**SUMMARY**

The rice variety Jiajing3768 is susceptible to neck blast at the booting stage (BS) but resistant at the full heading stage (FHS). This variety was used to analyze the inducible expression of defence genes and activities of antioxidant enzymes in the necks, at both the BS and FHS, after inoculation with *Magnaporthe oryzae*. We found that defence genes *PR1b* (pathogenesis-related class 1b), *PBZ1* (probenazole-inducible gene), *PAL* (phenylalanine ammonia-lyase), and *CHS* (chalcone synthase) may play roles in the resistance difference to neck blast between the BS and FHS in Jiajing3768. No significant differences were observed between the BS and FHS in the inducible expression of *PR1a*, *PR4*, and *JIOsPR10* (jasmonic acid induced PR 10). The antioxidant enzymes superoxide dismutase, peroxidase, catalase, glutathione reductase, and malondialdehyde coordinately participated in the stage-dependent resistance difference in Jiajing3768, and that oxidative damage is lower in the necks at the FHS than at the BS.

**Key words:** *Oryza sativa*, *Magnaporthe oryzae*, panicle stage, stage resistance, defence response.

Rice blast, caused by *Magnaporthe oryzae*, is the most devastating fungal disease of rice crops (Couch and Kohn, 2002). It has two forms, leaf blast and neck (or panicle) blast, the latter is the major cause of yield losses. In the field, there are three crucial stages that control neck blast: the booting stage (BS), the preliminary heading stage (PHS), and the full heading stage (FHS). Our unpublished work indicates that resistance to neck blast at the BS positively correlates with resistance to neck blast at the PHS or the FHS in many rice varieties. Nevertheless, a few varieties exhibit significantly higher or lower resistance to neck blast at one panicle stage. This form of resistance can be referred to as stage-dependent resistance the mechanism underlying it, however, remains largely unknown. Studies on rice resistance to neck blast mainly focus on the PHS (Pattama *et al.*, 2002; Tanee *et al.*, 2008), which is insufficient for investigating rice resistance in some varieties that exhibit stage-dependent resistance to this disease.

Plants defend themselves against pathogen challenges by the activation of defence response pathways (Staskawicz *et al.*, 1997). The recognition of pathogen avirulence gene products by plant resistance gene products leads to the rapid, coordinated expression of defence genes, whose products participate in fighting pathogen infection (Leo and Maarten, 2000). The speeds of activation and the expression levels of defence genes vary in different plant-pathogen interactions. Plants have also developed complex antioxidant defence systems that respond to biotic and abiotic stresses and mitigate the deleterious effects of reactive oxygen species (ROS) (Panda, 2007). The levels of ROS and the extent of oxidative damage depend largely upon the level of coordination among ROS-scavenging enzymes (Liang *et al.*, 2003). In this report, we examined the inducible expression of some known defence genes, the activities of some antioxidant enzymes, and malondialdehyde (MDA) levels in the necks at both the BS and FHS.

The rice variety Jiajing3768 (*Oryza sativa* subsp. *japonica*) is susceptible to neck blast at the BS but resistant at the FHS. Rice plants were grown under natural light in a greenhouse (20-30°C) for inoculation experiments. A 1×10⁵ conidia ml⁻¹ conidial suspension of the *M. oryzae* strain 06-257 (race ZG₃) was obtained according to Campbell and Ronald (2005).

The emerging panicles were collected at the BS and FHS (Rao *et al.*, 2005). Neck pieces 6 cm long, containing two or three nodes, were cut and placed onto the paper filters in glass dishes (10 cm diameter). The neck nodes were brushed with the conidial suspension and were incubated in a moist chamber at 26°C. The necks were collected at 1, 2, 3, 4, and 5 days post inoculation (dpi), frozen in liquid nitrogen and stored at -80°C. Necks treated with sterile water for 1, 2, 3, 4, and 5 days were used as controls.
The inducible expression of defence genes was analyzed by quantitative RT-PCR according to the manufacturer's instructions (ShineGene, China), using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, USA). TaqMan primers and probes were designed using the Primer Premier 3.0 software (Applied Biosystems, USA), according to gene sequences in the GenBank database (Table 1). PCR was carried out at 93°C for 2 min, followed by 40 cycles at 93°C for 5 sec (denaturation), and annealing at 60°C for 30 sec. The actin gene was used as the house-keeping gene.

Superoxide dismutase (SOD; EC 1.15.1.1), peroxi-

### Table 1. Primers and probes used for quantitative RT-PCR.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Accession*</th>
<th>Sequence</th>
<th>Product† (bp)</th>
</tr>
</thead>
</table>
| PR1a      | AJ278436   | F:5'-GGTGTCGAGAAGCAGTGTA-3'  
R:5'-GGCGAGATTTGAGGTAGGA-3'  
P:5'-CCGGAGGAGCTGTGC-3'      | 170          |
| PR1b      | U89895     | F:5'-AGGCGTTCGAGGAGAGACTA-3'  
R:5'-GAAGAGTTCGCGCAAAGGTT-3'  
P:5'-CAGCCAGAGGGCCAGACTG-3'   | 92           |
| PR4       | AY050642   | F:5'-CATATTACACACACACACAAAC-3'  
R:5'-GCACTCCCATGGGACCAAT-3'  
P:5'-TGGGACCTGAAAGGAGGCA-3'   | 71           |
| JIoPR10   | AF395880   | F:5'-GCAGGTCAGGAGAGGAATC-3'  
R:5'-GAAGAGTTCTGGGATGTA-3'   
P:5'-AGGACTCCTGCATGCTACATGC-3' | 68           |
| PBZ1      | D38170     | F:5'-ATGGCTACTATGGGCAAT-3'  
R:5'-CCTCTTCATCTAAGGGTGAT-3'  
P:5'-AGGACTCCTGTCGTCGACACCT-3' | 78           |
| PAL       | X16099     | F:5'-GGTGTTCGTGGATGTA-3'  
R:5'-GGGTGGTGCTTCAGTGT-3'   
P:5'-CAAGAGGAGGAAACCCGACCAC-3' | 73           |
| CHS       | AB000801   | F:5'-CGGCGGAAACTGCGTGAT-3'  
R:5'-CACATCTCTGGAACATCTCTC-3'  
P:5'-TAGAGATCAAGGAGGACAGCAT-3' | 101          |
| Actin     | X15865     | F:5'-GAGCTACGAGCTTCGATGGA-3'  
R:5'-CCTCAGGGCAGGGGAA-3'  
P:5'-AGGTATACCCCCATGGTGCTGAGC-3' | 65           |

*GenBank Accession numbers of genes; †Forward primers used for quantitative RT-PCR; ‡Reverse primers used for quantitative RT-PCR; ‡TaqMan probes used for quantitative RT-PCR; ‡Amplification product sizes of quantitative RT-PCR.

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**Fig. 1.** A. Necks of cv. Jiajing3768 at the booting stage, 10 days post-inoculation with *Magnaporthe oryzae* strain 06-257 (ZG1). Most of the necks display typical blast lesions with production of conidia, and etiolation. B. Necks of cv. Jiajing3768 at the full heading stage showing resistant-type lesions.
dase (POD; EC 1.11.1.7), catalase (CAT; EC 1.11.1.6), glutathione reductase (GR; EC 1.6.4.2), and MDA levels were measured with kits according to the manufacturer’s instructions (JianCheng, China).

Jiajing3768 showed significant differences between the BS and FHS in resistance to neck blast after inoculation with M. oryzae (Fig. 1). The inoculated necks were checked at 10 DPI, and the disease incidence, lesion length, and number of conidia in the necks at the BS were 97.1%, 48.5 mm, and 27,530 conidia ml⁻¹, respectively. The corresponding indexes at the FHS were 25.6%, 2.4 mm and 125 conidia ml⁻¹, respectively. We also confirmed that Jiajing3768 was susceptible to neck blast at the PHS (data not shown).

The expression levels of PR1a, JIOsPR10, PBZ1 and PAL were all significantly lower in the uninoculated necks at the BS than at the FHS (Fig. 2). At both stages, the PAL transcript showed the highest levels. The lowest levels were seen for the PR1a transcript at the BS, and the PR1b transcript at the FHS.

The induction of defence gene expression in the necks at the BS and FHS was analyzed at 1-5 dpi (Fig. 3). The PR1a transcript level increased until the 5th dpi at the BS, by only 1.3-fold compared with the control.

Although PR1a expression was up-regulated throughout the experimental period at the FHS, no significant differences were observed between the BS and FHS except at 5 dpi. OsPR1a, a rice acidic PR class 1 protein, is highly responsive to pathogen attack, wounding, and

![Fig. 2.](image)

**Fig. 2.** The expression of defence genes in the uninoculated necks at the booting stage (BS) and full heading stage (FHS) analyzed by quantitative RT-PCR. Transcript levels of defence genes were calculated relatively to the *actin* gene. Error bars represent the standard deviation of the mean from three independent experiments. Asterisks indicate a significant difference (*P*<0.05, *t*-test) in the necks between the booting and full heading stage within the same defence gene.

![Fig. 3.](image)

**Fig. 3.** Inducible expression of defence genes in the necks at the booting (BS) and full heading stage (FHS) in cv. Jiajing3768 after infection with the *Magnaporthe oryzae* strain 06-257 (ZG,) analyzed by quantitative RT-PCR. The necks were examined at 1, 2, 3, 4 and 5 days post-inoculation. Transcript levels of defence genes were normalized to those of the house-keeping gene *actin*. Expression levels of defence genes in inoculated samples were calculated relatively to those of controls treated with sterile water. Error bars represent the standard deviation of the mean from three independent experiments. Circles indicate a significant difference (*P*<0.05, *t*-test) between controls and inoculated samples. Asterisks indicate a significant difference (*P*<0.05) in the necks between booting and full heading stage at the same treatment time.
salicylic acid. The blast fungus infection can induce PR1α transcript accumulation in both compatible and incompatible interactions (Agrawal et al., 2000a). However, our data suggest that PR1α may not be involved in the resistance difference to neck blast in Jiajing3768 between the BS and FHS.

PR1β expression was up-regulated at both the BS and FHS during the entire duration of the experiment, except at 3 dpi at the BS. Interestingly, the inducible expression levels of PR1β at 3, 4 and 5 dpi compared to the level in control were significantly higher at the FHS than at the BS, indicating that this gene may play an important role in stage-dependent resistance to neck blast in Jiajing3768. The rice PR1β gene, which encodes a basic PR protein, is commonly used as a marker for systemic acquired resistance. The deduced amino acid sequence of OsPR1b has only 63.1% homology with the OsPR1a protein (Agrawal et al., 2000b), which may explain why PR1α and PR1β showed different expression patterns in this study.

OsPR4 was cloned from a differentially expressed cDNA library of rice leaves infected with the blast pathogen (Agrawal et al., 2003). In this report, PR4 expression was induced throughout most of the experimental period at both the BS and FHS, except at 1 dpi at the FHS. PR4 expression was highest at 2 dpi (2.1-fold) at the BS and at 4 dpi (2.3-fold) at the FHS, compared with the controls, respectively. This suggests that

Table 2. Activities of antioxidant enzymes and MDA levels in the uninoculated necks at the booting stage and full heading stage in Jiajing3768.

<table>
<thead>
<tr>
<th>Development stage</th>
<th>SOD (U mg⁻¹ protein)</th>
<th>POD (U mg⁻¹ protein min⁻¹)</th>
<th>CAT (U mg⁻¹ protein min⁻¹)</th>
<th>GR (U mg⁻¹ protein min⁻¹)</th>
<th>MDA (nmol mg⁻¹ protein)</th>
</tr>
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<tbody>
<tr>
<td>Booting stage</td>
<td>78.74 ± 3.18</td>
<td>58.16 ± 3.40</td>
<td>1.29 ± 0.62</td>
<td>17.13 ± 4.41</td>
<td>1.82 ± 0.17</td>
</tr>
<tr>
<td>Full heading stage</td>
<td>60.69 ± 11.36</td>
<td>64.71 ± 2.35</td>
<td>5.21 ± 1.00</td>
<td>21.22 ± 2.44</td>
<td>2.90 ± 0.32</td>
</tr>
</tbody>
</table>

Values represent the mean from three independent experiments ± standard deviation; * values represent a significant difference in the necks between the booting stage and full heading stage according to the Student’s t-test with P<0.05.

Fig. 4. SOD (a), POD (b), CAT (c) and GR (d) activities, and MDA levels (e) in the necks at the booting stage (BS) and full heading stage (FHS) in cv. Jiajing3768 after inoculation with Magnaporthe oryzae (DPI = days post-inoculation). Activities and levels are expressed relatively to controls (=100%, dashed line) at the respective times. Error bars represent the standard deviation of the mean from three independent experiments. Circles indicate a significant difference (P<0.05, t-test) between controls and inoculated samples. Asterisks indicate a significant difference (P<0.05) in the necks between the booting and full heading stage at the same treatment time.
PR4 may not be involved in the resistance difference between the BS and FHS to neck blast in Jiajing3768.

JIOsPR10, induced by treatment with jasmonic acid, salicylic acid, and pathogens, was discovered among five rice PR-10 genes (Kim et al., 2008). Our results suggest that JIOsPR10 had no effect on stage-dependent resistance to neck blast in Jiajing3768.

PBZ1 is induced by probenazole, and thus can be called OsPR10a (Liu and Ekramoddoullah, 2006). PBZ1 expression was up-regulated at both the BS and FHS throughout most of the experimental period, except at 1 dpi at the FHS. Furthermore, the inducible expression levels of PBZ1 at 4 and 5 dpi compared with the controls were higher at the FHS than at the BS, suggesting that PBZ1 could have an important function in the resistance difference to neck blast between the BS and FHS in Jiajing3768. Rapid induction and high levels of defence gene expression are necessary for plants to fight pathogens. In most cases, the inducible levels of gene expression in compatible interactions are lower than in incompatible interactions.

No significant induction of PAL expression was observed at the BS, but induction was significant at 4 dpi (3.6-fold) and 5 dpi (2.6-fold) at the FHS. However, CHS expression was down-regulated at the BS but up-regulated at the FHS throughout the experimental period. Therefore, we suggest that PAL and CHS could play important roles in stage-dependent resistance to neck blast in Jiajing3768. PAL is the first enzyme in the phenylpropanoid pathway, which has important functions in plants following exposure to environmental stresses and pathogen attack (Minami et al., 1989). CHS is the first enzyme in flavonoid and isoflavonoid biosynthesis, which is part of the phenylpropanoid pathway (Zabala et al., 2006), which may explain why PAL and CHS showed similar patterns of inducible expression after infection with M. oryzae at both growth stages.

CAT activity and MDA levels in the uninoculated necks were significantly higher at the FHS than at the BS (Table 2). SOD activity was lower at the FHS than at the BS. POD and GR had similar activities at both stages.

All antioxidant enzymes, except for SOD, showed different responses to M. oryzae in the necks at between the BS and FHS (Fig. 4). In active oxygen-scavenging systems, superoxide radicals generated in plants are converted to H2O2 by the action of SOD (Bowler et al., 1992). However, our data imply that SOD minimally participates in the resistance difference to neck blast between the BS and FHS in Jiajing3768 (Fig. 4 a).

POD is one of the most important enzymes active in elimination of ROS and catalyzes the oxidoreduction of various substrates using hydrogen peroxide. Many reports have suggested that POD plays a role in resistance to pathogens (Kawaoka et al., 2003; Caruso et al., 2001). POD activities increased slightly over controls at the FHS, but decreased at the BS (Fig. 4 b), suggesting that POD could be important in stage-dependent resistance to neck blast in Jiajing3768. POD and CAT, another major ROS-scavenging enzyme, showed similar trends (Fig. 4 c), thus these two enzymes might more efficiently clear ROS at the FHS than at the BS.

GR catalyzes the reduction of oxidized glutathione to reduced glutathione (GSH) with the accompanying oxidation of NADPH, and this reaction is important to the ascorbate (AsA)-GSH cycle, the main mechanism for the detoxification of ROS in plants (Noctor and Foyer, 1998). However, few studies have been done to analyze the functions of GR in plant-pathogen interactions. Our data indicate that Jiajing3768 exhibits similar changes in GR activity as POD, at both stages (Fig. 4 d), hinting that it may also play an important role in the resistance difference between the BS and FHS to neck blast in Jiajing3768.

Active oxygen radicals may induce chain-like peroxidation of unsaturated fatty acids in the membranes, leading to the formation of lipid peroxidation products such as malondialdehyde (MDA) (Mishra et al., 2008). However, no significant changes in MDA levels were observed in either stage, except that MDA was significantly higher at 2 dpi at the FHS than at the BS (Fig. 4 e). Therefore, we propose that POD, CAT and GR participate to different degrees in stage-dependent resistance to neck blast in Jiajing3768. As a result, in Jiajing3768 oxidative damage is lower in the necks at the FHS than at the BS after infection by M. oryzae.

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