Responses of Rice to *Rhizoctonia solani* and its Toxic Metabolite in Relation to Expression of *Osmyb*4 Transcription Factor

Singh POOJA and Subramanian BABU*

School of Bio Sciences and Technology, VIT University, Vellore, India
*Corresponding author: babu.s@vit.ac.in

Abstract

Pooja S., Babu S. (2017): Responses of rice to *Rhizoctonia solani* and its toxic metabolite in relation to expression of *Osmyb*4 transcription factor. Plant Protect. Sci., 53: 208–215.

The reaction of IR 50, TRY 3, and IR 36 cultivars of rice to *R. solani* challenge, the causal agent of sheath blight, and its toxic metabolite was studied. Differential response of these cultivars to the pathogen and/or toxin inoculation was observed in detached leaf sheaths and greenhouse-grown plants. The observations were based on disease scoring, electrolyte leakage, and also microscopic views of infection cushions. The *Osmyb*4 gene expression was studied in the tissues from all these experiments and a correlation between the level of expression and disease response of the varieties was found at least in some experiments. The mechanisms regulated by *Osmyb*4 might have a lower but significant contribution to the tolerance of rice cultivars to sheath blight.

Keywords: disease; *Oryza sativa*; resistance; *Rhizoctonia*; sheath blight; transcription factor

Sheath blight is a major fungal disease of rice caused by *Rhizoctonia solani* Kuhn leading to significant yield losses in all the rice growing regions of the world. Every year, sheath blight causes up to a 50% decrease in rice yield under favourable conditions (Groth 2008; Bernardes *et al.* 2009). In Asia, it affects approximately 15–20 million ha of irrigated rice fields and causes a yield loss of 6 million tons of rice grains per year (Bernardes *et al.* 2009). Until now the control measure practiced to combat the disease has been the use of fungicides. According to the reports, IR 50 is a variety known to be susceptible to sheath blight (Nandakumar 2002). TRY 3 (parentage: ADT43/Jeeraga samba) is a sheath blight tolerant variety released from Tamil Nadu Agricultural University, Coimbatore, India in 2010. IR 36 is a variety resistant to multiple diseases (Khush & Virk 2005) including blast, bacterial blight and viral disease. However, the molecular mechanisms behind the field observed

resistance of TRY 3 to sheath blight are unknown. With the availability of the whole genome sequence of rice and the advent of omics technologies, it is now possible to understand the mechanism of several complex physiological processes including disease resistance. While the pathogenic mechanism of *R. solani* is studied based on the whole genome sequencing of the fungus (Su'di *et al.* 2013; Zheng *et al.* 2013), on the other hand we attempted to study the role of rice transcription factors in sheath blight resistance. With the long-term objective of using the understanding in molecular breeding for sheath blight resistance, transcription factors are believed to be useful targets to investigate as they regulate and network many physiological processes in plants.

The rice *Osmyb*4 gene belonging to the R2/R3 subgroup of Myb transcription factors is known to be involved in response to both abiotic and biotic stresses. Homologous as well as heterologous overex-

pression of *Osmyb*4 in monocot and dicot plants have been reported to improve stress tolerance through direct or indirect activation/repression of several downstream genes. This includes expression in crops and plants like rice (Park *et al.* 2010), barley (Soltesz *et al.* 2012), *Arabidopsis* (Vannini *et al.* 2004, 2006; Mattana *et al.* 2005), tomato (Vannini *et al.* 2007), apple (Pasquali *et al.* 2008), and *Osteospermum ecklonis* (Laura *et al.* 2010). Docimo *et al.* (2013) performed an ectopic expression of *Osmyb*4 in tobacco and *Salvia sclarea* plants to produce specific bioactive compounds, the objective of which was different from the aforesaid studies on other plants. The *Osmyb*4 subfamily was found to be highly conserved among monocots and putative homologues are predicted in both monocots and dicots (Baldoni *et al.* 2013). They also observed *Osmyb*4 regulating its own expression by an autoregulatory mechanism. Altogether, the results of these studies indicate the pivotal role of *Osmyb*4 as a candidate gene for engineered stress tolerance/resistance in plants. It is obvious that most observations are oriented towards abiotic stress and the direct role of this gene in biotic stress response remains largely unknown.

We reported naturally existing levels of *Osmyb*4 expression in rice varieties relating to sheath blight and bacterial leaf blight resistant/susceptible genotypes (Singh *et al.* 2013). Recently, we reported an induction of this gene upon the sheath blight pathogen challenge and its correlation with other disease resistance genes like aminotransferase, ankyrin, and WRKY 12 (Pooja *et al.* 2015).

In this study, we used artificial inoculation of *Rhizoctonia solani*, the rice sheath blight pathogen and its toxic metabolite in rice plants to demonstrate the role of *Osmyb*4 in sheath blight pathogen response.

MATERIAL AND METHODS

Rice varieties and growth of seedlings. Rice (*Oryza sativa* L.) seeds of cultivars TRY 3 (tolerant to sheath blight), IR 50 (susceptible to sheath blight), and IR 36 (resistant to multiple diseases of rice excluding sheath blight) were obtained from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India. The seedlings were grown in germination paper in Hoagland's solution at room temperature for a period of two weeks. For a pot culture experiment, seedlings of the same varieties were raised in plastic pots (3.75 inches high and 5 nches at the

opening). The pots contained wetland soil with organic carbon 0.23%, pH 6.64, electrical conductivity of 0.06 dS/m (non-saline), and available N, P, K 168, 13.0, and 188 kg/ha, respectively.

Pathogen. *Rhizoctonia solani* Kuhn (strain RS 7, anastomosis group AG1, subgroup 1A) was obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The culture was maintained on potato dextrose agar (PDA).

Partial purification and inactivation of R. solani toxin. *R. solani* was grown in Richard's medium (50 g of sucrose, 10 g of KNO_3, 5 g of K_2HPO_4, 2.5 g of $MgSO_4$, 0.02 g of $FeCl_3$, and 1000 ml of distilled water, pH 7.0) in 500 ml Erlenmeyer flask by inoculating 4–5 mycelial discs (5 mm in diameter) grown in PDA and incubated at room temperature for 21 days. Mycelia were removed by filtering through Whatman filter paper No. 1 and the culture filtrate was treated with an equal volume of methanol, it was further stored overnight at 4°C. Further the filtrate was condensed to 15 ml volume in a rotary evaporator (Superfir™ Rotavap; Genser Scientific Instruments, Rothenburg ob der Tauber, Germany) at 65°C. Partial purification of the toxin was done with hexane, ethyl acetate, and chloroform in succession in a separating funnel. Gel permeation chromatography (Vidhyasekaran *et al.* 1997) was done for obtaining the partially purified toxin. The column contained Sephadex G-75 (Sigma Chemical Co., St. Louis, USA) as the gel matrix. Several 5-ml fractions were collected and the elutions corresponding to the peak absorbance were pooled and condensed in a rotary evaporator and reconstituted in sterile water. Chemical inactivation of toxin was done by mixing 2 ml of 35 mM potassium periodate with 10 ml of the partially purified toxin and incubated at 5°C for 22 hours. Further, the mixture was dialysed against sterile water for 24 hours.

Electrolyte leakage study. Rice sheath tissue pieces of cultivars TRY 3, IR 50, and IR 36 (2 mm size) were prepared from 45-days-old plants and tied in muslin cloth (100 mg/cloth bag). Three sets of the tubes were made each containing 3 ml of active toxin, inactive toxin, and water (control). Tissue pieces were infiltrated *in vacuo* for 30 min at room temperature followed by rinsing in distilled water several times and further leaching against 10 ml of distilled water for 45 minutes. The solution was used to evaluate the mean values of conductivity (μS/cm) at 0, 15, and 30 min (Vidhyasekaran *et al.* 1997). The experiment was performed with ten replications.

Artificial inoculation and disease scoring. A sheath blight infection assay was performed in de-

tached sheath as well as greenhouse-grown plants. Seedlings were raised in pot culture as described by Sɪɴɢʜ *et al.* (2013). For the detached sheath bioassay, sheaths were obtained from the actively growing tillers of 45-days-old plants. Inoculation of the pathogen was done by the pin prick method on the surface of the detached sheath and sheath portions of greenhouse-grown plants. Mycelial discs were cut from the active growing edges of *R. solani* culture in PDA plates using a cork borer.

Detached sheath bioassay with mycelial disc – Rice sheaths collected from 45-days-old plants of all the three varieties were uniformly cut into 7 cm. Each sheath was placed on a glass slide and its end was fixed with tape to avoid the disturbance of the sheath position. An injury was made using a sterile needle and the mycelial disc was placed over it. Plain PDA disc without culture served as control. The slides were kept in sterile Petri plates lined with 3 layers of Whatman filter paper No. 1 wetted with sterile water. The Petri plates were incubated at room temperature under laboratory conditions (12 h light and 12 h of darkness ± 2). Ten sheaths (replications) were used per treatment per variety and the entire experiment was done twice.

Detached sheath bioassay with active/inactive toxin – The experiment set up and disease scoring were similar as described for the bioassay done with mycelial disc. Instead of the mycelial disc, a volume of 50 μl of toxin (50 μg glucose equivalence) and inactive toxin (used to check the effect on *Osmyb*4 expression) were placed on the needle pricked region of the sheath. Inoculation with sterile water served as control. Ten sheaths (replications) were used per treatment per variety and the entire experiment was done twice.

Bioassay in greenhouse-grown plants – Seedlings were grown in a greenhouse for four weeks at 28–30°C under natural light conditions. Artificial inoculation using *R. solani* mycelial discs was done on replicated sets (ten plants for each variety and two sheaths per plant). Moisture was maintained everyday by applying sterile distilled water at the site of infection. Plants were covered with polythene bags sprayed with sterile water 24 h before and after inoculation.

Disease scoring was done following the standard protocol (Nᴀɴᴅᴀᴋᴜᴍᴀʀ *et al.* 2002) at 120 h and percentage disease index (PDI) was calculated based on the grades of 0–9 scale. For every treatment, scoring was done on two sheaths per plant on ten plants. Twenty score points were totalled to get the total grade points:

Disease index = (Total grade point/No. of sheath observed)
× (100/Max. grade)

Observation of infection cushions. The experimental setup was similar as described for the bioassay done with *R. solani* mycelial discs. A region of 2.5 cm long each from the infected and uninfected sheath from the three cultivars TRY 3, IR 50, and IR 36 was cut and the epidermal layer along with tissues was dissected using a sterile needle. This was stained with 0.5% trypan blue and placed on a glass slide. The number of infection cushions was counted in each square at 100× using a light microscope and reported as the number of infection cushions per cm^2 (Cʜᴏᴘᴘᴀᴋᴀᴛʟᴀ *et al.* 2006).

Semi-quantitative RT-PCR analysis. After disease scoring (120 h after inoculation) in the detached sheaths as well as sheaths of greenhouse-grown plants, a region of 1 cm long each from above and below the pin pricked site of infected and uninfected sheaths was cut from all samples and frozen for the gene expression analysis. Tissue samples collected from ten sheaths for each treatment and variety were pooled and ground to a fine powder using liquid nitrogen. Total RNA was isolated from all preserved samples of the infection assay experiment, using the Raflex total RNA isolation kit (Genei, Bangalore, India) as per the manufacturer's protocol. Semi-quantitative reverse transcription PCR was carried out following the protocol as we already reported (Sɪɴɢʜ *et al.* 2013) with the following primers: forward primer – 5'GCGTCGACTATGAAGCGGAAGCGGCCGGC'3 and reverse primer – 5'CGCGGATCCGCTTACT-GTTTGGATTCCATGA'3. Amplification of the *Osmyb*4 gene was carried out with initial denaturation at 95°C for 2 min and 20 cycles of denaturation at 95°C for 1 min, 48°C annealing for 1 min and 72°C extension for 1 minute. The rice actin gene was used as an internal control and amplification was performed using the primers 5'GTATCCATGAGACTACATA-CAAC'3 and 5'TACTCAGCCTTGGCAATCCACA'3 with an annealing temperature of 54°C. The experiment was repeated twice to confirm the results.

Statistical analysis. Statistical analysis was performed using ANOVA and the results of infection assays and electrolyte leakage study were expressed as mean ± SD with error bars in the graphs. $P < 0.05$ implies significance.

RESULTS

Infection assay on detached sheath. Artificial inoculation using the mycelial discs of *R. solani* was done

on three varieties, TRY 3, IR 50, and IR 36 (Figure 1A). Cv. IR 50 recorded a higher percentage disease index compared to cvs TRY 3 and IR 36 (Figure 1B). A similar experiment was performed with active and inactive toxin for inoculation and the PDI was calculated (Figure 2). Although the cultivar differences in response to active toxin were not drastic in terms of the PDI, inoculation with inactive toxin revealed cultivar differences. Overall, inoculation with inactive toxin recorded very negligible lesions and correspondingly the lower PDI. Inoculation with the active toxin of *R. solani* resulted in a PDI of 17.66 in cv. IR 50 where the inoculation of inactive toxin in the same variety recorded PDI 0.70. The sheath blight PDI was 0.35 and 0.62 in cvs TRY 3 and IR 36, respectively, when inoculated with inactive toxin.

Electrolyte leakage study. The ability to cause the leakage of electrolytes from rice sheath tissues was studied with the three cultivars TRY 3, IR 50, and IR 36 using active and inactive toxin with water as control (Figure 3). The conductivity was measured at 0, 15, and 30 minutes. Active toxin showed rapid electrolyte 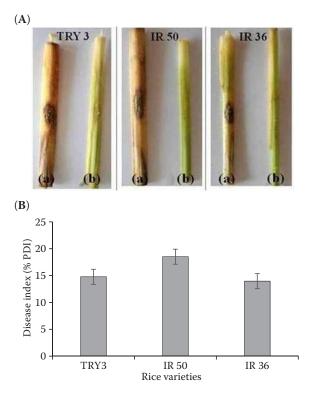leakage as compared to inactive toxin and control where the cv. TRY 3 showed a maximum with 2.95 µS/cm followed by cvs IR 50 and IR 36 with the lowest leakage. Inactive toxin was able to cause maximum electrolyte leakage in the cv. IR 50 (1.17 µS/cm) followed by cv. IR 36 and the lowest leakage was exhibited by cv. TRY 3.

Observation of infection cushions. Development of *R. solani* hyphae on the inoculated sheath tissue of the rice cultivars was microscopically observed and the number of infection cushions produced by the fungus was counted. In general, the growth of the hyphae was observed to be random (Figure 4). The severity of the infection could be correlated with the number of infection cushions formed, i.e. the susceptible cv. IR 50 showed the maximum number of infection cushions (225 cm^{-2}) followed by tolerant/resistant cv. IR 36 (218 cm^{-2}), and cv. TRY 3 (185 cm^{-2}) after 72 h of infection.

Infection assay in greenhouse-grown plants. Artificial inoculation with *R. solani* in the sheaths resulted in the PDI of 23.44 for cv. IR 50. The PDI values of the other two varieties TRY 3 and IR 36 were found to be 16.03 and 12.96, respectively. The results of the experiment are shown in Figure 5.

(**A**)

(**B**)

Figure 1. Infection assay on detached sheaths of different varieties using *R. solani* mycelial discs: (**A**) Symptom development on stems inoculated with *R. solani* mycelial discs (a) and plain agar discs (b); (**B**) Disease severity of the rice varieties upon inoculation with *R. solani* mycelial discs
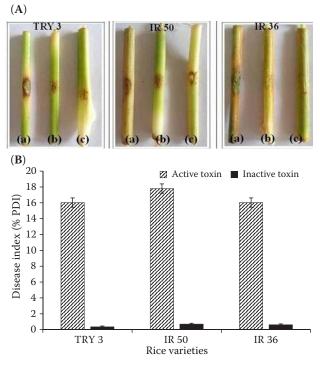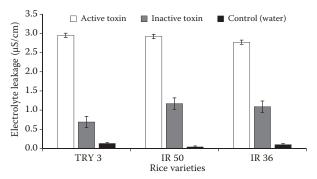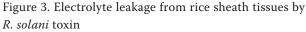
(**A**)

(**B**)

Figure 2. Infection assay on detached sheaths of different varieties using *R. solani* active or inactive toxin: (**A**) Sheath blight lesions produced on different varieties inoculated with active toxin (a), inactive toxin (b), and sterile water (c); (**B**) Disease severity in rice varieties upon treatment with *R. solani* active or inactive toxin
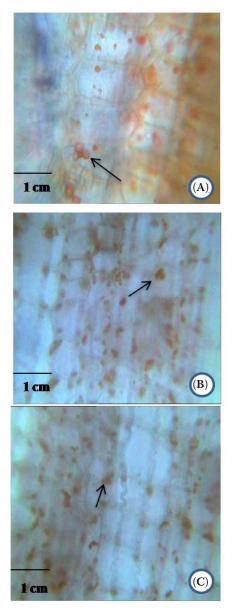
Figure 3. Electrolyte leakage from rice sheath tissues by *R. solani* toxin

Figure 4. Microscopic view (100×) of infection cushions developed on detached sheats of three cultivars: Lateral sections of sheath tissues of (**A**) TRY 3, (**B**) IR 50, and (**C**) IR 36 showing *R. solani* infection cushions

***RT-PCR analysis of Osmyb*4 *expression*. *Osmyb*4 was not found to be expressed in the control sheaths of IR 50, whereas after *R. solani* infection the variety showed the gene expression although it was lower as compared to the other two cultivars. On the other hand, cv. TRY 3 was observed to have the expression of the gene in both control and infected sheaths, but the expression was found to be higher with infection. There was not a large difference observed in the expression level of this gene and it was found to be more or less similar in cv. IR 36, with or without infection (Figures 6a–d). The RT-PCR analysis done on RNA samples obtained from the infection assay experiment using toxin revealed varietal differences in *Osmyb*4 expression (Figures 6e–j). The TRY 3 variety displayed a reduction in the expression level of the gene. Comparing the inoculation of inactive toxin and active toxin, similar results were obtained for cv. IR 36. In cv. IR 50, inactive toxin was able to induce
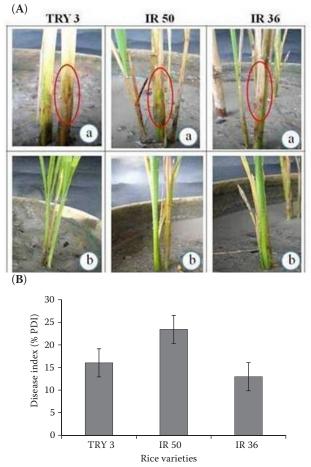

Figure 5. Pathogenicity assay on intact plants grown under greenhouse conditions: (**A**) Sheath blight symptoms on stems of different varieties inoculated with *R. solani* (a) or uninoculated (b); (**B**) Disease severity of different varieties upon artificial inoculation
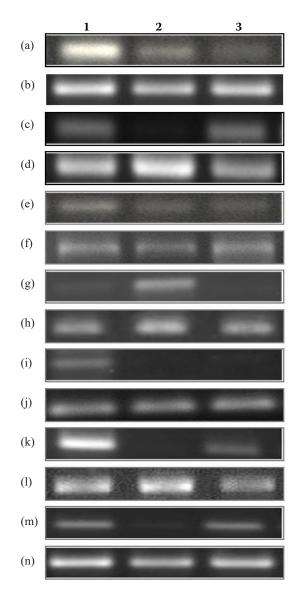
Figure 6. *Osmyb*4 expression analysis in rice cultivars under different experimental conditions

Lane 1 –TRY 3; Lane 2 – IR 50; Lane 3 – IR 36; (a) Detached sheath inoculated with *R. solani* mycelial disc (*Osmyb*4); (b) Detached sheath inoculated with *R. solani* mycelial disc (rice actin); (c) Detached sheath inoculated with plain agar disc (*Osmyb*4); (d) Detached sheath inoculated with plain agar disc (rice actin); (e) Detached sheath inoculated with *R. solani* active toxin (*Osmyb*4); (f) Detached sheath inoculated with *R. solani* active toxin (rice actin); (g) Detached sheath inoculated with *R. solani* inactive toxin (*Osmyb*4); (h) Detached sheath inoculated with *R. solani* inactive toxin (rice actin); (i) Detached sheath inoculated with sterile water (*Osmyb*4); (j) Detached sheath inoculated with sterile water (rice actin); (k) Greenhouse-grown plants inoculated with *R. solani* (*Osmyb*4); (l) Greenhouse-grown plants inoculated with *R. solani* (rice actin); (m) Greenhouse-grown plants – uninoculated control (*Osmyb*4); (n) Greenhouse-grown plants – uninoculated control (rice actin)

the expression of the gene which was not found in the case of active toxin inoculation. *Osmyb*4 expression was induced in the greenhouse-grown cv. TYR 3 plants upon inoculation with *R. solani* compared to other varieties (Figures 6 h–n). The expression level was undetectable in cv. IR 50 in both inoculated and uninoculated plants.

DISCUSSION

Homologous expression in rice was found to produce a similar induction of stress tolerance like in heterologous species (Park *et al.* 2010). In previous work, we demonstrated that the naturally existing levels of the *Osmyb*4 gene expression in rice seedlings correlate with the known genotypic reaction of rice cultivars to sheath blight disease (Singh *et al.* 2013). The susceptible IR 50 variety had a lower level of expression than cvs TRY3 and IR 36.

In the present study, we have explored the reaction of these varieties to *R. solani* challenge, the causal agent of sheath blight, and its toxic metabolite. Differential response of these cultivars to the pathogen and/or toxin inoculation was observed in detached leaf sheaths and greenhouse-grown plants. The observations were based on disease scoring, electrolyte leakage, and also microscopic views. The *Osmyb*4 gene expression was studied in the tissues from all these experiments and a correlation between the level of expression and disease response of the varieties was found at least in some experiments.

The clear statistical difference observed between varieties in reaction to the inoculation of mycelial discs of *R. solani* in either detached sheaths or greenhouse-grown plants was not seen in the experiments with toxin. The results of mycelial disc inoculation studies were also supported by the observations on infection cushions of *R. solani* in the inoculated tissues. The number was higher in susceptible cv. IR 50 than in the other two varieties. These results indicate that either the inoculation of toxin does not exactly mimic the pathogen infection or the quantity of toxin (we used 50 µl containing 50 µg glucose equivalents) used in the study was not sufficient to induce any response in rice tissues.

The gene expression analysis in detached sheaths inoculated with mycelial discs of *R. solani* revealed interesting results. TRY 3 and IR 36 varieties exhibited the gene expression even without pathogen challenge. Increased expression upon challenging

with the pathogen was observed only in cv. TRY 3 compared to cv. IR 36. IR 36 is a variety which is known for its resistance to multiple diseases including blast, bacterial leaf blight, grassy stunt, and tungro (Khush & Virk 2005). This variety was selected in our study to compare sheath blight resistant cv. TRY 3 with a non-resistant variety in addition to a susceptible variety. The difference observed between cvs TRY 3 and IR 36 with respect to the *Osmyb*4 expression indicated that *Osmyb*4 mediated resistance may be specific to sheath blight. The reason behind observed resistance to sheath blight in cv. IR 36 in the artificial inoculation studies needs further investigations. Cv. IR 50 showed the gene expression after infection and the level was comparable with the uninoculated cvs TRY 3 and IR 36. In other words, *Osmyb*4 is induced in a susceptible variety by the pathogen infection to a level which is similar to the natural level in a tolerant variety. The amount of *Osmyb*4 transcripts in rice plants determines the susceptibility or resistance to *R. solani.*

In the experiment with toxin inoculation, the expression of *Osmyb*4 remained more or less unaltered in TRY 3 both in toxin inoculated and uninoculated sheaths. Inactive toxin also suppressed the expression of the gene. This correlates with our results in electrolyte leakage and disease scoring experiments involving toxin. In addition, inactive toxin was found to elicit the expression of *Osmyb*4 in the susceptible IR 50 variety. Based on these two experiments it can be concluded that *R. solani* mycelia as well as its toxin are able to induce *Osmy*b4 transcripts in detached sheaths of susceptible variety, however to a level which is not sufficient to demonstrate a tolerance or resistance reaction. In the whole plants (greenhouse experiment), there was no detectable gene expression in cv. IR 50 either with or without pathogen challenge indicating that the gene could have a role in resistant varieties and not in susceptible varieties. The results from the greenhouse-grown plants were similar to those observed in detached sheath experiment (inoculated with mycelial discs). Overall, considering the gene expression results in detached sheaths and greenhouse plants inoculated with mycelial discs, in the experiments which closely resemble natural infection in field conditions compared to toxin experiments there is a clear induction of the gene in the sheath blight tolerant variety TRY 3 upon pathogen challenge.

Since electrolyte leakage is a measure of the effect of *R. solani* toxin on rice plants and an indicator of susceptibility/resistance (Vidhysekaran *et*

al. 1997; Sriram *et al.* 2000), we tried to observe electrolyte leakage in the varieties under study and also the expression of *Osmyb*4 upon toxin treatment. Although the results obtained with toxin inoculation and electrolyte leakage are not convincing, it also leads to an understanding that the *Osmyb*4 mediated resistance might involve a direct effect on the pathogen rather than its toxic metabolite. In other words, *Osmyb*4 mediated sheath blight resistance might not be based on a detoxification mechanism. This is supported by our recent observation on the induction of *Osmyb*4 along with aminotransferase and ankyrin proteins which have a direct role in pathogen resistance through the cell wall thickening and callose deposition (Pooja *et al.* 2015).

The mechanisms regulated by *Osmyb*4 might have a significant contribution to the tolerance of rice cultivars to sheath blight. Vannini *et al.* (2004) reported that the expression level of the *Osmyb*4 transcription factor is known to be low in normal conditions and strongly induced at 4°C. The natural promoter of this gene in rice genome is cold inducible. Sheath blight is a major disease in tropical rice grown under hot humid weather. The low level of the natural expression of *Osmyb*4 could be one of the reasons why most *indica* varieties are susceptible to this disease. This can also be interpreted as follows: the cold inducible nature of the gene could be the reason why sheath blight is not a serious disease in temperate countries/regions. From this point of view, the engineered expression of *Osmyb*4 by replacing the cold inducible promoter with a constitutively expressing promoter remains a valid strategy for developing a transgenic rice variety with sheath blight resistance.

References

Baldoni E., Genga A., Medici A., Coraggio I., Locatelli F. (2013): The *Osmyb*4 gene family: stress response and transcriptional auto-regulation mechanisms. Biologia Plantarum, 57: 691–700.

Bernardes-de-Assis J., Storari M., Zala M., Wang W., Jiang D., Shidong L., Jin M., McDonald B.A., Ceresini P.C. (2009): Genetic structure of populations of the rice-infecting pathogen *Rhizoctonia solani* AG-1 1A from China. Phytopathology, 99: 1090–1099.

Choppakatla V., Melouk H.A., Hunger R.M. (2006): Infection cushion formation by *Rhizoctonia* spp. on peanut and wheat root systems. Phytopathology, 96: S24.

Docimo T., Mattana M., Fasano R., Consonni R., de Tommasi N., Corragio I., Leone A. (2013): Ectopic expression of the *Osmyb*4 rice gene enhances synthesis of hydroxycinnamic acid derivatives in tobacco and clary sage. Biologia Plantarum, 57: 179–183.

Groth D.E. (2008): Effects of cultivar resistance and single fungicide application on rice sheath blight, yield and quality. Crop Protection, 27: 1125–1130.

Khush G.S., Virk P.S. (2005): IR Varieties and their Impact. Los Banos (Philippines), International Rice Research Institute. Available at http://books.irri.org/9712202062_content.pdf

Laura M., Consonni R., Locatelli F., Fumagalli E., Allavena A., Coraggio I., Mattana M. (2010): Metabolic response to cold and freezing of *Osteospermum ecklonis* overexpressing *Osmyb*4. Plant Physiology and Biochemistry, 48: 764–771.

Mattana M., Biazzi E., Consonni R., Locatelli F., Vannini C., Provera S., Coraggio I. (2005): Overexpression of *Osmyb*4 enhances compatible solute accumulation and increases stress tolerance of *Arabisopsis thaliana*. Physiologia Plantarum, 125: 212–213.

Nandakumar R., Babu S., Radjacommare R., Raguchander T., Samiyappan R. (2002): *Pseudomonas fluorescens* mediated antifungal activity against *R. solani*, sheath blight pathogen. Phytopathologia Mediterrania, 41: 109–119.

Park M.R., Yun K.Y., Mohanty B., Herath V., Xu F., Wijaya E., Bajic V.B., Yun S.J., De Los Reyes B.G. (2010): Supra optimal expression of the cold-regulated *Osmyb*4 transcription factor in transgenic rice changes the complexity of transcriptional network with major effects on stress tolerance and panicle development. Plant Cell and Environment, 33: 2209–2230.

Pasquali G., Biricolti S., Locatelli F., Baldoni E., Mattana M. (2008). *Osmyb*4 expression improves adaptive responses to drought and cold stress in transgenic apples. Plant Cell Reports, 27: 1677–1686.

Pooja S., Kumar S., Mohanapriya A., Sundandiradoss C., Siva R., Gothandam K.M., Babu S. (2015): Homotypic clustering of OsMYB4 binding site motifs in promoters of the rice genome and cellular-level implications on sheath blight disease resistance. Gene, 561: 209–218.

Singh P., Siva R., Gothandam K.M., Babu S. (2013): Naturally existing levels of *Osmyb*4 gene expression in rice cultivars correlates with their reaction to fungal and bacterial pathogen. Journal of Phytopathology, 161: 730–734.

Soltesz A., Vagujfalvi A., Rizza F., Kerepesi I., Galiba G., Cattivelli I., Coraggio I., Crosatti C. (2012): The rice *Osmyb*4 gene enhances tolerance to frost and improves germination under unfavorable conditions in transgenic barley plants. Journal of Applied Genetics, 53: 133–143.

Sriram S., Raghuchander T., Babu S., Nandkumar R., Shanmugam V., Vidhyasekaran P., Balasubramanian P., Samiyappan R. (2000): Inactivation of phytotoxin produced by rice sheath blight pathogen *Rhizoctonia solani*. Canadian Journal of Microbiology, 46: 520–524.

Su'udi M., Park J.M., Kang W.R., Hwang D.J., Kim S., Ahn I.P. (2013): Quantification of rice sheath blight progression caused by *Rhizoctonia solani*. Journal of Microbiology, 51: 380–388.

Vannini C., Locatelli F., Bracale M., Magnani E., Marsoni M., Osnato M., Mattana M., Baldoni E., Coraggio I. (2004): Overexpression of the rice *Osmyb*4 gene increases chilling and freezing tolerance of *Arabidopsis thaliana* plants. Plant Journal, 37: 115–127.

Vannini C., Iriti M., Bracale M., Locatelli F., Faoro F., Croce P., Pirona R., Maro A.D., Coraggio I., Genga A. (2006): The ectopic expression of the rice *Osmyb*4 gene in *Arabidopsis* increases tolerance to abiotic, environmental and biotic stress. Physiological and Molecular Plant Pathology, 69: 26–42.

Vannini C., Campa M., Iriti M., Genga A., Faoro F., Carravieri S., Rotino G.L., Rossoni M., Spinardi A., Bracale M. (2007): Evaluation of transgenic tomato plants ectopically expressing rice *Osmyb*4 gene. Plant Science, 173: 231–239.

Vidhyasekaran P., Ponmalar R., Samiyappan R., Velazhahan R., Vimala R., Ramanathan A., Paranidharan V., Muthukrishanan S. (1997): Host-specific toxin production by *Rhizoctonia solani*, the rice sheath blight pathogen. Phytopathology, 87: 1258–1263.

Zheng A., Lin R., Zhang D., Qin P., Xu L., Ai P., Ding L., Wang Y., Cheng Y., Liu Y., Sun Z., Feng H., Liang X., Fu F., Tang C., Li Q., Zhang J., Xie Z., Deng Q., Li S., Wang S., Zhu J., Wang L., Liu H., Li P. (2013): The evolution and pathogenic mechanisms of the rice sheath blight pathogen. Nature Communications, 4: 1424.