer E, Heller W, Ernst D (2000) A simple and efficient protocol for isolation of functional RNA from plant tissues rich in secondary metabolites. Plant Mol Biol Rep 18:33–39

Li HR, Wu BC, Yan SQ (1998) Aetiology of *Rhizoctonia* in sheath blight of maize in Sichuan. Plant Pathol 47(1):16–21

Liu RJ (1995) Effect of vesicular-arbuscular mycorrhizal fungi on verticillium wilt of cotton. Mycorrhiza 5:293–297

Liu JH, Wang XM, Fang ZW, Li YL (1999) Breeding and cultivated technology of high quality and high yield new corn variety Yuenong-9. Guangdong Agricultural Sciences 4:13–14, in Chinese

Tollrian R, Harvell CD (1999) The ecology and evolution of inducible defenses. Princeton University Press, Princeton

Liu J, Maldonado-Mendoza I, Lopez-Meyer M, Cheung F, Town CD, Harrison MJ (2007) Arbuscular mycorrhizal symbiosis is accompanied by local and systemic alterations in gene expression and an increase in disease resistance in the shoots. Plant J 50:529–544

Maldonado-Bonilla LD, Betancourt-Jiménez M, Lozoya-Gloria E (2008) Local and systemic gene expression of sesquiterpene phytoalexin biosynthetic enzymes in plant leaves. Eur J Plant Pathol 121:439–449

Mauch-mani B, Slusarenko AJ (1996) Production of salicylic acid precursors is a major function of phenylalanine ammonia-lyase in the resistance of *Arabidopsis* to *Peronospora parasitica*. Plant Cell 8:203–212

Morant AV, Jorgensen K, Jorgensen C, Paquette SM, Sánchez-Pérez R, Møller L, Bak S (2008) β-Glucosidases as detonators of plant chemical defense. Phytochemistry 69:1795–1813

Morse S, Wratten SD, Edwards PJ, Niemeyer HM (1991) Changes in the hydroxamic acid content of maize leaves with time and after artificial damage: implications for insect attack. Ann Appl Biol 119:239–249

Mukerji KG, Manoharachary C, Chamola BP (2002) Techniques in mycorrhizal studies. Kluwer Academic Publishers, Dordrecht

Niemeyer HM (1988) Hydroxamic acids (4-hydroxy-1,4-benzoxazin-3-ones), defence chemicals in the Gramineae. Phytochemistry 27:3349–3358

Niemeyer HM, Perez FJ (1995) Potential of hydroxamic acids in the control of cereal pests, diseases and weeds. In: Inderjit KM, Dakshini M, Einhellig FA (eds) Allelopathy organisms, processes, and applications. American Chemical Society, Washington DC, pp 260–270

Niemeyer HM, Pesel E, Copaja SV, Bravo HR, Franke S, Francke W (1989) Changes in hydroxamic acid levels of wheat plants induced by aphid feeding. Phytochemistry 28:447–449

Pozo MJ, Azcon-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398

Pozo MJ, Cordier C, Dumas-Gaudot E, Gianinazzi S, Barea JM, Azcon-Aguilar C (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophtora* infection in tomato plants. J Exp Bot 53:525–534

Rostás M (2007) The effects of 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one on two species of *Spodoptera* and the growth of *Setosphaeria turcica* in vitro. J Pest Sci 80:35–40

Ruiz-Lozano JM, Azcón R, Palma JM (1996) Superoxide dismutase activity in arbuscular mycorrhizal *Lactuca sativa* plants subjected to drought stress. New Phytol 134:327–333

Safir G (1968) The influence of vesicular mycorrhiza on the resistance of onion to *Pyrenochaeta terrestris*. MS. Thesis, University of Illinois, Urbana, USA

Saunders M, Kohn LM (2008) Host-synthesized secondary compounds influence the in vitro interactions between fungal endophytes of maize. Appl Environ Microb 74:136–142

Schaller F, Schaller A, Stintz A (2005) Biosynthesis and metabolism of jasmonates. J Plant Growth Regul 23:179–199

Sicker D, Frey M, Schulz M, Gierl A (2000) Role of natural benzoxazinones in the survival strategy of plants. Int Rev Cytol 198:319–346

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, Cambridge

Søltoft M, Jørgensen LN, Svensmark B, Fomsgaard IS (2008) Benzoxazinoid concentrations show correlation with Fusarium Head Blight resistance in Danish wheat varieties. Biochem Syst Ecol 36:245–259

Sriram S, Raguchander T, Vidhyasekaran P, Muthukrishnan S, Samiyappan R (1997) Genetic relatedness with special reference to virulence among the isolates of *Rhizoctonia solani* causing sheath blight in rice. J Plant Dis Prot 104:260–271

St-Arnaud M, Vujanovic V (2007) Effect of the arbuscular mycorrhizal symbiosis on plant diseases and pests. In: Hamel C, Plenchette C (eds) Mycorrhizae in crop production: applying knowledge. Haworth, Binghampton, pp 67–122

Ton J, D'Alessandro M, Jourdie V, Jakab G, Karlen D, Held M, Mauch-Mani B, Turlings TC (2006) Priming by airborne signals boosts direct and indirect resistance in maize. Plant J 49:16–26

Toussaint JP, Smith FA, Smith SE (2007) Arbuscular mycorrhizal fungi can induce the production of phytochemicals in sweet basil irrespective of phosphorus nutrition. Mycorrhiza 17:291–297

van Hulten M, Pelser M, Van Loon LC, Pieterse CMJ, Ton J (2006) Costs and benefits of priming for defense in Arabidopsis. Proc Natl Acad Sci USA 103:5602–5607

van Loon LC, Rep M, Pieterse CMJ (2006) Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol 44:135–162

Varma A, Hock B (1995) Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin

Weibull J, Niemeyer HM (1995) Changes in dihydroxymethoxybenzoxazinone glycoside content in wheat plants infected by three plant pathogenic fungi. Physiol Mol Plant Pathol 47:201–212

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227

Wilkes MA, Marshall DR, Copeland L (1999) Hydroxamic acids in cereal roots inhibit the growth of take-all. Soil Biol Biochem 31:1831–1836

Zhu HH, Yao Q (2004) Localized and systemic increase of phenols in tomato roots induced by *Glomus versiforme* inhibits *Ralstonia solanacearum*. J Phytopathol 152:537–542

Zubek S, Stojakowska A, Anielska T, Turnau K (2010) Arbuscular mycorrhizal fungi alter thymol derivative contents of *Inula ensifolia* L. Mycorrhiza 20:497–504