Acta Physiol Plant (2013) 35:3465–3475
DOI 10.1007/s11738-013-1382-y

ORIGINAL PAPER

Arbuscular mycorrhizal fungi-enhanced resistance against *Phytophthora sojae* infection on soybean leaves is mediated by a network involving hydrogen peroxide, jasmonic acid, and the metabolism of carbon and nitrogen

Yuanjing Li · Zhilei Liu · Hongyan Hou · Hong Lei · Xiancan Zhu · Xuhui Li · Xingyuan He · Chunjie Tian

Received: 21 May 2013 / Revised: 13 August 2013 / Accepted: 30 August 2013 / Published online: 17 September 2013
© Franciszek Górski Institute of Plant Physiology, Polish Academy of Sciences, Kraków 2013

Abstract The arbuscular mycorrhizal fungi (AMF) enhance the resistance to pathogen infection in host plant. However, it is unclear how the AMF are involved in the systemic acquired resistance of host plant against pathogen. Here, an experiment was carried out to clarify the role of the AMF in soybean's defense against the infection from pathogen *Phytophthora sojae*. It was found that the AMF contributed to the resistance of soybean against *Phytophthora sojae* by the release of hydrogen peroxide and by the accumulation of jasmonic acid in response to pathogenic invasion. Furthermore, the trade of nitrogen (N) from the fungus for carbon from the host was accelerated in the AM symbiosis in the defense reaction, which was indicated by the increased soluble sugar level, NO content and enzyme activities involved in N metabolism in the AM symbiosis.

Keywords Arbuscular mycorrhizal fungi (AMF) · *Phytophthora sojae* · Hydrogen peroxide (H_2O_2) · Jasmonic acid (JA) · Carbon · Nitrogen

Communicated by O. Ferrarese-Filho.

Y. Li · Z. Liu · H. Hou · X. Zhu · X. Li · X. He · C. Tian (✉)
Laboratory of Soil Microbiology and Nutrient Cycle, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun 130102, People's Republic of China
e-mail: tiancj@neigae.ac.cn

Y. Li · Z. Liu
University of Chinese academy of sciences, Beijing, China

H. Lei
Key Laboratory of Molecular Biology, College of Life Sciences, Heilongjiang University, Harbin 150080, People's Republic of China

Abbreviations

AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi
SAR	Systemic acquired resistance
H_2O_2	Hydrogen peroxide
JA	Jasmonic acid
NO	Nitric oxide
NR	Nitrate reductase
GS	Glutamine synthetase

Introduction

Most crops throughout the world suffer from pathogen-induced diseases. Soybean (*Glycine max*), a major source of vegetable proteins worldwide, is damaged badly upon infection by *Phytophthora sojae* (Canaday and Schmitthenner 2010; Allen et al. 2009; Wrather and Koenning 2006). Pathogen infection often interrupts plant hormone signaling pathways. For example, proteins released by some pathogens activate plant jasmonic acid (JA)-mediated stomatal opening processes, promoting pathogen invasion (Zheng et al. 2012). Simultaneously, host plant combats positively pathogen invasion by regulating hormones such as JA and salicylic acid (SA) (Grant and Jones 2009; Spoel and Dong 2008). In addition to plant hormones, other small molecules, such as hydrogen peroxide (H_2O_2) and nitric oxide (NO), play important roles in regulating signal transduction pathways that mediate systemic pathogen responses (Liu et al. 2013; Kamran Qureshi et al. 2013; Piererse et al. 2009; Besson-Bard et al. 2008; Miller et al. 2008; Glazebrook 2005). These responses are accompanied by a series of plant self-defense reactions, including the adjustment of membrane permeability by

phospholipase-degraded unsaturated fatty acids, the import or export of cations or ions through membrane, and the expression of intracellular defense genes that manipulate plant hypersensitivity (Piffanelli et al. 1999). Through a series of metabolic processes such as dehydrogenation, reduction and β-oxidation, linolenic acid released through the cell membrane is finally converted into JA, which is then metabolized into jasmonate acid ester and methyl jasmonate to activate the expression of plant defense genes (Farmer and Ryan 1992). JA is known to play an important role in plant systemic acquired resistance (SAR) to disease by affecting genes related to pathogen infection (Wasternack 2007; Van Loon et al. 2006; Ryan and Moura 2002). Furthermore, H_2O_2 may function in conjunction with plant hormones, resulting in the coadjutant regulation of SAR. For example, SA may promote the accumulation of intracellular H_2O_2 by inhibiting catalase activity and increasing self-transformation into radicals for lipid peroxidation (Van Loon et al. 1994; Chen et al. 1993) or by acting on NADPH oxidase in the plasma membrane to produce H_2O_2 in response to pathogen infection (Shirasu et al. 1997). The peroxidase and active oxygen radicals produced serve as messengers that assist methyl jasmonate-mediated systemic pathogen resistance in the host plant. NO is an additional small molecule that affects H_2O_2 release; NO affects the regulation of plant secondary metabolites and the inhibition of NADPH oxidase for H_2O_2 production (Yun et al. 2011). Accordingly, H_2O_2, NO, and JA are closely interconnected in the host plant network against pathogen infection.

Arbuscular mycorrhiza, which is one of the most widespread symbioses worldwide, is formed by the arbuscular mycorrhizal fungi (AMF) with more than 80 % of vascular land plants (Smith and Read 2008). The special fungal structures of the arbuscular and the vesicles formed by the intraradical mycelium (IRM) colonized in the root cells are primarily responsible for nutrient transfer and energy cycling between the fungus and the host plant (Bonfante and Genre 2010; Harrison 2005). The host plant acquires mineral nutrients, such as nitrogen (N) and phosphate (P), through symbiotic AMF; the AMF gain carbohydrate (C) from the host plant's photosynthetic processes (Campos-Soriano and Segundo 2011; Bonfante and Genre 2010; Parniske 2008; Pozo and Azcon-Aguilar 2007; Parniske 2004). The transfer and metabolism of C from the host plant to the fungus satisfies the fungal needs for materials and energy in its life cycle and acts as one means of maintaining a beneficial balance for both participants. In addition, this relationship may stimulate the host plant's self-protective responses, preventing the excessive colonization of the AMF (Campos-Soriano and Segundo 2011) because nearly 20 % of the carbohydrates from plant photosynthesis are transferred to symbiotic AMF (Allen and Shachar-Hill 2008; Jakobsen and Rosendahl 1990). By improving the host plant's nutrient uptake through the extended large soil mycelium, the AMF enhance the capability of the host plant to combat pathogens; they also trigger the plant's systemic resistance responses against pathogens through an unclear mechanism by which the immune resistance can be transferred and communicated among plants through the mycelium network (Song et al. 2010). Interestingly, AMF-induced resistance is effective against underground pathogen infection and aboveground (leaf) infections (Campos-Soriano et al. 2012; Liu et al. 2007; Fritz et al. 2006; Whipps 2004). However, the mechanism by which the AMF perceive pathogen infection signaling and then participate in the plant's systemic responses against the pathogenic invasion remains unclear.

The trade of N (or P) for C between the AMF and host plant is the driving force that maintains a balanced communication in the symbiotic relationship. Moreover, this exchange may also act as a signal, triggering JA-mediated acquired systemic responses in the host plant; further, JA may regulate the soluble sugar content in the symbiosis by regulating enzymes involved in C metabolism (Schaarschmidt et al. 2006; Hause et al. 2002; Pieterse et al. 1998). The increased soluble sugar content in an infected host plant is often associated with an altered JA content, suggesting a key role of JA in sugar metabolism and its corresponding impacts on pathogen resistance. In addition to improved nutrient uptake through the AMF, the direct competition between the AMF and pathogens for ecological root niches also contributes to the plant's enhanced pathogen resistance. However, the AMF-regulated signal transduction against *Phytophthora sojae* infection in soybean remains unclear. Accordingly, mycorrhizal soybeans treated with *Phytophthora sojae* were used in this study. The release of H_2O_2 and its induction on JA-mediated host response regulation were evaluated, and the elucidation of NO dynamics and the transfer and metabolism of C and N were studied.

Materials and methods

Soybean and arbuscular mycorrhizal fungus used for experiment

The cultivated soybean variety (*Glycine max*) used for the experiment was NEAU-D50 (National validation number: black trial bean 2007022), which is sensitive to *Phytophthora sojae* infection. The arbuscular mycorrhizal fungus used for inoculation was *Glomus intraradices* (BGC BJ09); the fungus was provided by the Center of Plant Nutrition and Natural Resources, Beijing Academy of Agriculture, China.

Culture of soybean seedlings and inoculation with the AMF

Healthy soybean seeds were sterilized with 75 % ethanol and 0.5 % sodium hypochlorite. The sterilized seeds were rinsed several times with distilled water and then incubated on the germination paper at 28 °C in the growth chamber. Three days later, germinated seeds were inoculated with the AMF and transplanted into pots containing 1 kg of an autoclaved mixture of sand, pearlite and vermiculite in a volume ratio of 1:1:1; the plants were placed into a growth chamber with 75 % humidity and 16 h light and 8 h dark at 25 °C. The plants were watered with 100 mL Hoagland's nutrition solution every other day.

The agents containing 3,000 *Glomus intraradices* spores were used for mycorrhizal fungal inoculation; the same amount of an autoclaved agent was used as the control (Zhu et al. 2010).

Strain used as pathogen and its infection on soybean leaves

The variety of *Phytophthora sojae* (CGMCC 3.14914) that is distributed dominantly in northern China was selected for the soybean leaf infection. The strain was cultured on V8 solid culture medium at 25 °C in the dark until the colony reached a diameter of approximately 9 cm and was ready for treatment.

Plants cultured in a chamber for 50 days were infected with the pathogen. Fresh leaves were wounded at a location 2–3 mm from the main veins. Pathogen colonies in 10 mm × 10 mm squares on the culture medium were applied to the wounds, sprayed with sterilized water and covered with plastic to maintain moisture. The plants were then returned to the chamber for 3 additional days, at which point the pathogenic infection was evaluated. The isolated leaves were also simultaneously treated with the pathogen in a similar manner. Wounded leaves lacking the *Phytophthora sojae* infection were used as the control (Zhang et al. 2010; Sandhu et al. 2005).

To quantify the plant injury, the percentage of the disease lesion area on the leaf was assessed by using Assess2.0 software (Lamari 2008).

Detection of H_2O_2 and NO

The H_2O_2 concentration was determined by chemiluminescence in a ferricyanide-catalyzed oxidation of luminol as described by Schwacke and Hager (1992). Leaf sample (0.2 g) was ground in liquid nitrogen, and 1 mL of $HClO_4$ (0.2 M) was added for 5 min. After centrifugation, the supernatant was collected (Hu et al. 2009). One hundred microliters of the supernatant, 50 μL of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), and 800 μL of phosphate-buffered saline were mixed and added to 100 μL of $K_3[Fe(CN)_6]$ to initiate the reaction. One unit of H_2O_2 was defined as the chemiluminescence that resulted from the internal standard of 1 μM H_2O_2 per gram fresh weight.

The NO content was detected as described by Ding et al. (1988) with slight modifications. Leaf sample (0.6 g) was added to 3 mL of a pre-cooled acetate solution (50 mM, pH 3.6, containing 4 % zinc diacetate), and the mixture was then homogenized by grinding and centrifuged at 4 °C to collect the supernatant. One milliliter of the supernatant was added to 1 mL of Greiss (1 % sulfanilamide/0.1 % *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 5 % phosphate acid), and the solution was incubated for 30 min at room temperature before it was measured at 540 nm.

High-performance liquid chromatography (HPLC) analysis for JA

Based on the method described by Kramell et al. (1997) and Royo et al. (1999), 1 g of leaf sample was homogenized by grinding in liquid nitrogen and was added to 10 mL of methanol (80 %); the solution was allowed to incubate overnight. The JA concentration was determined by high-performance liquid chromatography (HPLC), with a Nova-pak C18 column (Waters 3.9 × 150 mm, 10 μm) at 35 °C. The wavelength was set to 210 nm, the flow rate was set to 1 mL/min, and the injection volume was 10 μL; methanol and phosphate buffer (0.05 mol L^{-1}, pH 7.0) were mixed with the solvent at a volume ratio of 40:60 (Hause et al. 2002).

Determination of soluble sugar and total nitrogen

The soluble sugar content was determined as described by Yemm and Willis (1954). Root sample (0.1 g) was extracted with ethanol (70 %) in a final volume of 50 mL. From this solution, 1 mL was added to 5 mL of anthrone–H_2SO_4 and incubated for 10 min in a boiling water bath before the solution was measured at 620 nm.

The determination of total nitrogen was described by Nelson and Sommers (1973); concentrated H_2SO_4–H_2O_2 was used in conjunction with a Smart-Chem Discrete Auto Analyzer (Italy, AMS).

Measurement of enzymatic activities

The detection of the activities of catalase (CAT, EC 1.11.1.6) and glutathione reductase (GR, EC 1.8.1.10) was previously described by Chance and Maehly (1955) and Halliwell and Foyer (1978), respectively. Leaf sample ground in liquid nitrogen was added to extraction buffer [sodium phosphate (50 mM, pH 7.5), sucrose (250 mM),

EDTA (1.0 mM), KCl (10 mM), $MgCl_2$ (1 mM), phenylmethylsulfonyl fluoride (PMSF, 0.5 mM), dithiothreitol (DTT, 0.1 mM) and 1 % (w/v) polyvinylpolypyrrolidone (PVPP)] in a 1:6 (w/v) ratio. After the solution was homogenized at 4 °C for 4 h and then centrifuged at 25,000*g* for 20 min, the supernatant was collected and brought to 80 % saturation by the addition of solid ammonium sulfate and gentle shaking at 4 °C for 4 h. Following centrifugation at 28,000*g* for 45 min, the supernatant was collected and used for the enzymatic assay. CAT and GR activities were measured by a spectrophotometer at 240 and 340 nm, respectively, according to Zhang and Kirkham (1996). One unit enzymatic activity was defined as the absorption value which decreased 0.1 in 1 min at OD 240 nm for CAT and at OD 340 nm for GR.

Nitrate reductase (NR) activity was measured as described by Yu and Zhang (2012) with slight modifications. Root sample (0.2 g) was ground and homogenized in 4.0 mL of an extraction solution consisting of phosphate buffer (25 mM, pH 7.5, mixed with K_2HPO_4 and KH_2PO_4), cysteine (5 mM) and EDTA-Na_2 (5 mM). After centrifugation at 4,000*g* at 4 °C, 0.4 mL of the supernatant was collected and added to 1.6 mL of reaction reagent (KNO_3–phosphate buffer and NADH). The solution was incubated for 30 min at 30 °C. Next, 1.0 mL of 1 % 4-aminobenzene sulfonic acid and 1.0 mL of 0.2 % 1-naphthylamine were used to terminate the reaction. After a centrifugation step at 4,000*g* for 10 min, the supernatant was collected and used for detection at 540 nm. One unit enzymatic activity was defined as the absorption value which decreased 0.1 in 1 min at OD 540 nm for NR.

Glutamine synthetase (GS) activity was measured as described by Yu and Zhang (2012) with modifications. Root sample (0.2 g) was ground into a homogenate in 3.0 mL of extraction buffer consisting of Tris–HCl (0.05 M, pH 8.0), $MgSO_4$ (2 mM), DTT (2 mM) and sucrose (0.4 M) at 4 °C. After centrifugation at 15,000*g* for 20 min, 1.0 mL of crude enzyme solution was collected and added to 0.6 mL of imidazole–HCl buffer (0.25 M, pH7.5), 0.4 mL of sodium hydrogen glutamate (0.30 M), 0.4 mL of ATP-Na (30 mM) and 0.2 mL of $MgSO_4$ (0.5 M); the solution was incubated for 5 min at 25° C. Next, 0.2 mL of hydroxylamine hydrochloride was added, and the solution incubated for 15 min. Finally, 0.8 mL of $FeCl_3$ solution (10 % $FeCl_3$, 24 % trichloroacetic acid and 50 % HCl in a volume ratio of 1:1:1) was added to terminate the reaction. Following centrifugation at 4,000*g* for 10 min, the supernatant was collected, and absorbance was detected at 540 nm. One unit enzymatic activity was defined as the absorption value which decreased 0.1 in 1 min at OD 540 nm for GS.

Statistical analysis

Student's *t* test, one-way ANOVA, and Duncan's test were performed to analyze the data using the SPSS software program (13.0). The values were based on three biological replicates for each treatment, and $P < 0.05$ was considered to be significant.

Results

AMF decreased the H_2O_2 content and increased the NO and JA content in soybean

The *Phytophthora sojae* infection of non-mycorrhizal and mycorrhizal soybean plants was performed 3 days before the H_2O_2 content and antioxidant enzyme activities were determined. As shown in Fig. 1a, the pathogen infection initiated a substantial increase in the H_2O_2 content in soybean leaves. After pathogenic application, the mycorrhizal soybean leaves contained lower H_2O_2 levels than the non-mycorrhizal soybean leaves ($P < 0.05$), suggesting that AMF may work to prevent the excessive pathogen-induced accumulation of H_2O_2 in the leaves, thereby reducing damage. The pathogen infection triggered the accumulation of JA in soybean (Fig. 1b). Compared with the non-mycorrhizal soybean, the mycorrhizal soybean contained higher JA levels regardless of the pathogenic condition, indicating that AMF inoculation also increases JA accumulation. The NO content in the non-mycorrhizal soybean under the pathogenic condition was increased significantly (Fig. 1c). The NO content in the mycorrhizal soybean was higher than that in the non-mycorrhizal soybean, and the mycorrhizal soybean under the pathogenic condition displayed the highest NO content.

AMF increased the antioxidant activities of GR in soybean

To verify the antioxidant capacity of soybean after pathogenic application, the activities of antioxidant enzymes were measured. Among the reported antioxidant enzymes, CAT and GR have been reported to be effective in decreasing the accumulation of H_2O_2 (Sarvajeet and Narendra 2010). Accordingly, the activities of CAT and GR were detected, and it was found that CAT and GR were upregulated after the pathogen infection in both the mycorrhizal and non-mycorrhizal soybean plants compared to the control (Fig. 2). However, compared with the activity of CAT, the activity of GR was significantly higher under mycorrhizal condition than that under non-mycorrhizal condition after the pathogen infection, suggesting that mycorrhizal soybean has a stronger level of antioxidant protection and that the GR function is stronger than that of

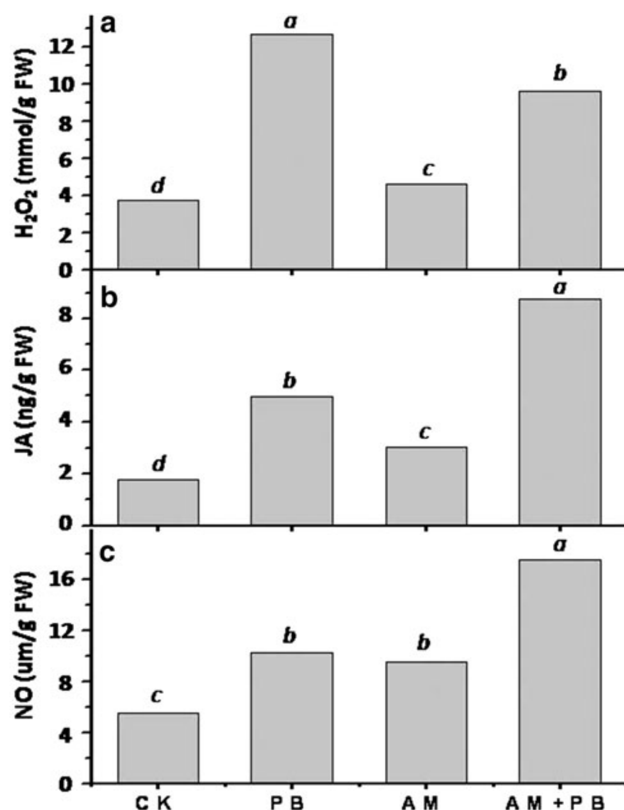

Fig. 1 The content of H_2O_2 (**a**), JA (**b**) and NO (**c**) in soybean leaves in response to *Phytophthora sojae* infection. The seedlings were treated as the follows: CK, control; PB, infected by *Phytophthora sojae*; AM, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices*; AM + PB, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* and infected by *Phytophthora sojae*. The significant difference among treatments were represented by *a*, *b*, *c* and *d marked on the column* using Duncan's multiple range tests ($P < 0.05$)

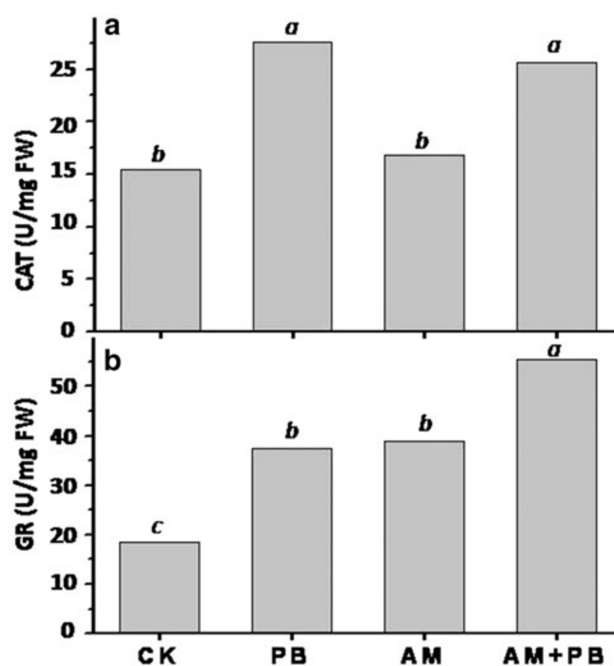

Fig. 2 The activities of antioxidant enzymes including catalase (CAT) (**a**) and glutathione reductase (GR) (**b**) in soybean leaves in response to *Phytophthora sojae* infection. The seedlings were treated as the follows: CK, control; PB, infected by *Phytophthora sojae*; AM, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices*; AM + PB, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* and infected by *Phytophthora sojae*. The significant difference among treatments were represented by *a*, *b* and *c marked on the column* using Duncan's multiple range tests ($P < 0.05$)

CAT. Under the non-pathogenic condition, no significant change was observed in the activity of CAT in mycorrhizal soybean, while the activity of GR was higher than that in the non-mycorrhizal soybean.

AMF enhanced the metabolism of N and C involved in pathogen resistance

Figure 3a shows that both the mycorrhizal and non-mycorrhizal soybeans displayed increased soluble sugar contents under the pathogenic condition, while the mycorrhizal soybean displayed higher concentrations in comparison with the non-mycorrhizal soybean. With respect to the non-mycorrhizal soybean, the pathogen infection status did not affect the total nitrogen (Fig. 3b); however, the activities of enzymes involved in N metabolism, including nitrate reductase (NR) and glutamine synthetase (GS), were up-regulated by the pathogen infection (Fig. 4). The total nitrogen, NR and GS activities in the mycorrhizal soybean were all increased regardless of the pathogenic condition compared to the non-mycorrhizal soybean. The total nitrogen in the mycorrhizal soybean was similar between the pathogenic and non-pathogenic conditions (Fig. 3b), while activities of NR and GS were different (Fig. 4).

Pathogen-induced H_2O_2 and JA in isolated leaves

The AM colonization rate of mycorrhizal soybean with or without pathogen infection was 62.43 and 64.09 %, respectively, which suggested that the pathogen infection had no effect on the AM colonization at the current test condition. It is unclear whether the AMF placed a "pre-stored" pathogen-resistant substance into the plant or stimulated pathogenic signaling upon infection, thereby enhancing subsequent pathogenic signaling processes. Therefore, the leaves of mycorrhizal and non-mycorrhizal soybean plants were obtained and inoculated with *Phytophthora sojae*. The H_2O_2 and JA contents and pathogen resistance abilities were determined in these leaves. As shown in Fig. 5, the H_2O_2 and JA levels were both increased in the isolated leaves under the pathogenic condition (Fig. 5a, b). Regardless of the mycorrhizal status, the

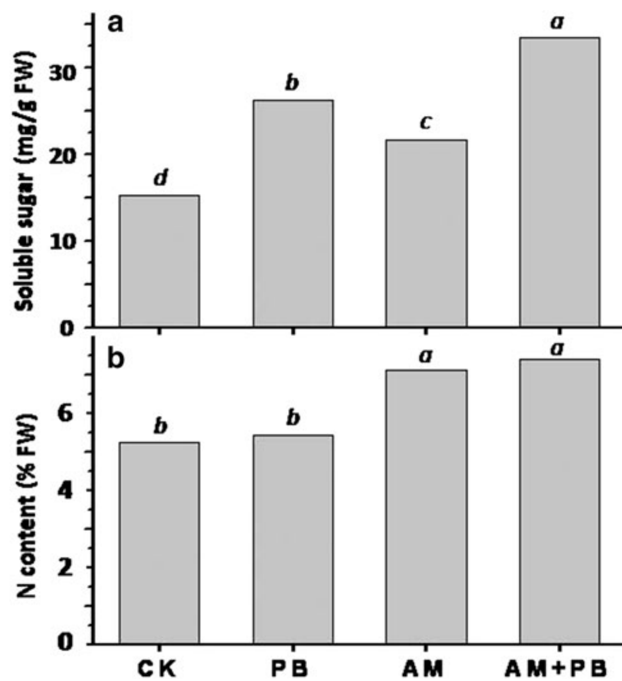

Fig. 3 The content of soluble sugar (**a**) and total nitrogen content (**b**) in the roots of soybean. The seedlings were treated as the follows: CK, control; PB, infected by *Phytophthora sojae*; AM, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices*; AM + PB, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* and infected by *Phytophthora sojae*. The significant difference among treatments were represented by *a*, *b*, *c* and *d marked on the column* using Duncan's multiple range tests ($P < 0.05$)

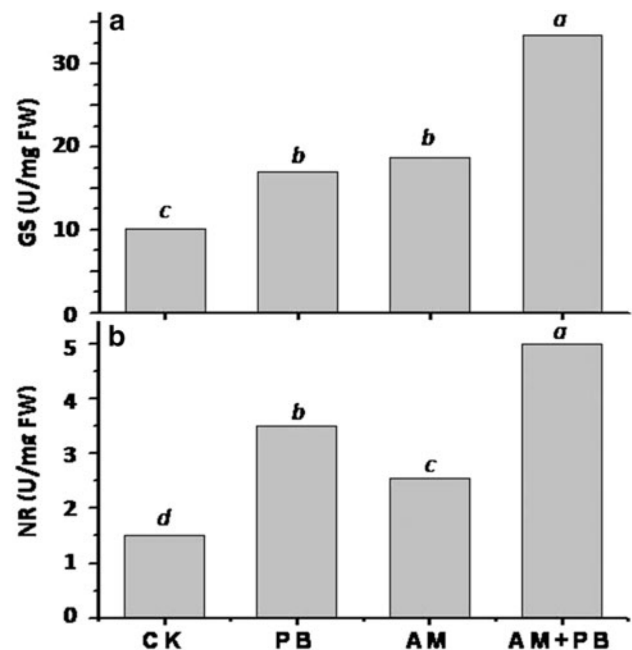

Fig. 4 The activities of enzymes involved in N metabolism in the roots of soybean, including glutamine synthetase (GS) (**a**) and nitrate reductase (NR) (**b**). The seedlings were treated as the follows: CK, control; PB, infected by *Phytophthora sojae*; AM, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices*; AM + PB, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* and infected by *Phytophthora sojae*. The significant difference among treatments were represented by *a*, *b*, *c* and *d marked on the column* using Duncan's multiple range tests ($P < 0.05$)

isolated leaf H_2O_2 content was higher than that in the entire plant system under the pathogenic condition (Fig. 5a), while JA displayed the opposite pattern (Fig. 5b). This result may have been caused by the more serious injury that was inflicted on the isolated leaves compared to the entire plant system. Under the pathogenic condition, the isolated leaves from the mycorrhizal soybean displayed lower H_2O_2 levels than in the non-mycorrhizal soybean, indicating that the mycorrhizal isolated leaves sustained less injury compared to the non-mycorrhizal isolated leaves (Fig. 5a). The JA level remained consistent, illustrating that no pathogenic resistance difference existed between the mycorrhizal and non-mycorrhizal soybean isolated leaves (Fig. 5b). However, the isolated leaves from the mycorrhizal soybean had lower H_2O_2 contents than that in the non-mycorrhizal soybean, suggesting that AMF may alter soybean physiology and enhance the resistance of the structure by increasing nutrient absorption, thereby reducing pathogen infection (Fig. 5a). Under the pathogenic condition, the JA content in the mycorrhizal soybean was significantly higher and the H_2O_2 content was significantly lower than that in the mycorrhizal detached leaves, indicating that AMF may participate in the process of JA accumulation following pathogen infection and then enhance the active defense of mycorrhizal soybean to reduce damage, which was indicated by the comparison of the percentage of disease lesion area between leaf samples from the entire plant or isolated leaves (Fig. 5c, d).

Discussion

The colonization of AMF in root cells contributes to resistance to soil-borne pathogens, such as *Phytophthora sojae*, which can cause *Phytophthora* root rot of soybean (Wehner et al. 2010). However, the mechanism by which the AMF are involved in soybean resistance to *Phytophthora* infection in leaves remains unknown.

Involvement of H_2O_2, JA and NO in the pathogen resistance of mycorrhizal soybean

Upon plant pathogen infection, NADPH oxidase activity is up-regulated, resulting in H_2O_2 accumulation, which is one response of the antioxidant system (Siegmund et al. 2013).

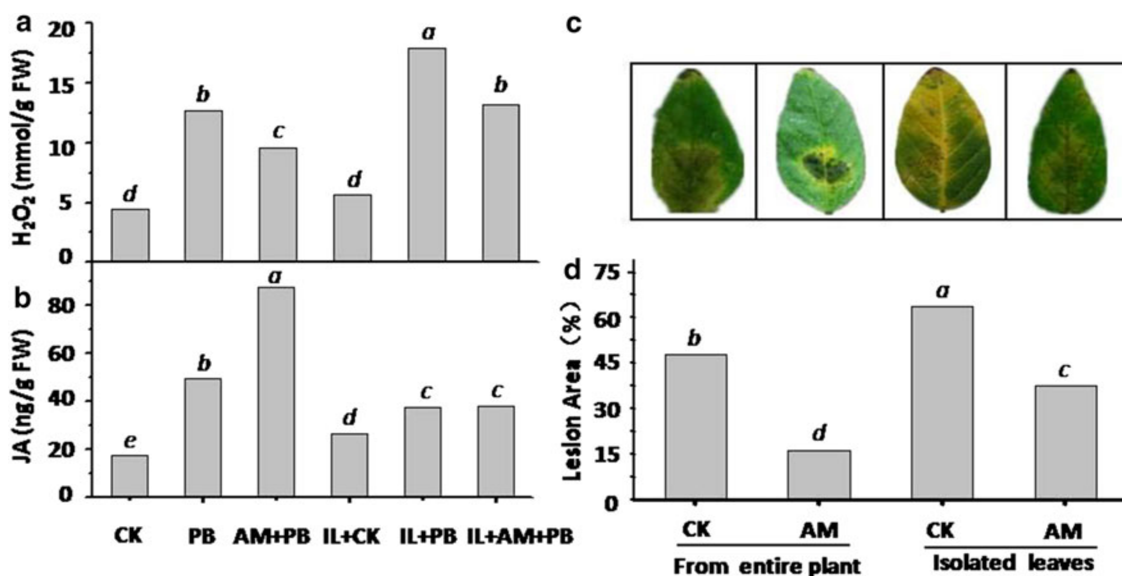

Fig. 5 The content of H_2O_2 (**a**) and JA (**b**) in isolated leaves and the comparison of pathogen lesions between the entire plant and the isolated leaves (**c**, **d**). The seedlings were treated as the follows: CK, control; PB, infected by *Phytophthora sojae*; AM + PB, inoculated with the arbuscular mycorrhizal fungus *Glomus intraradices* and infected by *Phytophthora sojae*. IL represented that the evaluation was based on the isolated leaves treated with pathogen, while the others were based on the entire plant after treatment. The significant difference among treatments were represented by *a*, *b*, *c* and *d marked on the column* using Duncan's multiple range tests ($P < 0.05$)

The generation of reactive oxygen radicals, such as H_2O_2 and O_2^-, is a positive defense against pathogenic invasion, although excessive radical production may destroy the cells. This study revealed that *Phytophthora sojae*-infected non-mycorrhizal soybean contained increased H_2O_2 levels, while the AMF inoculation suppressed H_2O_2 accumulation to a certain extent (Fig. 1a). The treatment also activated the antioxidant enzymatic activities of CAT and GR for plant self-protection (Fig. 2a, b), resulting in the plant's enhanced antioxidant capability and pathogen resistance. Under the pathogenic condition, the AMF-suppressed H_2O_2 production and enhanced activities of oxidative enzymes suggest that H_2O_2 can act as a signaling molecule for defense responses against pathogen infection in mycorrhizal soybean. The AMF-involved network appears to be more sensitive when perceiving the release of pathogen-induced H_2O_2 to activate plant antioxidant protection. Under stress conditions, the AMF have been reported to significantly reduce H_2O_2 accumulation. For example, when inoculated with AMF, the H_2O_2 in rice roots can be reduced by 60 % under water stress (Ruiz-Sánchez et al. 2010) and by 40 % in tomato roots under salt stress (Hajiboland et al. 2010). Accordingly, it has been accepted that the AMF can reduce H_2O_2 generation in host plants as one strategy to protect the host plant against stresses (Fester and Hause 2005; Ramu et al. 2002). Conversely, in the absence of stress, the presence of the AMF in the root may slightly increase the accumulation of H_2O_2 (Huang et al. 2008), which may result from the "invasive" colonization of the AMF at the beginning of the symbiotic formation as confirmed by diaminobenzidine (DAB) staining techniques (Salzer et al. 1999). The result may also explain why the AMF-involved system can sensitively perceive pathogenic invasion through a H_2O_2-related mechanism, through which the AMF-involved network is rapidly stimulated to enhance the activities of antioxidant enzymes (Lambais et al. 2003; Blilou et al. 2000). The activated antioxidant enzymatic activities of CAT and GR for plant self-protection (Fig. 2a, b) suggested that one of the strategies that provide the best benefit in mycorrhizal symbiosis is protecting plants from the injury of oxidative stresses, and the protective capability depends greatly on activities of antioxidant enzymes.

Pathogen-induced reactive oxygen radicals have been reported to lead to increased JA contents in mycorrhizal plants (Hause et al. 2002). Further, the JA signaling molecule can provoke the expression of plant defense genes (Ren and Dai 2012; Wasternack and Hause 2002). A *Phytophthora* protein elicitor initiates H_2O_2 accumulation coupled with increased JA contents by upregulating the transcription of JA synthesis genes (Hu et al. 2009), and the clean of generated H_2O_2 can inhibit JA accumulation (Hu et al. 2009). We demonstrated that pathogen infection increased the levels of JA. Compared with the non-mycorrhizal soybean, the mycorrhizal soybean displayed a higher JA content regardless of the pathogenic condition (Fig. 1b), indicating that AMF inoculation also increases JA accumulation as a reaction for the pathogen resistance.

It has been accepted that JA plays an important role in the transfer and metabolism of C and N in the AM symbiosis. However, the crosstalk between H_2O_2 and JA after pathogenic infection in the AM symbiosis still needs a further investigation.

NO is reportedly involved in the process of AMF root cell colonization (Meilhoc et al. 2011; Stohr and Stremlau 2006). Calcagno et al. (2012) used fluorescent probe detection to determine that NO accumulation is related to mycorrhizal colonization. NO accumulation in *Medicago truncatula* was shown to be attributed to the function of NO synthase (NOS) (Besson-Bard et al. 2009) and NR (Horchani et al. 2011). Consistent with these reports, we found that the AMF could increase the NO content and NR activity (Fig. 1c, Fig.4b). As a signaling molecule, NO facilitates plant disease resistance by mediating reactive oxygen metabolism (Rasul et al. 2012; Delledonne et al. 1998). H_2O_2 can activate mitogen-activated protein kinases (MAPKs) and affect subsequent NO accumulation as a result of increased NR activity (Wang et al. 2010); the released NO can inhibit the accumulation of H_2O_2 by suppressing NADPH oxidase (Boscari et al. 2013; Yun et al. 2011). It was confirmed that the pathogen infection resulted in the up-regulation of NR activity in both the mycorrhizal and non-mycorrhizal plants (Fig. 4b), and the increased enzymatic activities were associated with pathogen-related H_2O_2 accumulation. NO acts as a signaling molecule to up-regulate the activity of antioxidant enzymes, such as CAT and ascorbate peroxidase (APX), to remove reactive oxygen radicals, thereby avoiding cellular injury (Besson-Bard et al. 2009). However, NO also functions as an antioxidant that directly scavenges some reactive oxygen radicals (Zheng et al. 2009; Beligni and Lamattina 1999).

Enhanced transfer and metabolism of C and N in the AM symbiosis after the pathogen infection in mycorrhizal soybean

In mycorrhizal symbiosis, the host plant donates nearly 20 % of its photosynthetic production to the symbiotic fungi (Allen and Shachar-Hill 2008; Jakobsen and Rosendahl 1990). In the arbuscular mycorrhizal symbiosis, C and N metabolism is interconnected because C transferred from the host plant to the fungus stimulates N gene expression, promoting increased trading between both sides (Tian et al. 2010; Fellbaum et al. 2012). For example, C transferred from the host plant to the fungus regulates the delivery of N or P from the fungus to the host plant (Hammer et al. 2011; Bucking and Shachar-Hill 2005). As shown in Figs. 3 and 4, the pathogen infection led to the increased soluble sugar content in the mycorrhizal soybean and the simultaneous up-regulation of NR and GS activity. These results were consistent with a recent study regarding the exchange of C and N between the AMF and host plant, which was determined to represent a positive reaction from the fungal side (Fellbaum et al. 2012). When inoculated with the AMF, the host plant had a significantly higher JA content than that of non-mycorrhizal soybean, indicating that JA is probably related to the pathogenic condition and partially to the presence of the AMF, as reported by Hause et al. (2002), who proposed that the AMF inoculation was associated with increased JA content. JA regulates the metabolism of sugar, which acts as signaling molecule for carbon reallocation in mycorrhizal plants. As a result, the sugar-mediated osmotic pressure induced JA accumulation by upregulating JA synthesis genes (Hause et al. 2002). The soluble sugar content in the mycorrhizal soybean was significantly higher than that in the non-mycorrhizal soybean (Fig. 3), and the JA content in the mycorrhizal pathogenic condition was significantly increased as previously described (Fig. 1). These results suggest that the elevated JA levels may mediate the soluble sugar levels and affect pathogen resistance. Mycorrhizal plants under the pathogenic condition may provoke increased JA and soluble sugar contents (Fig. 1b,3a). Increased soluble sugar leads to a high osmotic pressure, which signals the induction of JA synthesis genes (Hause et al. 2002). JA accumulation stimulates the expression of extracellular invertase, such as sucrose aminotransferase (Schaarschmidt et al. 2006; Thoma et al. 2003), which results in an increased osmotic potential and increased carbohydrate synthesis and transport to root cells (Hause et al. 2007).

H_2O_2 and JA accumulation in isolated leaves and corresponding pathogen resistance

Under the pathogenic condition, the isolated leaves had higher levels of H_2O_2, lower JA levels and more serious lesions than the entire plant (Fig. 5a, b), suggesting that the pathogenic condition in the isolated leaves may not effectively stimulate the JA-mediated systemic resistance pathway in which H_2O_2 was involved. Furthermore, in isolated leaves under the pathogenic condition, the H_2O_2 content was lower in the mycorrhizal leaves compared to the non-mycorrhizal leaves, while the JA contents remained relatively consistent (Fig. 5a, b). This result suggests the potential for a "pre-stored" AMF contribution even in the isolated leaves of mycorrhizal plants. Mycorrhizal isolated leaves had less H_2O_2 content and smaller lesions compared with the non-mycorrhizal isolated leaves, suggesting that the mycorrhizal soybean isolated leaf structure is slightly more resistant than the non-mycorrhizal isolated leaves (Fig. 5a, c, d). The disease resistance of the mycorrhizal plant is based on both the strengthened leaf physiological structure and the regulation of plant

hormone-mediated signal transduction pathways in which plant metabolites and enzymes are involved in active pathogen defense. However, additional studies must be completed to determine the detailed mechanism by which this result occurs.

Conclusion

AMF inoculation improves soybean resistance to *Phytophthora sojae* infection in the leaves, in which the H_2O_2-participated signaling networks and JA-mediated regulation of plant defense responses are involved. Furthermore, the transfer and metabolism of N and C through the mycorrhizal symbiosis is also involved, which is interconnected with the generation of NO acting as a signaling molecule for the pathogen resistance of mycorrhizal soybean. In addition, some physiological characters, such as the improved nutrients and biomass and strengthened structures, are also meaningful for the enhanced pathogen resistance. However, more details must be investigated to thoroughly understand the AMF-involved network by which the host plant gains effective pathogen resistance.

Author contribution C. Tian and Y. Li designed the research; Y. Li, Z. Liu and H. Hou performed the experiment; H. Lei gave materials and technical support; X. Zhu, and X. Li gave technical support; X. He gave theoretical support; Y. Li and C. Tian finished the manuscript.

Acknowledgments We are thankful to Dr. Shengqun Liu, Xiaoying Chen, Lixing Wei and Zhiqiang Deng for the help on some experiments, and Hongwen Yu Lab, Fengbin Song and Yang Wang Lab, and Zhengwei Liang Lab for help of some equipment. Especially thanks to Dr. Shuzhen Zhang who kindly provided the strain for pathogen infection. This is supported by "One Hundred Talents Program in CAS", "1.2.4 Program in NEIGAE", CAS project (KZZ-EW-TZ-16), NSFC (31370144) and Jilin NSFC (20130101080JC).

References

Allen JW, Shachar-Hill Y (2008) Sulfur transfer through an arbuscular mycorrhiza. Plant Physiol 149:549–560

Allen DK, Ohlrogge JB, Shachar-Hill Y (2009) The role of light in soybean seed filling metabolism. Plant J 58:220–234

Beligni MV, Lamattina L (1999) Is nitric oxide toxic or protective? Trends Plant Sci 4:299–300

Besson-Bard A, Courtois C, Gauthier A, Dobrowolska G, Jeandroz S, Pugin A, Wendehenne D (2008) Nitric oxide in plants: production and cross-talk with Ca^{2+} signaling. Mol Plant 1:218–228

Besson-Bard A, Astier J, Rasul S, Wawer I, Dubreuil-Maurizi C, Jeandroz S, Wendehenne D (2009) Current view of nitric oxide-responsive genes in plants. Plant Sci 177:302–309

Blilou I, Bueno P, Ocampo JA, García-Garrido JM (2000) Induction of catalase and ascorbate peroxidase activities in tobacco roots inoculated with the Arbuscular mycorrhizal fungus *Glomus mosseae*. Mycol Res 104:722–725

Bonfante P, Genre A (2010) Mechanisms underlying beneficial plant-fungus interactions in mycorrhizal symbiosis. Nat Commun 1:48

Boscari A, Del Giudice J, Ferrarini A, Venturini L, Zaffini AL, Delledonne M, Puppo A (2013) Expression dynamics of the Medicago truncatula transcriptome during the symbiotic interaction with *Sinorhizobium meliloti*: which role for nitric oxide ? Plant Physiol 161:425–439

Bucking H, Shachar-Hill Y (2005) Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus Glomus intraradices is stimulated by increased carbohydrate availability. New Phytol 165:899–912

Calcagno C, Novero M, Genre A, Bonfante P, Lanfranco L (2012) The exudate from an arbuscular mycorrhizal fungus induces nitric oxide accumulation in Medicago truncatula roots. Mycorrhiza 22:259–269

Campos-Soriano L, Segundo BS (2011) New insights into the signaling pathways controlling defense gene expression in rice roots during the arbuscular mycorrhizal symbiosis. Plant Signal Behav 6:553–557

Campos-Soriano L, Garcia-Martinez J, Segundo BS (2012) The arbuscular mycorrhizal symbiosis promotes the systemic induction of regulatory defence-related genes in rice leaves and confers resistance to pathogen infection. Mol Plant Pathol 13:579–592

Canaday CH, Schmitthenner AF (2010) Effects of chloride and ammonium salts on the incidence of phytophthora root and stem rot of soybean. Plant Dis 94:758–765

Chance B, Maehly AC (1955) Assay of catalases and peroxidases. Method Enzymol 2:764–775

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science 262:1883–1886

Delledonne M, Xia Y, Dixon RA, Lamb C (1998) Nitric oxide functions as a signal in plant disease resistance. Nature 394:585–588

Ding AH, Nathan CF, Stuehr DJ (1988) Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. J Immunol 141:2407–2412

Farmer EE, Ryan CA (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 4:129–134

Fellbaum CR, Gachomo EW, Beesetty Y, Choudhari S, Strahan GD, Pfeffer PE, Kiers ET, Bucking H (2012) Carbon availability triggers fungal nitrogen uptake and transport ini arbuscular mycorrhizal symbiosis. Proc Natl Acad Sci USA 109:2666–2671

Fester T, Hause G (2005) Accumulation of reactive oxygen species in arbuscular mycorrhizal roots. Mycorrhiza 15:373–379

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

Glazebrook J (2005) Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu Rev Phytopathol 43:205–227

Grant MR, Jones JD (2009) Hormone (Dis) harmony moulds plant health and disease. Science 324:750–752

Hajiboland R, Aliasgharzadeh N, Laiegh SF, Poschenrieder C (2010) Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. Plant Soil 331:313–327

Halliwell B, Foyer CH (1978) Properties and physiological function of a glutathione reductase purified from spinach leaves by affinity chromatography. Planta 139:9–17

Hammer EC, Pallon J, Wallander H, Olsson PA (2011) Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. FEMS Microbiol Ecol 76:236–244

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42

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

Hause B, Mrosk C, Isayenkov S, Strack D (2007) Jasmonates in arbuscular mycorrhizal interactions. Phytochemistry 68:101–110

Horchani F, Prevot M, Boscari A, Evangelisti E, Meilhoc E, Bruand C, Raymond P, Boncompagni E, Aschi-Smiti S, Poppo A, Brouquisse R (2011) Both plant and bacterial nitrate reductases contribute to nitric oxide production in Medicago truncatula nitrogen-fixing nodules. Plant Physiol 155:1023–1036

Hu X, Neiell SJ, Yang Y, Cai W (2009) Fungal elicitor Pep-25 increases cytosolic calcium ions, H2O2 production and activates the octadecanoid pathway in arabidopsis thaliana. Planta 229:1201–1208

Huang LL, Yang C, Zhao Y, Xu X, Xu Q, Li GZ, Cao J, Herbert SJ, Hao L (2008) Antioxidant defenses of mycorrhizal fungus infection against SO_2-induced oxidative stress in Avena nuda seedlings. B Environ Contam Tox 81:440–444

Jakobsen I, Rosendahl L (1990) Carbon flow into soil and external hyphae from roots of mycorrhizal cucumber plants. New Phytol 115:77–83

Kamran Qureshi M, Sujeeth N, Gechev TS, Hille J (2013) The zinc finger protein ZAT11 modulates paraquat-induced programmed cell death in Arabidopsis thaliana. Acta Physiol Plant 35:1863–1871

Kramell R, Miersch O, Hause B, Ortel B, Parthier B, Wasternack C (1997) Amino acid conjugates of jasmonic acid induce jasmonate-responsive gene expression in barley (*Hordeum vulgare* L.) leaves. FEBS Lett 414:197–202

Lamari L (2008) Assess 2.0: Image Analysis Software for Plant Disease Quantification. St. Paul, MN: APS Press, USA

Lambais MR, Ríos-Ruiz WF, Andrade RM (2003) Antioxidant responses in bean (Phaseolus vulgaris) roots colonized by arbuscular mycorrhizal fungi. New Phytol 160:421–428

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

Liu ZL, Li YJ, Hou HY, Zhu XC, Rai V, He XY, Tian CJ (2013) Differences in the arbuscular mycorrhizal fungi-improved rice resistance to low temperature at two N levels: aspects of N and C metabolism on the plant side. Plant Physiol Bioch 71:87–95

Meilhoc E, Boscari A, Bruand C, Puppo A, Brouquisse R (2011) Nitric oxide in legume–rhizobium symbiosis. Plant Sci 181:573–581

Miller G, Shulaev V, Mittler R (2008) Reactive oxygen signaling and abiotic stress. Physiol Plantarum 133:481–489

Nelson NW, Sommers LE (1973) Determination of Total Nitrogen in Plant Material. Agron J 65:109–112

Parniske M (2004) Molecular genetics of the arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 7:414–421

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775

Piererse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM (2009) Networking by small-molecule hormones in plant immunity. Nat Chem Biol 5:308–316

Pieterse CMJ, Van Wees SCM, Van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ, van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in Arabidopsis. Plant Cell 10:1571–1580

Piffanelli P, Devoto A, Schulze-Lefert P (1999) Defence signaling pathways in cereals. Curr Opin Plant Biol 2:295–300

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

Ramu SK, Peng HM, Cook DR (2002) Nod factor induction of reactive oxygen species production is correlated with expression of the early nodulin gene rip1 in Medicago truncatula. Mol Plant Microbe In 15:522–528

Rasul S, Dubreuil-Maurizi C, Lamotte O, Koen E, Poinssot B, Alcaraz G, Wendehenne D, Jeandroz S (2012) Nitric oxide production mediates oligogalacturonide-triggered immunity and resistance to Botrytis cinerea in *Arabidopsis thaliana*. Plant, Cell Environ 35:1483–1499

Ren CG, Dai CC (2012) Jasmonic acid is involved in the signaling pathway for fungal endophyte-induced volatile oil accumulation of *Atractylodes lancea* plantlets. BMC Plant Biol 12:128

Royo J, Leon J, Vancanneyt G, Albar JP, Rosahl S, Ortego F, Castanera P, Sanchez-Serrano JJ (1999) Antisense-mediated depletion of a potato lipoxygenase reduces wound induction of proteinase inhibitors and increases weight gain of insect pests. Proc Natl Acad Sci USA 96:1146–1151

Ruiz-Sánchez M, Aroca R, Muñoz Y, Polón R, Ruiz-Lozano JM (2010) The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. J Plant Physiol 167:862–869

Ryan CA, Moura DS (2002) Systemic wound signaling in plants: a new perception. Proc Natl Acad Sci USA 99:6519–6520

Salzer P, Corbiere H, Boller T (1999) Hydrogen peroxide accumulation in Medicago truncatula roots colonized by the arbuscular mycorrhiza-forming fungus Glomus intraradices. Planta 208: 319–325

Sandhu D, Schallock KG, Rivera-Velez N, Lundeen P, Cianzio S, Bhattacharyya MK (2005) Soybean Phytophthora resistance gene Rps8 maps closely to the Rps3 region. J Hered 96:536–541

Sarvajeet SG, Narendra T (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Bioch 48:909–930

Schaarschmidt S, Roitsch T, Hause B (2006) Arbuscular mycorrhiza induces gene expression of the apoplastic invertase LIN6 in tomato (*Lycopersicon esculentum*) roots. J Exp Bot 57:4015–4023

Schwacke R, Hager A (1992) Fungal elicitors induce a transient release of active oxygen species from cultured spruce cells that is dependent on Ca^{2+} and protein-kinase activity. Planta 187: 136–141

Shirasu K, Nakajima H, Rajasekhar VK, Dixon RA, Lamb C (1997) Salicylic acid potentiates an agonist dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. Plant Cell 9:261–270

Siegmund U, Heller J, van Kann JA, Tudzynski P (2013) The NADPH oxidase complexes in Botrytis cinerea: evidence for a close association with the ER and the tetraspanin Pls1. PLoS ONE 8:e55879

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier Academic Publishers, New York

Song YY, Zeng RS, Xu JF, Li J, Shen X, Yihdego WG (2010) Interplant communication of tomato plants through underground common mycorrhizal networks. PLoS One 10:e13324

Spoel SH, Dong X (2008) Making sense of hormone crosstalk during plant immune responses. Cell Host Microbe 3:348–351

Stohr C, Stremlau S (2006) Formation and possible roles of nitric oxide in plant roots. J Exp Bot 57:463–470

Thoma I, Loeffler C, Sinha A, Gupta M, Krischke M, Steffan B, Roitsch T, Mueller MJ (2003) Cyclopentenone isoprostanes induced by reactive oxygen species trigger defense gene activation and phytoalexin accumulation in plants. Plant J 34:363–375

Tian C, Kasiborski B, Koul R, Lammers P, Bucking H, Shachar-Hill Y (2010) Regulation of the nitrogen transfer pathway in the arbuscular mycorrhizal symbiosis: gene characterization and the coordination of expression with nitrogen flux. Plant Physiol 153:1175–1187

Van Loon LC, Pierpoint WS, Boller TH, Conejero V (1994) Recommendation for naming plant pathogenesis-related proteins. Plant Mol Biol Rep 12:245–264

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

Wang P, Du Y, Li Y, Ren D, Song CP (2010) Hydrogen peroxide-mediated activation of MAP kinase 6 modulates nitric oxide biosynthesis and signal transduction in Arabidopsis. Plant Cell 22:2981–2998

Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot 100:681–697

Wasternack C, Hause B (2002) Jasmonates and octadecanoids: signals in plant stress responses and development. Prog Nucleic Acid Res 72:165–221

Wehner J, Antunes PM, Powell JR, Mazukatow J, Rillig MC (2010) Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? Pedobiologia 53:197–201

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

Wrather JA, Koenning SR (2006) Estimates of disease effects on soybean yields in the United States 2003–2005. J Nematol 38:173–180

Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514

Yu XZ, Zhang FZ (2012) Activities of nitrate reductase and glutamine synthetase in rice seedlings during cyanide metabolism. J Hazard Mater 225–226:190–194

Yun BW, Feechan A, Yin M, Saidi NB, Le Bihan T, Yu M, Moore JW, Kang JG, Kwon E, Spoel SH, Pallas JA, Loake GJ (2011) S-nitrosylation of NADPH oxidase regulates cell death in plant immunity. Nature 478:264–268

Zhang J, Kirkham MB (1996) Lipid peroxidation in sorghum and sunflower seedlings as affected by ascorbic acid, benzoic, and propyl gallate. J Plant Physiol 149:489–493

Zhang S, Xu P, Wu J, Chen C, Li W, Chen W (2010) Races of phytophthora sojae and their virulences on commonly grown soybean varieties in Heilongjiang, China. Plant Dis 94:87–91

Zheng C, Jiang D, Liu F, Dai T, Liu W, Jing Q, Cao W (2009) Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. Environ Exp Bot 67:222–227

Zheng X, Spivey NW, Zeng W, Liu P, Fu ZQ, Klessig DF, He SY, Dong X (2012) Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. Cell Host Microbe 11:587–596

Zhu X, Song F, Xu H (2010) Arbuscular mycorrhizae improves low temperature stress in maize via alterations in host water status and photosynthesis. Plant Soil 331:129–137