Journal of Plant Protection Research ISSN 1427-4345

ORGINAL ARTICLE

Defense responses of rice plant to *Monographella albescens* attack

Dariush Ebadi Almas*, Atefeh Rahmani Kamrodi

Plant Breeding and Biotechnology Department, Agricultural Sciences & Natural Resources University, Gorgan, Iran

Vol. 59, No. 4: 535–543, 2019

DOI: 10.24425/jppr.2019.131268

Received: April 5, 2019
Accepted: June 27, 2019

*Corresponding address:
d_ebadi@yahoo.com

Abstract

Leaf scald, caused by the necrotrophic fungus *Monographella albescens,* is one of the main threats to rice (*Oryza sativa* L.) around the world. This disease decreases yields in rice by up to 30% because of dead leaf tissue, damaged seeds, and sterile flowers. Currently, there is limited knowledge about the molecular mechanisms involved in rice plant resistance against this pathogen. For this purpose, six commercial cultivars of rice were primarily screened for *M. albescens* infection and development. Dasht and Salari were found to be the most resistant and susceptible to *M. albescens* infection, respectively. The plants were kept in a greenhouse at 29 ± 2°C during the day and 26 ± 2°C at night with a relative air humidity of 85 ± 5%. Forty-five days after sowing, the plants with three biological replications were inoculated by transferring a PDA disc (0.3 cm^2) containing *M. albescens* mycelia to the middle third of the 7th, 8th, and 9th completely open leaves. The leaves were collected 24, 48, 72, 96 and 120 hai. Leaf samples were also collected from the non-inoculated plants (0 h) to serve as controls. Real-time quantitative PCR (RT-qPCR) showed rapid induction and significant accumulation of jasmonic acid (JA) and ethylene (ET) responsive genes such as lipoxygenase (*LOX*), allene oxide synthase 2 (*Aos*2), jasmonic acid carboxyl methyltransferase 1 (*JMT1*) and ACC synthase 1 (*ACS1*) in the resistant Dasht cultivar after infection with *M. albescens*. Furthermore, the transcripts of salicylic acid (SA) responsive phenyl alanine ammonia lyase 1 (*PAL1*) and nonexpressor of pathogenesis-related genes 1 (*NPR1*) genes were induced in the incompatible interaction. The activities of the defense enzymes superoxide dismutase (SOD), peroxidase (POX) and glutathione reductase (GR) increased strongly in Dasht in response to *M. albescens* infection. In addition, there was an increase in the H_2O_2 levels in the leaves of the Dasht cultivar during the infectious period of *M. albescens* associated with the enhancement of catalase (CAT) activity as well as higher levels of malondialdehyde (MDA). This is the first study on the interaction between rice and *M. albescens* at the molecular level. It can contribute to understanding how rice responds to pathogen infection, as well as assist with future research plans of molecular breeding regarding the tolerance to leaf scald disease.

Keywords: defense-signaling genes, enzyme activities, *Monographella albescens*, rice, RT-qPCR

Introduction

Rice plants (*Oryza sativa* L.) can be affected by a number of fungal diseases in all rice producing countries in the world. Rice leaf scald has an extensive global distribution and it is present in several American, African and Asian countries (Filippi *et al.* 2005). This disease reduces rice yields by up to 30% because of dead leaf tissue, damaged seeds and sterile flowers (Prabhu and Filippi 2006). The disease is caused by the necrotrophic fungus *Monographella albescens* (Hashiola and Yokogi) Samuels and I. C. Hallett (=*Rhynchosporium oryzae* Hashioka and Yokogi). The first symptoms of leaf scald include zonate or oblong colored olive lesions with light brown halos and without well-defined margins on the leaf tips or edges. As the disease develops, large parts of the leaf blades become blighted and dry out very quickly giving the leaf a scalded appearance (Ou

1985; Filippi *et al.* 2005). The most suitable conditions for the occurrence of leaf scald epidemics are periods of intense rain, prolonged foliar dew, temperatures greater than 25°C, excess nitrogen and close spacing (Ou 1985; Groth 1992). Fungicides are widely used to manage leaf scald [International Rice Research Institute (IRRI) 1983; Groth 1992)], but this leads to the death of natural enemies and environmental pollution (Uphoff and Dazzo 2016). Therefore, host resistance breeding is known as one of the most effective, economic and environmentally-friendly approaches for management of rice leaf scald disease.

Plant responses to pathogens are regulated through a complex network of signaling pathways that involve three phytohormones: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). Synergistic and/or antagonistic interactions between these three pathways allow the plant to finely tune responses to a specific pathogen (Denance *et al.* 2013; Ma and Ma 2016). While SA dependent signaling is specifically associated with resistance to biotrophic and hemibiotrophic pathogens, JA and ET synergistically regulate defense against necrotrophic fungi (Gimenez-Ibanez and Solano 2013). Plants also defend themselves against fungal infection through the production of reactive oxygen species (ROS) (Magbanua *et al.* 2007). Production of ROS such as the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), as one of the earliest responses to pathogen attack, can trigger hypersensitive cell death. Abnormally high production of ROS causes damage to biomolecules, whereas ROS at low/moderate concentrations acts as a second messenger in signaling cascades that mediates several responses in plant cells including programed cell death (Sharma *et al.* 2012). The hypersensitive response (HR), as an early defense response, restricts pathogen infection to the site of attempted ingress by necrosis and cell death. Plants utilize efficient, antioxidative enzymatic and non-enzymatic protective mechanisms to scavenge excess ROS. Several antioxidative enzymes including superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and glutathione reductase (GR) are involved in detoxification of ROS (Debona *et al.* 2012). Superoxidas dismutase is one of the most important scavenging enzymes and catalyzes the dismutation of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2) (Bowler *et al.* 1992). The accumulation of H_2O_2 is prevented by either CAT or the ascorbate-glutathione cycle, in which GR and ascorbate peroxidase (APX) reduce H_2O_2 to H_2O (Foyer *et al.* 1994). POX is responsible for the degradation of lipid peroxides (Shri *et al.* 2009). Active oxygen radicals may stimulate the chain-like peroxidation of unsaturated fatty acids in the plasma membranes, leading to the formation of lipid peroxidation products such as malondialdehyde (MDA) (Mishra *et al.* 2008). Generally, the levels of ROS and the degree of oxidative damage mainly depend upon the level of coordination among the ROS-scavenging enzymes (Liang *et al.* 2003).

Currently, information about rice defense response mechanisms to *M. albescens*, a typical necrotrophic fungus, is very limited. Therefore, elucidating the interaction between rice and *M. albescens* would be helpful to better understand the molecular basis for plant resistance to leaf scald diseases. In this paper, real-time quantitative PCR (RT-qPCR) was used to analyze differential expression of genes involved in the SA- and JA/ET-mediated defense pathways at different time points when two rice cultivars were infected by *M. albescens*. Defense enzyme activities, H_2O_2 levels and MDA levels were also assayed after *M. albescens* inoculation. An initial screening was done earlier with six cultivars to arrive at a resistant cultivar and another susceptive cultivar against leaf scald disease.

Materials and Methods

Screening of rice cultivars against leaf scald disease

An isolate of *M. albescens* (RV7.1) that was obtained from paddy fields in Gilan Province, Iran was used to inoculate the plants. This isolate was grown in Petri dishes containing potato dextrose agar (PDA) medium and incubated at 25°C for 15 days with a continuous photoperiod. Six commercial cultivars of rice were screened against leaf scald disease: Domsiah, Salari, Khazar, Nemat, Dasht and Neda. The susceptible rice cultivar Anbarboo was also included as a negative control. Seed lots of all rice cultivars were provided by the Rice Research Institute of Iran (RRII), Rasht, Iran. Rice seeds were surface sterilized in 70% (v/v) EtOH and 2% (v/v) NaOCl for 1 min and rinsed in distilled water. Five seeds were sown in plastic pots containing 3 kg of field soil. For soil fertilization, 6 g of $Ca(H_2PO_4)_2 \cdot H_2O$, 5 g $(NH_4)_2SO_4$ and 3 g KCl were added for each 3 kg of soil at the time of sowing. The plants were kept in a greenhouse at 29 ± 2°C during the day and 26 ± 2°C at night with a relative air humidity of 85 ± 5%.

Rice leaves were inoculated as previously described by Tatagiba *et al.* (2016). Forty-five days after sowing, five plants with three biological replications were inoculated by transferring a PDA disc (0.3 cm^2) containing *M. albescens* mycelia to the middle third of the 7th, 8th, and 9th completely open leaves. Immediately after inoculation, the plants were transferred to a plastic mist growth chamber (28 ± 4°C, 90% relative humidity) inside a greenhouse for the duration of the

experiments. The non-inoculated plants were kept in a separate plastic mist growth chamber and exposed to the same environmental conditions as the inoculated plants. Six days after inoculation, expansion of scald lesions (mm) was measured on the adaxial surfaces from the inoculation site with an electronic digital caliper (Mitutoyo, Japan).

Transcriptional and biochemical analysis

The 7th, 8th and 9th leaves (from base to apex) of Dasht and Salari cultivars from each replication per treatment were collected 24, 48, 72, 96 and 120 hours after inoculation (hai). Leaf samples were also collected from the non-inoculated plants (0 h) to serve as controls. The leaves were individually stored in aluminum foil, rapidly frozen in liquid nitrogen and stored in an ultrafreezer (–80°C) for further transcriptional and biochemical analysis.

RNA isolation and cDNA synthesis

Total RNA was extracted from frozen samples using Trizol reagent (Takara, Japan) and treated with DNAse I (Fermentase, Germany) as per manufacturer's instructions. A NanoDrop 2000 Spectrophotometer was used to determine the concentration of total RNA. The quality of the extracted RNA was assessed with 1% agarose gel. For each sample, 1 µg of total RNA was used for first strand complementary DNA (cDNA) synthesis according to the manufacturer's protocol (HiScript® 1st Strand cDNA Synthesis Kit, Vazyme, China). The cDNA concentration was measured using a NanoDrop 2000 spectrophotometer and then diluted to 5 ng · µl^{-1}.

RT-qPCR

Real-time quantitative PCR analysis was performed using a 7500 Real-Time PCR System (Applied Biosystem). The primer pairs listed in Table 1 were used to amplify the corresponding nine genes of interest. Each reaction mixture contained 0.4 µl of each primer, 10 µl of SYBR Green I Master Mix (Takara, Shiga, Japan), 2 µl of diluted cDNA, and 7.2 µl of double distilled water to a final volume of 20 µl. PCRs were carried out as follows: 95°C for 5 min, 40 cycles at 95°C for 10 s and 60°C for 30 s. The specificity of amplifications was verified by an additional melt curve program (95°C for 15 s, 60°C for 15 s, followed by a slow ramp from 60°C to 95°C). Cycle threshold (*Ct*) values were measured with three biological replicates for each sample with three technical replicates. The housekeeping gene actin (*Actin*) was used as an internal control. The expression level of the genes in the control sample was set to one and the relative gene expression was estimated using the comparative $2^{-\Delta\Delta CT}$ Method (Livak and Schmittgen 2001).

Determination of antioxidant enzymes and MDA levels

Leaf samples (0.5 g) were homogenized in 0.05 M sodium phosphate buffer (pH 7.8) comprising 1 mM EDTA.Na_2 and 2% (w/v) polyvinylpolypyrrolidone (PVPP). Homogenates were centrifuged at 12,000 ×*g* for 30 min at 4°C. The supernatant was used for assays of antioxidant enzymes and MDA levels, which were measured with the SOD (A001), CAT (A007-2), POX (A084-3), GR (A062) and MDA (A003-1) commercial kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions. Protein content was measured according to Bradford (1976) and the standard curve for total proteins with bovine serum albumin (BSA) was obtained with different concentrations (0–1 mg · ml^{-1}). All spectrophotometric analyses were carried out with an Ultraviolet-Visible spectrophotometer (UV-160, Shimadzu, Japan).

Determination of H_2O_2 levels

H_2O_2 levels were measured according to the methods of Velikova *et al.* (2000). Leaf tissues (0.4 g) were homogenized in 1 ml of 0.1% (w/v) trichloroacetic acid (TCA) on ice and centrifuged at 12,000 ×*g* for 15 min. One milliliter of potassium phosphate buffer and 1 ml of potassium iodide (KI) were added to 0.5 ml aliquot of the supernatant. The absorbance of the supernatant was measured at 390 nm and H_2O_2 levels were calculated using a standard curve.

Statistical analysis

Data from the experiments were submitted to analysis of variance (ANOVA) by using SAS software (Release 8.02 Level 02M0 for Windows; SAS Institute, Inc., 1989, Cary, NC, USA). The statistical significance was determined at $p < 0.05$.

Results

Evaluation of rice cultivars against leaf scald

Of six cultivars screened against leaf scald disease, Dasht was the most resistant (lesion expansion 6.5 mm), while Salari turned out to be the most susceptible (lesion expansion 61.3 mm). The other tested cultivars showed intermediate levels of resistance (Fig. 1). Phenotypic differences in Dasht and Salari inoculated with *M. albescens* made it possible to select the two cultivars for further transcriptional and biochemical studies.

Table 1. Primers used for gene expression studies

Gene name	Locus ID	Primer sequence (5′→3′)
LOX	GenBank: D14000	F-AGATGAGGCGCGTGATGAC R-CATGGAAGTCGAGCATGAACA
Aos2	RAP-DB: Os03t0225900	F-GCGAGAGACGGAGAACCC R-CGACGAGCAACAGCCTTC
JMT1	RAP-DB: Os06g0314600	F-CACGGTCAGTCCAAAGATGA R-CTCAACCGTTTTGGCAAACT
ACS1	RAP-DB: Os03g51740	F-GATGGTCTCGGATGATCACA R-GTCGGGGGAAAACTGAAAAT
PAL1	RAP-DB: Os02g41630	F-TGTGCGTGCTTCTGCTGCTG R-AGGGTGTTGATGCGCACGAG
NPR1	RAP-DB: Os01t0194300	F-AGAAGTCATTGCCTCCAG R-ACATCGTCAGAGTCAAGG
ICS1	RAP-DB: Os09g19734	F-TGTCCCCACAAAGGCATCCTGG R-TGGCCCTCAACCTTTAAACATGCC
PAD4	RAP-DB: Os11t0195500	F-TCAGAGGCAAGGCAGTAGTG R-ACCGCTCACGCAGGATAG
Actin	GenBank: AK058421	F-CAGCCACACTGTCCCCATCTA R-AGCAAGGTCGAGACGAAGGA

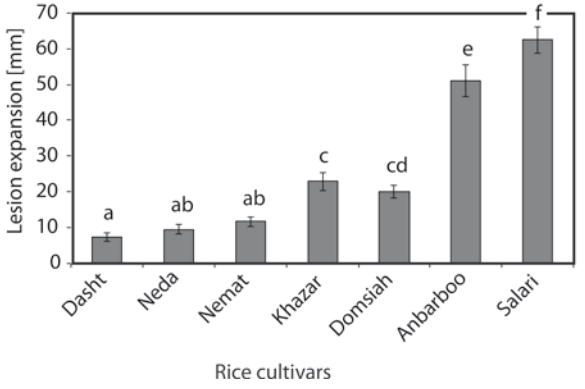

Fig. 1. Rice cultivars screened for resistance to *Monographella albescens*. Lesion expansion of leaf scald in rice leaves of cultivars was evaluated 6 days after inoculation. Error bars represent the standard deviation of the mean from three in dependent biological experiments. Different letters indicate significant differences (Duncan's Multiple Range Test, $p < 0.05$)

Gene expressions in JA/ET signal pathway

The results revealed a constitutive expression of lipoxygenase (*LOX*), allene oxide synthase 2 (*Aos2*) and jasmonic acid carboxyl methyltransferase 1 (*JMT1*) genes that are associated with JA biosynthesis. Nevertheless, a significant difference could be seen in the relative expression of the three genes under compatible and incompatible interactions. In the resistant cultivar Dasht, *LOX* was remarkably up-regulated as early as 24 hai (4.9 fold) which gradually increased and reached a peak at 72 hai (17.6 fold) which was significantly higher than the compatible cultivar Salari. Thereafter, the expression declined from 72 hai to 120 hai where it was still three fold higher than the transcript expression in the susceptible cultivar (Fig. 2). Likewise, the transcript accumulation for *Aos2* gene in Dasht reached a peak at 72 hai (25.6 fold) before gradually decreasing from 96 hai (16.1 fold) to 120 hai (14.6 fold) (Fig. 2). For *JMT1*, the transcript levels increased by 35.3 fold in the resistant genotype as early as 24 hai. A second peak of 38.4 fold was observed at 96 hai before decreasing to 21.6 fold at 120 hai, which was still significantly higher than the susceptible cultivar (Fig. 2). ACC synthase 1 (*ACS1*), the ethylene biosynthetic gene, revealed significant transcript accumulation by 24 hai (2.5 fold) which gradually increased by 96 hai (9.4 fold) and subsequently decreased by 120 hai (5.2 fold) in the Dasht cultivar. However, *ACS1* transcripts were only slightly accumulated under compatible interaction in the Salari cultivar (Fig. 2).

Gene expressions in the SA signal pathway

Phenyl alanine ammonia lyase 1 (*PAL1*), a key gene of the phenylpropanoid pathway, demonstrated significant up-regulation as early as 24 hai (12.4 fold) and 48 hai (16.6 fold). It further increased 72 hai (21.7 fold) in the resistant cultivar. However, at the later stages of infection (96 hai and 120 hai), the expression pattern for *PAL1* was found to be similar in both Dasht and Salari (Fig. 2). Similarly, the transcript levels of non-expressor of pathogenesis-related genes 1 (*NPR1*), a prominent mediator of systemic acquired resistance (SAR) was significantly up-regulated in the resistant cultivar at 24 and 48 hai (Fig. 2). The isochorismate

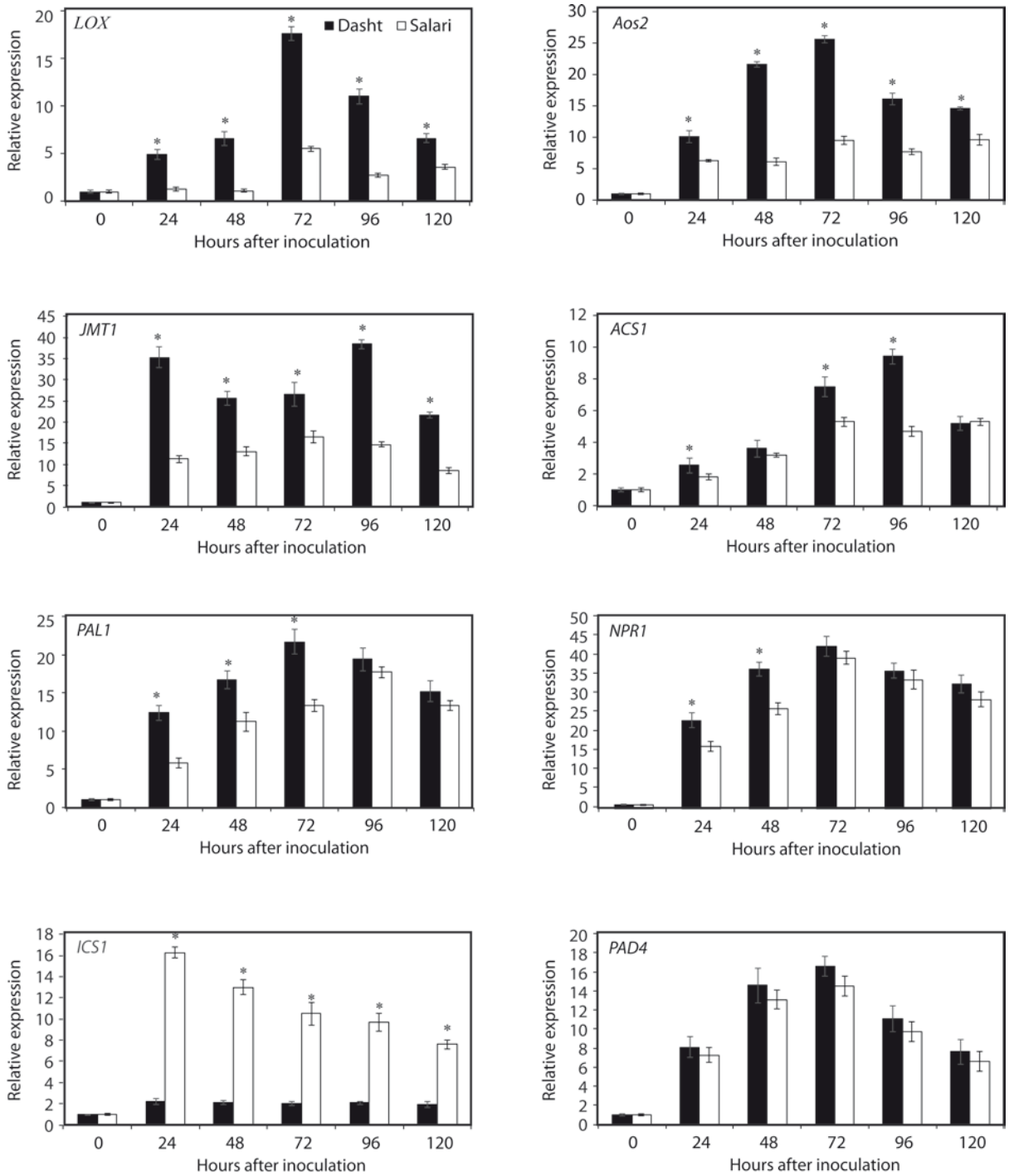

Fig. 2. Differential expression patterns of jasmonic acid (JA), ethylene (ET) and salicylic acid (SA) marker genes in Dasht and Salari cultivars upon infection with *Monographella albescens.* Error bars represent the standard deviation of the mean from three independent biological experiments. Asterisks indicate a significant difference between Dasht and Salari cultivars (*t* test, $p < 0.05$)

synthase 1 (*ICS1*) gene was significantly induced in the Salari cultivar within 24 hai (16.2 fold) before gradually reducing from 48 hai (13 fold) to 120 hai (7.6 fold) (Fig. 2). In the Dasht cultivar, *ICS1* was marginally induced during the whole period of infection. The phytoalexin deficient 4 (*PAD4*) gene demonstrated a similar pattern of high constitutive expression at 72 hai that gradually declined from 96 hai to 120 hai in both the resistant and susceptible cultivars (Fig. 2).

Enzyme activities, H_2O_2 levels and MDA levels

The SOD activity significantly increased by 113, 83, 157 and 180%, at 48, 72, 96 and 120 hai, respectively, in Dasht compared to Salari (Fig. 3). Likewise, significant increases in the CAT activity of 60, 90, 64, 73 and 85%, at 24, 48, 72, 96 and 120 hai, respectively, occurred in Dasht compared to Salari (Fig. 3). The POX activity significantly increased by 55, 11 and 9%, respectively, at 48, 96 and 120 hai (Fig. 3), and the GR activity significantly

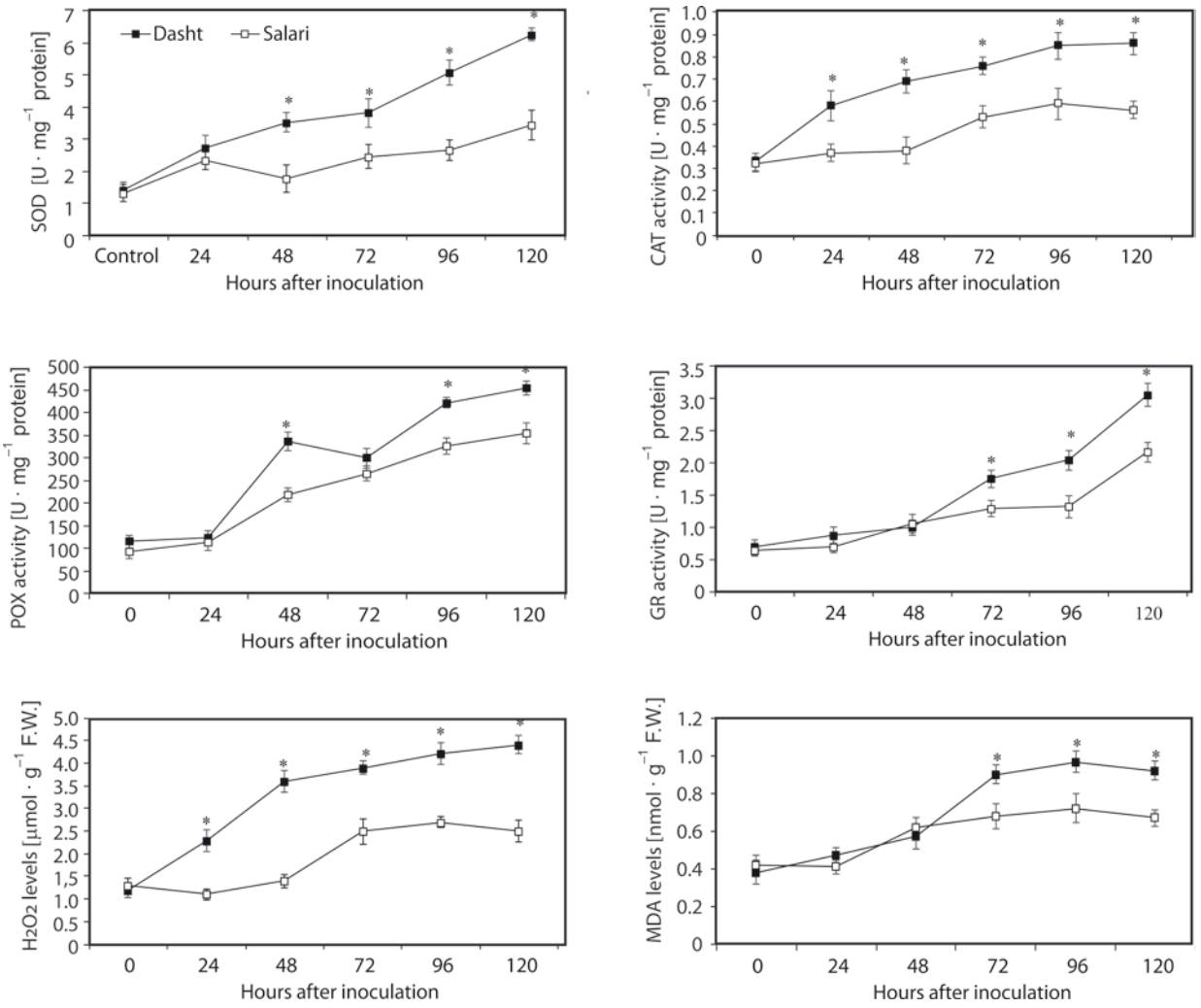

Fig. 3. Superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR) activities, H_2O_2 levels, and malondi-aldehyde (MDA) levels in Dasht and Salari cultivars upon infection with *Monographella albescens*. Error bars represent the standard deviation of the mean from three independent biological experiments. Asterisks indicate a significant difference between Dasht and Salari cultivars (*t* test, $p < 0.05$); F.W. – fresh weight

increased by 56, 91 and 108%, respectively, at 72, 96 and 120 hai in Dasht compared to Salari (Fig. 3).

For the H_2O_2 levels, significant increases of 107, 192, 132, 142 and 175%, respectively, at 24, 48, 72, 96 and 120 hai were observed in Dasht compared to Salari (Fig. 3).

The MDA levels were higher in Dasht 72, 96 and 120 hai than in Salari. At 72, 96 and 120 hai, the MDA levels significantly increased by 75, 82 and 81% in Dasht compared to Salari (Fig. 3).

Discussion

Plant defense responses against insects and pathogens involve global variations in gene expression mediated by several signaling pathways. These defense pathways are mainly mediated by small, chemical modules such as SA, JA and ET (Ma and Ma 2016). A large number of defense-related genes are activated by these signaling molecules after infection with the pathogens to trigger an innate immune response (Andolfo and Ercolano 2015). Leaf scald, caused by *M. albescens,* is one of the major threats to rice around the world. However, there is limited knowledge about the molecular mechanisms involved in rice plant resistance against this pathogen. In the present study, we analyzed the transcriptional levels of eight defense-signaling genes that possibly contribute to the establishment of leaf scald resistance in rice plants.

The transcripts of six defense-signaling genes encoding *LOX*, *Aos2*, *JMT1*, *ACS1, PAL1* and *NPR1* accumulated significantly at a greater level and earlier in the resistant cultivar Dasht than in the susceptible cultivar Salari leaves upon inoculation with *M. albescens*.

Lipoxygenase (*LOX*) is the first in the biosynthesis of jasmonates, and catalyzes the production of jasmonic

acid from linolenic acid (Westernack and Hause 2013). Jasmonic acid and its components are known to play a central role in plant resistance by providing front-line defense against necrotrophic pathogen-induced damage (Antico *et al.* 2012). The *LOX* transcript has been shown to be strongly expressed in the resistant rice cultivar after infection with *Rhizoctonia solani* (Sayari *et al.* 2014). Allene oxide synthase (AOS) is a key enzyme in the JA biosynthetic pathway and catalyzes the dehydration of 13-hydroperoxy octadecatrienoic acid to an unstable epoxide, which is then converted by allene oxide cyclase to a direct precursor of JA (Mei 2006). Overexpression of an *OsAos2* gene leads to induction of *PR*-genes and enhanced resistance to *Magnaporthe grisea* as well as an increased endogenous level of JA (Mei 2006). Jasmonic acid can be converted into volatile methyl jasmonate by the enzyme jasmonic methyl transferase (JMT). Exogenous application of methyl jasmonate (MeJA) has been shown to enhance resistance against several necrotrophic fungal species (Antico *et al.* 2012). ACC synthase (ACS) is the rate--limiting enzyme that regulates ethylene biosynthesis in response to pathogen attack. Upon challenge with necrotrophic fungal pathogens, there is activation of the MAPK signaling cascades which positively regulate ACS to increase ET production in plants (Pandey *et al.* 2016). Moreover, a recent report also confirmed that a JA-ET hormonal interplay is critical for the expression of plant defensins during necrotrophic infection (Pandey *et al.* 2016). Surprisingly, the transcripts of *PAL1* and *NPR1* genes, marker genes of SA signaling pathway, were induced by *M. albescens* infection in resistant Dasht cultivar. This suggested that both JA/ET and SA signaling pathways were involved in the interaction between rice and *M. albescens.* The co-activation in SA- and JA-mediated defense signaling pathways may be due to a high endogenous SA concentration in rice plants under normal conditions that lead to a rise in the JA level and can consequently cause enhanced expression of JA-pathway genes (Tamaoki *et al.* 2013; Sayari *et al.* 2014). Furthermoe, gene expression analysis showed the stable expression of defense related genes in a resistant cultivar, so that it sometimes increased up to 72 hai or 96 hai and then it decreased to 120 hai. This fluctuation was somewhat different in the susceptible cultivar. The stable over-expression of the *TaPERO* peroxidase gene in wheat resulted in enhanced resistance against *Blumeria graminis* f. sp. *tritici* (Altpeter *et al.* 2005). In this situation, it seems that plants spend less metabolic energy to deal with the disease.

In ROS-scavenging systems, superoxide radicals generated in plants are converted to H_2O_2 by the action of SOD (Bowler *et al.* 1992). In the present study, the SOD activity increased in the leaves of rice plants in response to *M. albescens* infection. However, the SOD activity increased in the leaves of the inoculated plants of both cultivars. Its activity in Dasht showed a more prominent increase than in Salari. The SOD activity increased in the leaves of rice plants in response to *M. albescens* infection. In tomato, the peroxisomal SOD activity increased during the initial stage of *Botrytis cinerea* infection but decreased as the necrotic lesions appeared (Kuzniak and Sklodowska 2005). However, in the present study, the SOD activity increased in the leaves of the inoculated plants of both cultivars. Its activity in Dasht showed a more prominent increase than in Salari. The SOD activity was also shown to increase in faba bean leaves infected by the necrotrophic fungus *Botrytis fabae* but the SOD activity for the resistant cultivars was greater than for the susceptible ones (El-Komy 2014). Some fungal pathogens could benefit from an increase in ROS levels generated in the host cells during the defense against their infection as a facilitating factor to maximize their tissue colonization and nutrient uptake (Govrin and Levine 2000). Thus, significantly higher SOD activity in resistant cultivar Dasht could be a strategy of the plant to restrict *M. albescens* colonization because the excess ROS can be removed (Govrin and Levine 2000; Ehsani-Moghaddam *et al.* 2006).

Among the enzymes involved in the removal of excess H_2O_2 generated spontaneously or by O_2^- dismutation via SOD, CAT plays a key role (Mittler 2002). In the present study, the increase in H_2O_2 levels observed in the leaves of the Dasht cultivar during the infectious period of *M. albescens* was associated with an enhancement of CAT activity, which confirms reports for many plant-pathogen interactions. According to Debona *et al.* (2012) a less efficient enzymatic ROS-scavenging system, mainly a decrease in CAT activity, explains the high level of damage caused by *Pyricularia oryzae*. Additionally, Na *et al.* (2018) reported that the leaves of a resistant sunflower cultivar infected with *Sclerotinia sclerotiorum* accumulated more CAT than susceptible cultivars. Therefore, the difference in the CAT activity between cultivars suggests that this enzyme plays a major role in rice resistance to *M. albescens*.

In the inoculated plants of both cultivars, there was a consistent increase in POX activity during the infectious period of *M. albescens*. However, the increase was more pronounced for Dasht than for Salari at 48, 96 and 120 hai. POX is involved in the removal of H_2O_2, and this enzyme plays a central role in plant defense against pathogens due to its participation in lignin biosynthesis (Yoshida *et al.* 2003). A close relationship was found between resistance in date palm against *Fusarium oxysporum* f. sp. *albedinis* and the activation of POX enzyme (Jaiti *et al.* 2009). The POX activity was also more important for resistant than for susceptible wheat cultivars when infected by necrotrophic fungus *Rhizoctonia cerealis* (Hong-xia *et al.* 2011). These reports are in agreement with the results of this study,

which indicated that the POX activity was retained at a higher level in the leaves of rice plants of Dasht than in Salari against *M. albescens* infection.

Glutathione reductase catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) with the accompanying oxidation of NADPH. This reaction is important in the ascorbate AsA-GSH cycle, which is responsible for the detoxification of ROS in plants (Noctor and Foyer 1998). Glutathione reductase activity has been shown to be enhanced in the resistant genotypes of apricot and wheat after infection with fungal phytopathogens (Hernández *et al.* 2001; Debona *et al.* 2012). In the present study, the GR activity increased in the leaves of Dasht and Salari cultivars but was greater for Dasht from 48 hai to 120 hai. Little research has been done to investigate the functions of GR in plant-pathogen interactions. However, the different responses in Dasht and Salari suggest that GR may play an important role in resistance of rice plants to leaf scald.

An increase in the H_2O_2 levels in the leaves of the Dasht cultivar during the infectious period of *M. albescens* contributed to the higher levels of MDA. The high levels of ROS in the roots of tomato plants infected by *F. oxysporum* f. sp. *lycopersici* resulted in increased lipid peroxidation (Mandal *et al.* 2008). The extent of the cellular damage caused by the oxidative stress related to the plant response against pathogen infection can be estimated by the products of the peroxidation of membrane lipids (Aly *et al.* 2012).

Conclusions

In conclusion, a comprehensive RT-qPCR based strategy demonstrated significant differential expression of defense-signaling genes in rice during compatible and incompatible interactions with the leaf scald pathogen *M. albescens*. Our results showed that multiple phytohormone responsive defense genes such as *LOX*, *Aos2*, *JMT1*, *ACS1*, *PAL1* and *NPR1* were significantly induced in the resistant cultivar suggesting a coordinated activation and cross talk between JA/ET and SA mediated signaling pathways during resistance responses of rice to *M. albescens*. In addition, the results of this study indicate that simultaneous enhancement of hydrogen peroxide levels and defense enzyme activities effectively contribute to resistance to rice leaf scald disease. This is the first study of interaction between rice and *M. albescens* at the molecular level, contributing to understanding how rice responds to pathogen infection, as well as to future plans of molecular breeding regarding tolerance to leaf scald disease.

Acknowledgements

The authors would like to thank the Agricultural Sciences & Natural Resources University for providing laboratory facilities.

References

Altpeter F., Varshney A., Abderhalden O., Douchkov D., Sautter C., Kumlehn J., Dudler R., Schweizer P. 2005. Stable expression of a defense-related gene in wheat epidermis under transcriptional control of a novel promoter confers pathogen resistance. Plant Molecular Biology 57 (1): 271–283. DOI: https://doi.org/10.1007/s11103-004-7564-7

Aly A.A., Mansour M.T.M., Mohamed H.I., Abd-Elsalam K.A. 2012. Examination of correlations between several biochemical components and powdery mildew resistance of flax cultivars. The Plant Pathology Journal 28 (2): 149–155. DOI: 10.5423/PPJ.2012.28.2.149

Andolfo G., Ercolano M.R. 2015. Plant innate immunity multicomponent model. Frontiers in Plant Science 987 (6): 1–6. DOI: https://doi.org/10.3389/fpls.2015.00987

Antico C.J., Colon C., Banks T., Ramonell K.M. 2012. Insights into the role of jasmonic acid-mediated defenses against necrotrophic and biotrophic fungal pathogens. Frontiers in Biology 7 (1): 48–56. DOI: https://doi.org/10.1007/s11515-011-1171-1

Bowler C., Montagu M.V., Inze D. 1992. Superoxide dismutase and stress tolerance. Annual Review of Plant Physiology and Plant Molecular Biology (43): 83–116. DOI: https://doi.org/10.1146/annurev.pp.43.060192.000503

Bradford M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72 (1–2): 248–254. DOI: https://doi.org/10.1016/0003-2697(76)90527-3

Debona D., Rodrigues F.Á., Rios J.A., Nascimento K.J.T. 2012. Biochemical changes in the leaves of wheat plants infected by *Pyricularia oryzae*. Phytopathology 102 (12): 1121–1129. DOI: http://dx.doi.org/10.1094/PHYTO-06-12-0125-R

Denance N., Sanchez-Vallet A., Goffner D., Molina A. 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness cost. Frontiers in Plant Science 155 (4): 1–12. DOI: https://doi.org/10.3389/fpls.2013.00155

Ehsani-Moghaddam B., Charles M.T., Carisse O., Khanizadeh S. 2006. Superoxide dismutase responses of strawberry cultivars to infection by *Mycosphaerella fragariae*. Journal of Plant Physiology 163 (2): 147–153. DOI: https://doi.org/10.1016/j.jplph.2005.04.025

El-Komy M.H. 2014. Comparative analysis of defense responses in chocolate spot-resistant and -susceptible faba bean (*Vicia faba*) cultivars following infection by the necrotrophic fungus *Botrytis fabae*. The Plant Pathology Journal 30 (4): 355–366. DOI: http://dx.doi.org/10.5423/PPJ.OA.06.2014.0050

Filippi M.C., Prabhu A.S., Silva G.B. 2005. Escaldadura Do Arroz E Seu Controle. Goiânia, Goiás, Brazil, EMBRAPA-CNPAF, Circular Técnica.

Foyer C.H., Lelandais M., Kenert K.J. 1994. Photooxidative stress in plants. Physiologia Plantarum 92 (4): 696–717. DOI: https://doi.org/10.1111/j.1399-3054.1994.tb03042.x

Gimenez-Ibanez S., Solano R. 2013. Nuclear jasmonate and salicylate signalling and crosstalk in defense against pathogens. Frontiers in Plant Science 72 (4): 1–11. DOI: https://doi.org/10.3389/fpls.2013.00072

Govrin E.M., Levine A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis*

cinerea. Current Biology 10 (13): 751–757. DOI: https://doi.org/10.1016/S0960-9822(00)00560-1

Groth D. 1992. Leaf scald. In: "Compendium of Rice Diseases" (R. Webster, P. Gunnel P., eds.). Saint Paul, MN, USA, The American Phytopatological Press, 18 pp.

Hernández J.A., Talavera J.M., Martínez-Gómez P., Dicenta F., Sevilla F. 2001. Response of antioxidative enzymes to plum pox virus in two apricot cultivars. Physiologia Plantarum 111 (3): 313–321. DOI: https://doi.org/10.1034/j.1399-3054.2001.1110308.x

Hong-xia L., Zhi-yong X., Zeng-yan Z. 2011. Changes in activities of antioxidant-related enzymes in leaves of resistant and susceptible wheat inoculated with *Rhizoctonia cerealis*. Agricultural Sciences in China 10 (4): 526–533. DOI: https://doi.org/10.1016/S1671-2927(11)60033-3

International Rice Research Institute (IRRI). 1983. Field Problems of Tropical Rice. The Philippines, Manila, 172 pp.

Jaiti F., Verdeil J.L., El Hadrami I. 2009. Effect of jasmonic acid on the induction of polyphenoloxidase and peroxidase activities in relation to date palm resistance against *Fusarium oxysporum* f. sp. *albedinis*. Physiological and Molecular Plant Pathology 74 (1): 84–90. DOI: https://doi.org/10.1016/j.pmpp.2009.09.005

Kuzniak E., Sklodowska M. 2005. Fungal pathogen-induced changes in the antioxidant systems of leaf peroxisomes from infected tomato plants. Planta 222 (1): 192–200. DOI: https://doi.org/10.1007/s00425-005-1514-8

Liang Y.C., Chen Q., Liu Q., Zhang W.H., Ding R.X. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). Journal of Plant Physiology 160 (10): 1157–1164. DOI: https://doi.org/10.1078/0176-1617-01065

Livak K.J., Schmittgen T.D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ Method. Methods 25 (4): 402–408. DOI: https://doi.org/10.1006/meth.2001.1262

Ma K.W., Ma W. 2016. Phytohormone pathways as targets of pathogens to facilitate infection. Plant Molecular Biology 91 (6): 713–725. DOI: 10.1007/s11103-016-0452-0

Magbanua Z.V., De Moraes C.M., Brooks T.D., Williams W.P., Luthe D.S. 2007. Is catalase activity one of the factors associated with maize resistance to *Aspergillus flavus*? Molecular Plant-Microbe Interactions 20 (6): 697–706. DOI: https://doi.org/10.1094/MPMI-20-6-0697

Mandal S., Mitra A., Mallick N. 2008. Biochemical characterization of oxidative burst during interaction between *Solanum lycopersicum* and *Fusarium oxysporum* f. sp. *lycopersici*. Physiological and Molecular Plant Pathology 72 (1–3): 56-61. DOI: https://doi.org/10.1016/j.pmpp.2008.04.002

Mei C., Qi M., Sheng G., Yang Y. 2006. Inducible overexpression of rice Allene oxide synthase gene increases the endogenous jasmonic acid level, PR gene expression and host resistance to fungal infection. Molecular Plant-Microbe Interactions 19 (10): 1127–1137. DOI: https://doi.org/10.1094/MPMI-19-1127

Mishra S., Srivastava S., Tripathi R.D., Trivedi P.K. 2008. Thiol metabolism and antioxidant systems complement each other during arsenate detoxification in *Ceratophyllum de mersum* L. Aquatic Toxicology 86 (2): 205–215. DOI: https://doi.org/10.1016/j.aquatox.2007.11.001

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7 (9): 405–410. DOI: https://doi.org/10.1016/S1360-1385(02)02312-9

Na R., Luo Y., Bo H., Zhang J., Jia R., Meng Q., Zhou H., Hao J., Zhao J. 2018. Responses of sunflower induced by *Sclerotinia sclerotiorum* infection. Physiological and Molecular Plant Pathology 102: 113–121. DOI: https://doi.org/10.1016/j.pmpp.2017.12.004

Noctor G., Foyer C.H. 1998. Ascorbate and glutathione: keeping active oxygen under control. Annual Review of Plant Physiology and Plant Molecular Biology 49 (1): 249–279. DOI: 10.1146/annurev.arplant.49.1.249

Ou S.H. 1985. Rice Diseases. Slough, UK, Commonwealth Agricultural Bureau.

Pandey D., Rajendran S.R.C.K., Gaur M., Sajeesh P.K., Kumar A. 2016. Plant defense signaling and responses against necrotrophic fungal pathogens. Journal of Plant Growth Regulation 35 (4): 1159–1174. DOI: https://doi.org/10.1007/s00344-016-9600-7

Prabhu A.S., Filippi M.C.C. 2006. Brusone em arroz: controle genético, progresso e perspectivas. Embrapa Arroz e Feijão. Santo Antônio de Goiás, GO, p. 388.

Sayari M., Babaeizad V., Ghanbari M.A.T., Rahimian H. 2014. Expression of the pathogenesis related proteins, NH-1, PAL, and lipoxygenase in the Iranian Tarom and Khazar rice cultivars, in reaction to *Rhizoctonia solani* – the causal agent of rice sheath blight. Journal of Plant Protection Research 54 (1): 36–43. DOI: https://doi.org/10.2478/jppr-2014-0006

Sharma P., Jha A.B., Dubey R.S., Pessarakli M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of Botany 2012: 217037 DOI: http://dx.doi.org/10.1155/2012/217037

Shri M., Kumar S., Chakrabarty D., Kumar Trivedi P., Mallick S., Misra P. 2009. Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedlings. Ecotoxicology and Environmental Safety 72 (4): 1102–1110. DOI: https://doi.org/10.1016/j.ecoenv.2008.09.022

Tamaoki D., Seo S., Yamada S., Kano A., Miyamoto A., Shisido H., Miyoshi S., Taniguchi S., Akimitsu K., Gomi K. 2013. Jasmonic acid and salicylic acid activate a common defense system in rice. Plant Signaling & Behavior 8 (6): e24260. DOI: 10.4161/psb.24260

Tatagiba S.D., DaMatta F.M., Rodrigues F.A. 2016. Magnesium decreases leaf scald symptoms on rice leaves and preserves their photosynthetic performance. Plant Physiology and Biochemistry 108 (1): 49–56. DOI: https://doi.org/10.1016/j.plaphy.2016.07.002

Uphoff N., Dazzo F.B. 2016. Making rice production more environmentally-friendly. Environments 3 (12): 1–7. DOI: 10.3390/environments3020012

Velikova V., Yordanov I., Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Science 151 (1): 59–66. DOI: https://doi.org/10.1016/S0168-9452-(99)00197-1

Westernack C., Hause B. 2013. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in *annals* of botany. Annals of Botany 111 (6): 1021–1058. DOI: 10.1093/aob/mct067

Yoshida K., Kaothien P., Matsui T., Kawaoka A., Shinmyo A. 2003. Molecular biology and application of plant peroxidase genes. Applied Microbiology and Biotechnology 60 (6): 665–670. DOI: https://doi.org/10.1007/s00253-002-1157-7