RESEARCH ARTICLE

Microarray Analysis of bacterial blight resistance 1 mutant rice infected with Xanthomonas oryzae pv. oryzae

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ABSTRACT We analyzed the transcriptional profile of the Xoo infected bbr1 mutant using a commercial rice gene chip containing 51,279 transcripts. Microarray revealed 92 genes with increased levels of expression and 22 genes with decreased levels of expression in bbr1. Some of the differentially expressed genes were validated by qRT-PCR. Higher expression of defense-related genes and AP2 domain containing transcription factors along with lower expression of reactive oxygen scavenging enzymes may be responsible for defense signaling in the bbr1 upon Xoo infection. The putative target genes of AP2 domain containing transcription factors also showed differential gene expression during Xoo infection, some of which encoded bacterial pathogen resistance-related protein. Induction of AP2 domain containing transcription factors along with up-regulation of their putative target genes during Xoo infection may inhibit pathogen spread in the bbr1. This observation supports the hypothesis that AP2 domain containing transcription factors is involved in the regulation of differentially expressed genes in bbr1.

Keywords Bacterial blight resistant rice, Gamma-ray mutant, Xanthomonas oryzae pv. oryzae, ERF and DREB/CFB

INTRODUCTION

Rice (Oryza sativa L.) is the most important crop in the world as it feeds half of the world’s population. The stable production of rice is affected by biotic and abiotic stress. The rice diseases caused by plant pathogenic fungi, bacteria and viruses are capable of causing heavy loss on rice crop, the global yields of which is annually decreasing by 10-15% (Dai et al. 2007). The use of resistant cultivars is one of the most important factors used to control diseases.

Because worldwide rice production has been severely affected by bacterial blight (BB) caused by Xanthomonas oryzae pv. oryzae (Xoo), early research efforts have been focused on the utilizing disease resistance (R) genes in rice. Since the first cloning of the R gene Xa21 which confers resistance to Xoo almost twenty years ago (Song et al. 1995), nearly 30 major R genes for resistance to Xoo have been identified (Kurata and Yamazaki 2006) and five of them: Xa1, Xa3/Xa26, xa5, xa13 and Xa27, have been cloned: (Yoshimura et al. 1998; Iyer and McCouch 2004; Sun et al. 2004; Gu et al. 2005; Chu et al. 2006; Jiang et al. 2006). Genetic studies of BB resistance have resulted in the development of donor lines carrying major R genes. A number of these donor lines have been used in rice breeding programs around the world. Using transgenic approach, agronomically important cultivars such as IR64 and IR72 have been transformed with the Xa21 gene and field trials of selected lines were successfully undertaken in China (Zhang et al. 1998; Tu et al. 2000). Further, the Xa21 gene has been introduced into a widely used restorer of hybrid rice in China, Minghui 63, in order to produce BB-resistant hybrid rice with elite agronomic characters (Zhai et al. 2002).

Mutation breeding is also an important tool for crop improvement. During the past 75 years, more than 3,100
mutant varieties have been produced worldwide (The FAO/IAEA Mutant Varieties Database, http://mvgs.iaea.org/AboutMutantVarities.aspx). More than 501 new varieties in rice have been obtained by applying different mutagenic agents. In Asia Pacific, there are approximately 343 mutants of rice released (Ahloowalia et al. 2004).

DNA microarray can measure the individual transcript level of tens of thousands of genes simultaneously, thus providing a high-throughput means for analyzing gene expression levels at the whole-genome scale (Schena et al. 1995; Chu et al. 1998) that may help elucidate the network of defense response. For example, microarray has been used to characterize the rice-pathogen interaction, such as the interaction of rice-Xoo (Li et al. 2006; Kottapalli et al. 2007), -flagellin (Fujisawa et al. 2004), -lipopolysaccharides (Desaki et al. 2006), -fungal elicitor (Kim et al. 2005), -fungal elicitor (Kim et al. 2005), -rice dwarf virus (Shimizu et al. 2007) and -plant hopper (Cho et al. 2005).

In previous study, six rice mutant lines (M5 generation); TILL300-534, TILL300-537, TILL300-1212, TILL300-793, TILL300-693 and TILL300-651, which present resistant phenotype against Xoo KXO85 were selected from 3000 lines of gamma-ray mutated M3 plants (Lim et al. 2010). Among selected six mutant lines TILL300-651 (Bacterial Blight Resistance 1; bbr1) showed relatively strong induction of rice R gene Xa21 which confers resistance to Xoo and other rice R genes such as Pi36 and Pi-Ta against Magnaporthe oryzae (Lim et al. 2010). To further understand defense response in the bbr1-Xoo interaction, we profiled the expression of rice genes involved in the phenotype of rice mutant, bbr1, using a commercial rice gene chip containing 51,279 transcripts representing two rice cultivars, indica and japonica.

**MATERIALS AND METHODS**

**Plant materials and pathogen inoculation**

‘Dongan’ seeds (Oryza sativa L. cv. japonica) were allowed to imbibe water overnight at 22°C and kept on moistened filter paper until germination. Germinated seeds were grown in soil in a greenhouse for 40 days prior to Xoo inoculation.

Bacterial cells of Xoo were suspended in sterile distilled water and the inoculation concentration was adjusted to OD600 = 1.0 (Song et al. 1995). Rice leaves were inoculated with the bacterial suspension using the leaf clipping method (Kauffman et al. 1973). For gene expression analysis, leaf samples were collected from untreated control and infected leaves at 10 days after inoculation. The samples were flash frozen in liquid nitrogen and stored at -80°C.

**RNA Isolation and qRT-PCR analysis**

Total RNA was isolated from the collected samples using RNasey mini kit (Qiagen) according to the manufacturer’s instruction. Approximately 1 µg DNA-free RNA was used for first-strand cDNA synthesis using the Moloney Murine Leukemia Virus (M-MuLV) reverse transcriptase for quantitative real-time polymerase chain reaction (qRT-PCR; Fermentas). The qRT-PCR reactions were performed using a Thermal Cycler Dice Real Time System TP850 (TaKaRa, http://www.takara-bio.com) and SYBR Premix Ex Taq (TaKaRa). Primer sets prepared at 0.1 µM final concentration were used for a final volume of 25 µL. The thermal profile of the qRT-PCR reactions was 10 min at 95°C, 40 cycles of 5 s at 95°C/20 s at 60°C. Subsequently, a dissociation curve was generated. All reactions were carried out in triplicate. Primers used for qRT-PCR are listed in the Table 1.

**Microarray analysis**

The microarray experiment was conducted by the DNA Link Corporation (Seoul, Korea) according to the Affymetrix technical manual (http://www.affymetrix.com/support/index.affx). Total RNAs were extracted from the samples stored at -80 °C and gene expression was compared between the wild type ‘Dongan’ and bbr1 mutant with two replicates. Hybridization, washing, staining and scanning procedures were performed as described in the Affymetrix technical manual. The analysis software was the Affymetrix Command Console, R affy-package (2.9.2), Expression Console1.1, DAVID.
Table 1. Summary table displaying Arabidopsis orthologous of differentially expressed genes with known roles in disease resistance (R- ), transcript regulation (TF- ) and oxidative stress (POD- ) and sequences of forward and reverse primers used in quantitative RT-PCR to validate the 17 selected gene expression changes determined by microarray analysis. TF, Transcription factor; R, Resistance; POD, Peroxidase.

| Primer name | Gene title | Arabidopsis orthologous | Forward-primer | Reverse-primer |
|-------------|------------|-------------------------|----------------|---------------|
| TF-1        | Os02g0527300 | AT2G26150               | gttgcaactagtcaagca | tacctcccaagctgccttt |
| TF-2        | Os06g0127100 | AT4G25480               | ctacgcgtactagcgaac | gaggacagcaagtgtggag |
| TF-3        | Os08g0474000 | AT4G34410               | gagaacaggagaccctct | ttcaattagacacagccttaa |
| TF-4        | Os04g0583900 | AT5G37260               | ccacacaaacaggagtgg | tggattcataagagccttt |
| TF-5        | Os03g0327800 | AT3G04070               | cgatgtctcgaatctgc | ccggctttatgatcttgac |
| TF-6        | Os07g0558100 | AT4G21440               | gcacaacacacacagctca | aagtctcgatcagcctgg |
| TF-7        | Os01g0975300 | AT5G59780               | cagcagagggagctgctg | gcgaataacccggagcag |
| TF-8        | Os01g0141000 | AT1G13260               | atcagctctcctgttca | tgcattgacacacagcaaa |
| R-1         | Os01g0944900 | AT4G16260               | gtttactacccggagcctaa | atgcagctagtgctgcttg |
| R-2         | Os02g0194700 | AT3G45140               | gctgacattggagcaggtt | atccgctcagctgacact |
| R-3         | Os03g0129100 | AT2G39200               | aaagggtgagctgaggtg | gcgctacacgctgtacact |
| R-4         | Os06g0698300 | AT4G31750               | ctgcaaaaaagctctcctcag | tgctggtggacacaagac |
| R-5         | Os10g0490800 | AT2G15130               | gggaactagcgaagcaggt | gtaacctccgccttcacag |
| POD-1       | Os03g0235000 | AT5G06720               | gcgaactacggcctggtagag | gcgcctccagcataataa |
| POD-2       | Os07g0677100 | AT5G05340               | atccgtctctctgaccaaa | cggctgacctacatggct |
| POD-3       | Os07g0677200 | AT5G05340               | agctgctccacggaact | atgggctgctgcttcacat |
| POD-4       | Os08g0113000 | AT4G33420               | ctgaaactgcgccccgtag | cctctccacgacaataaaa |
| ACTIN       | Os03g50890   | AT3G18780               | ggaaacttgtaggcagcc | atgcacctgtacaccccaag |

RESULTS AND DISCUSSION

Isolation of the bbr1 mutant with enhanced Xoo resistance

To examine whether the Xoo resistant phenotype can be maintained in the next generation, progeny from the TILL300-651 (M5) were tested for the Xoo resistance phenotype. Figure 1a shows a picture of typical leaves from each of the following inoculated wild type ‘Dongan’ and bbr1 mutant. While all bbr1 mutant progenies tested were resistant, showing relatively short lesions, inoculated leaves of wild type ‘Dongan’ developed water soaked long lesions (Fig. 1a, b). This result demonstrates that the bbr1 mutant enhanced resistance to Xoo.

Differential gene expression in the leaves of bbr1 mutant plants

Leaf samples were collected from the uninoculated (no treatment; NT) and Xoo-inoculated (10 DAI) wild type ‘Dongan’ (WT) and bbr1 mutant (M6), to detect differential gene expression under both conditions. To reduce experimental variation we separately pooled the leaf samples from eight inoculated/uninoculated WT and bbr1 mutant of individual biological replicate. Total RNA was then isolated from pooled samples and used for labeling. To identify the significantly differential expression of Xoo responsive genes in bbr1 compared to WT, we applied the following criteria; (a) the gene expression change occurred at the same direction (increase or decrease) in replication; and (b) the average ratio (fold change of bbr1/WT signal intensity) of expression levels in all microarray analyses was greater than 4 or less than -4. Applying these criteria, we detected 92 genes with increased (up-regulated) levels of expression and 22 genes with decreased (down-regulated) levels of expression in bbr1 (Fig. 2). These genes were considered as characteristics of the bbr1 mutant during Xoo infection.
Fig. 1. Phenotypic analysis of the rice bbr1 mutant. (a) Water-soaked disease lesions on three leaves from wild type ‘Dongan’ (WT) and bbr1 mutants (M6). 40-day-old plants were inoculated with Xoo. Image was taken two weeks after Xoo inoculation. (b) Leaf lesion lengths of eight bbr1 M6 progeny. Lesion lengths were measured at two weeks post Xoo inoculation. Experiments were repeated two times with similar results. Each data point represents the average and standard deviation of at least three leaves.

Fig. 2. Gene ontology classification of differentially expressed genes in rice leaves at 10 day post inoculation. Functional categories are derived from the primary annotation of biological process retrieved from the Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg). Differentially-regulated genes were expressed at 4-fold higher or lower levels in the bbr1 mutant compared to wild type from two independent microarray analyses. White bars indicated the number of four-fold down regulated genes and black bars indicated the number of four-fold up-regulated genes compared to the WT.
These 114 differentially expressed genes (DEGs) were further analyzed and annotated using public access databases (http://www.genome.jp/kegg). According to predicted functions of their homologous genes in *Arabidopsis*, these genes could be assigned to eleven biological process categories (Fig. 2), including defense and/or stress response, carbohydrate metabolic process, hormone responses, lipid metabolic process, nucleic acid metabolism, oxidation reduction, phosphate metabolism, protein metabolism, structure, transport and unknown function. Major categories

| Description | Gene Title | Average fold change $^a$ |
|-------------|------------|-------------------------|
| Transcription factor activity | | |
| TF-1; Similar to Heat shock transcription factor 31 | Os02g0527300 | 11.52 |
| TF-2; AP2, Similar to CBF-like protein | Os06g0127100 | 43.92 |
| TF-3; AP2, Similar to AP2 domain containing protein RAP2.6 | Os08g0474000 | 51.32 |
| TF-4; MYB, Similar to LHY protein | Os04g0583900 | 6.12 |
| TF-5; NAM, No apical meristem (NAM) domain containing protein | Os03g0327800 | 5.37 |
| TF-6; MYB, Similar to Myb-related transcription factor LBM1 | Os07g0558100 | 6.04 |
| TF-7; MYB, Similar to Typical P-type R2R3 Myb protein | Os01g0975300 | -7.52 |
| TF-8; AP2, RAV-like protein | Os01g0141000 | -4.17 |
| Response to biotic stress | | |
| R-1; Similar to Beta-1,3-glucanase-like protein | Os01g0944900 | 19.29 |
| R-2; Similar to Lipoxigenase 2.3, chloroplast precursor | Os02g0194700 | 10.9 |
| R-3; Seven transmembrane protein MLO2 | Os03g0129100 | 8.27 |
| R-4; Protein phosphatase 2C family protein | Os06g0698300 | 7 |
| R-5; Similar to NtPRp27 | Os10g0490800 | 14.22 |
| Similar to Iron-phytosiderophore transporter protein yellow stripe 1 | Os02g0649900 | -6.28 |
| Similar to Pathogen-related protein | Os01g0731100 | -8.17 |
| Response to oxidative stress | | |
| POD-1; Peroxidase | Os03g0235000 | -42.2 |
| POD-2; Peroxidase | Os07g0677100 | -7.73 |
| POD-3; Peroxidase | Os07g0677200 | -4.79 |
| POD-4; Similar to Peroxidase 47 precursor | Os08g0113000 | -14.6 |
| Response to abiotic stress | | |
| Late embryogenesis abundant (LEA) group 1 family | Os04g0589800 | 25.25 |
| Similar to Low-temperature induced protein lt101.2 | Os05g0122700 | 16.92 |
| Similar to Allyl alcohol dehydrogenase | Os04g0497000 | 11.48 |
| Similar to 1-Cys peroxiredoxin; | Os07g0638300 | 9.16 |
| Similar to Small heat stress protein class CIII | Os02g0782500 | 8.33 |
| GRAM domain containing protein; | Os12g0478100 | 7.53 |
| Similar to Acyl-CoA-binding protein 2 (ACBP 2) | Os06g0115300 | 6.89 |
| Hly-III related proteins family protein | Os06g0652200 | 6.54 |
| Similar to Dehydrin DHN1 (B8) | Os01g0702500 | 6.42 |
| Heat shock protein DnaJ, N-terminal domain containing protein | Os01g0606900 | 6.34 |
| EFA27 for EF hand, abscisic acid, 27kD | Os04g0511200 | 4.86 |
| Glycoside hydrolase, family 17 protein | Os01g0860800 | 4.44 |
| Similar to germin-like protein 8 | Os08g0189850 | -6.45 |
| Similar to germin-like protein 12 | Os08g0189900 | -6.92 |

$^a$ Average values of Xoo inoculated *bbr1* samples, compared to WT samples, from two independent microarray analysis. Numbers show the factor of change between wild type and mutant after Xoo inoculation; positive values represent up-regulation (e. g. 3 = 3-fold increase) negative values down-regulation (e. g. -3 = 3-fold decrease).
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of DEGs were biotic and abiotic stress related and transcription factors (Table 2). Thirty-three genes were classified into these categories and only considered for functions on specific pathways to further illustrate the differential response of WT and bbr1 mutant to Xoo infection (Table 2).

Defense-related genes induced in bbr1 mutant

Higher expression of β-1,3-glucanase-like protein (Os01g0944900; >19 fold), lipoxygenase 2.3, chloroplast precursor (Os02g0194700; >10 fold), seven transmembrane protein MLO2 (Os03g0129100; >8 fold), protein phosphatase 2C family protein (Os06g0698300; 7 fold) and basic secretory protein (Os10g0490800; >14 fold) genes were detected at 10 DAI in bbr1 mutant plant. On the other hand, lower expression of iron-phytosiderophore transporter protein yellow stripe 1 (Os02g0649900; >6 fold) and pathogen-related protein (Os01g0731100; >8 fold) were detected in Xoo-infected bbr1 leaf tissue (Table 1).

qRT-PCR analysis further confirmed the expression of

![Fig. 3. Altered expressions of genes related to Xoo inoculation in bbr1. The rice ACTIN gene was used as an internal positive control and transcript levels of the tested genes were normalized with that of ACTIN. Relative expressions of the tested genes were compared with that in WT plants. Bar represents standard deviation (three replicates)](image-url)
representative five genes (R-1 ~ R-5; Table 1) that were highly up-regulated in \textit{bbrl} mutant 10 DAI. Among the five genes, three [Os01g0944900 (β-1,3-glucanase-like protein; R-1), Os02g0194700 (lipoxygenase 2.3 chloroplast precursor (R-2); and Os03g0129100 (seven transmembrane protein MLO2; R-3)] were expressed at higher levels in \textit{bbrl} than in WT after \textit{Xoo} inoculation (Fig. 3a).

As a member of the PR-2 group of pathogenesis-related (PR) proteins, β-1,3-glucanase is induced by pathogen infection and plays an active antifungal role in hydrolyzing β-1,3-glucan, a major structural component of fungal cell walls (Sela-Buurlage \textit{et al}. 1993; Jach \textit{et al}. 1995). Lipid peroxidation, triggered by lipoxygenases and reactive oxygen species (ROS), is a hallmark of plant pathogen responses, both in signal transduction processes and during the execution of programmed cell death. Lipoxygenases oxidize free fatty acids in the cytosol or chloroplasts, thereby initiating several oxylipin pathways including the jasmonic acid and hydroperoxide lyase pathway (Mosblech \textit{et al}. 2009). Twelve predicted \textit{Mlo} homologs were identified in the rice genome (Goff \textit{et al}. 2002). The seven transmembrane MLO protein (Devoto \textit{et al}. 1999) is thought to mediate defense suppression against \textit{Blumeria graminis} f. sp. \textit{hordei} attack via direct Ca\textsuperscript{2+}-dependent interaction with calmodulin (Kim \textit{et al}. 2002). MLO-mediated defense suppression also likely involves one or several small GTP-binding proteins of the ROP (Rho-related GTPases from plants) family (Schultheiss \textit{et al}. 2002). Consistent with its involvement in plant-microbe interactions, MLO expression is induced upon biotic and abiotic stress stimuli (Piffanelli \textit{et al}. 2002). Induction of defense related genes during \textit{Xoo} infection may inhibit pathogen spread in the \textit{bbrl}.

Peroxidase genes reduced in \textit{bbrl} mutant

Four class III peroxidases genes [Os03g0235000 (POD-1), Os07g0677100 (POD-2), Os07g0677200 (POD-3) and Os08g0113000 (POD-4)] were expressed at lower levels in the \textit{bbrl} mutant than in WT 10 DAI (Table 2). qRT-PCR also confirmed lower level of four peroxidases expression (Fig. 3b). Peroxidases belong to well-known class of PR-9 proteins (van Loon \textit{et al}. 2006). They are expressed to limit cellular spreading of the infection through the establishment of structural barriers or generation of highly toxic environments by massively producing ROS and reactive nitrogen species (RNS) (Passardi \textit{et al}. 2005). Since POD scavenges the ROS, a reduced activity of this enzyme will increase the levels of ROS and result in hastened cell death. Down-regulation of PODs in \textit{bbrl} may therefore result in enhanced resistance against the invading pathogen. A lower expression of four PODs in \textit{bbrl} mutant implies the ROS involvement in defense against \textit{Xoo} invasion.

\textbf{Induced and suppressed genes encoding transcription factors}

WRKY, myeloblastosis (MYB), APETALA2 (AP2) domain containing transcription factors (ethylene-responsive element-binding proteins; EREBP, C-repeat-binding proteins; CBFs, related to ABI3/VP1; RAV), basic region/leucine zipper motif (bZIP), no apical meristem (NAM), zinc-finger proteins, and heat shock factors (HSFs) are encoded by large gene families and have been intensively studied for their roles in stress responses (Kagaya \textit{et al}. 1999; Singh \textit{et al}. 2002; Saibo \textit{et al}. 2009; Hirayama and Shinozaki 2010; Santos \textit{et al}. 2011; Scharf \textit{et al}. 2012).

Higher expression of heat shock transcription factor 31 (Os02g0527300; >11 fold), RAP2.6 (Os08g0474000; >50 fold) and CBF-like protein (Os06g0127100; >40 fold), MYB domain containing protein LHY (Os04g0583900; >6 fold) and LBM1 (Os07g0558100; >6 fold) and NAM domain containing protein (Os03g0327800; >5 fold) genes were detected in \textit{bbrl} mutant plant 10 DAI. On the other hand, lower expression of typical P-type R2R3 MYB protein (Os01g0975300; >7 fold) and AP2 domain containing protein RAV (Os01g0141000; >4 fold) were detected in \textit{Xoo}-infected \textit{bbrl} leaf tissue (Table 2). qRT-PCR analysis further confirmed the expression of eight representative transcription factor genes (TF1-1 ~ TF-8; Table 1) that were highly up- and down-regulated in \textit{bbrl} mutant 10 DAI. The result showed that expression levels of TF-1 (heat shock transcription factor 31), TF-2 (CBF-like protein) and TF-3 (RAP2.6) were increased in \textit{bbrl} whereas TF-7 (typical P-type R2R3 Myb protein) and TF-8 (RAV-like protein) were suppressed in \textit{bbrl} which are in agreement with the microarray results (Fig. 3c). Due to the central role of ERF and CBFs/DEBs in biotic and abiotic...
stress responses and their ability to regulate a large number of stress-responsive target genes, induction of these transcription factors are involved in the regulation of differentially expressed genes in \textit{bbr1}.

\textbf{AP2 domain containing transcription factors is involved in the regulation of differentially expressed genes in \textit{bbr1}}

On the basis of the number of AP2/ERF domains encoded and the gene function, the AP2/EREBP gene family has been divided into four subfamilies: AP2, RAV, DREB and ERF (Sakuma \textit{et al}. 2002). Both ERF and DREB/CBF subfamilies are of particular interest owing to their involvement in plant responses to stresses. ERF subfamily genes encode a large number of ERFs (Fujimoto \textit{et al}. 2000), which have been shown to participate in the plant response to biotic stress such as pathogens by recognizing the \textit{cis}-acting element GCCGCC, known as the GCC-box (Hao \textit{et al}. 1998). The DREB subfamily genes play an important role in the resistance of plants to abiotic stresses by recognizing the dehydration responsive element (DRE), which has a core motif CCGAC (Liu \textit{et al}. 1998). The transcription factors, RAV1 and RAV2, contain an AP2 domain in the N-terminal regions and a B3 domain in the C-terminal regions (Kagaya \textit{et al}. 1999). Using binding site selection assays, the AP2 and B3 domains of RAV1 were found to bind to the CAACA and CACCTG motifs (Kagaya \textit{et al}. 1999). Ectopic expression of the \textit{CaRAV1} gene in transgenic Arabidopsis plants resulted in the induction of some pathogenesis related (PR) genes, enhancing resistance against infection by bacterial pathogens, and tolerance to osmotic stresses caused by high salinity and dehydration conditions (Sohn \textit{et al}. 2006).

Among the differentially expressed transcription factor genes observed in this study, the average expression fold change of RAP2.6 (51.32 fold higher than wild type) and CBF like protein (43.92 fold higher than wild type) are the highest in the \textit{bbr1}. Another AP2 domain containing transcription factor RAV-like protein (4.17 fold lower than wild type) is also suppressed in the \textit{bbr1} after \textit{Xoo} infection (Table 2). The extent of induction or repression of an individual gene by \textit{Xoo} infection in \textit{bbr1} depends on a complex interaction between its transcription apparatus and associated regulation related sequences. To analyze the promoter region of target genes we selected 117 candidates which are more than two fold up- or down-regulated genes in \textit{bbr1} and used sequences located 2 kb upstream of the 5’ termini of each candidate genes. Among 117 candidate genes, GCC-box (GCCGCC; Table S1), DRE-related core motifs (CCGAC; Table S2) and AP2+B3 binding motif (CAACA and CACCTG; Table S3) are found in the promoter regions of 90 (73.77\%) putative target genes (Fig. 4). Among the genes we detected, several were reported to be related to disease resistance, such as F-BOX STRESS INDUCED 2 (Maldonado-Calderon \textit{et al}. 2012), PEN3 (Xin \textit{et al}. 2013), RPM1-INDUCED PROTEIN KINASE

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{venn_diagram.png}
\caption{Venn diagram of more than two folds up- and down-regulated genes with different AP2 domain binding motif in \textit{bbr1} mutant. A total 117 genes were differentially expressed before and after \textit{Xoo} infection, among which 90 putative target genes contain DRE-related core motifs (80 genes), GCC-box (36 genes) and AP2+B3 binding motif (51 genes) in their putative promoter regions.}
\end{figure}
(Feng et al. 2012), WRKY108 (Higashi et al. 2008) and BETA-1,3-GLUCANASE 2 (Silipo et al. 2005). This observation supports the hypothesis that AP2 domain containing transcription factors are involved in the regulation of differentially expressed genes in bbr1.

CONCLUSION

Japonica rice mutant bbr1 which is selected by mutant screening in this study was characterized. The up- and down-regulated genes identified in Xoo resistant bbr1 may play a role in resistance. To investigate the Xoo resistance of the gamma-ray irradiated mutant line, bbr1, we performed the commercial rice gene chip analysis. Through DNA microarray analysis, we found significantly elevated expression of AP2 domain containing transcription factor genes, RAP2.6 (Os08g0474000) and CBF (Os06g0127100). Because the primary role of ERF and CBFs/DREBs is the regulation of a large number of target stress-responsive genes in biotic and abiotic stress, the enriched expression of the transcription factors suggests that they might be involved in the regulation of differentially expressed genes in bbr1. Also, the suppression of ROS scavenging enzyme, class III POD genes (Os03g023500, Os07g0677100, Os07g0677200, and Os08g0113000) was detected from DNA microarray analysis of bbr1. Down-regulation of PODs in bbr1 may contribute to the resistance against the invading pathogen. A lower expression of four PODs in bbr1 mutant than wild-type implies that increased ROS may have influence on the defense against Xoo invasion. These observations support the hypothesis that the genes mentioned above, contribute to bacterial blight resistance in bbr1 mutant.

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| Gene Description | Gene Title | AGI Number | putative promoter region^a | Number of GCC-box in putative promoter region | Average fold change^b |
|------------------|------------|------------|-----------------------------|-----------------------------------------------|-----------------------|
|                  |            |            | GCCGCC GCCGCGC Total No     | NT Xoo                                         |                       |
| Transcription factor activity |            |            |                             |                                               |                       |
| MYB family transcription factor | Os01g0863300 | AT2G38090 | 3                           | 3 6                                           | 7.5 8.72              |
| MYB family transcription factor | Os02g0104500 | AT1G76890 | 5                           | 10 15                                         | -5.62 -2.1            |
| Response to stress |            |            |                             |                                               |                       |
| HSF-type DNA-binding domain containing protein | Os06g0553100 | AT3G24520 | 0                           | 4 4                                           | 2.69 24.93            |
| VQ domain containing protein | Os01g0278000 | AT2G41010 | 1                           | 1 2                                           | 4.84 7.29             |
| oxidoreductase/ transition metal ion binding protein | Os09g0445600 | AT5G19875 | 1                           | 5 6                                           | 3.57 6.87             |
| Similar to F-BOX STRESS INDUCED 2 | Os02g0561300 | AT4G21510 | 5                           | 3 8                                           | 3.64 6.57             |
| Calmodulin-related calcium sensor protein | Os01g0955100 | AT1G76640 | 1                           | 2                                            | 7.24 6.32             |
| phosphate carrier protein | Os09g0454600 | AT3G48850 | 4                           | 2 6                                           | 3.86 6.19             |
| diacylglycerol kinase 1 | Os12g0240000 | AT5G63770 | 1                           | 1 2                                           | 2.31 2.75             |
| PR (pathogenesis-related) peptide | Os12g0347800 | AT2G38870 | 12                          | 5 17                                          | -3.81 1.06            |
| Protein modification process |            |            |                             |                                               |                       |
| protein phosphatase 2C | Os03g0268600 | AT2G29380 | 1                           | 2 3                                           | 7.29 9.99             |
| STE_MEKK_ste11_MAP3K.6 - STE kinases receptor protein kinase CRINKLY4 precursor protein kinase | Os01g0699500 | AT5G55090 | 2                           | 0 2                                           | 9.48 9.47             |
| STE_MEKK_ste11_MAP3K.4 - STE kinases protein phosphatase 2C | Os01g0699100 | AT5G55090 | 2                           | 0 4                                           | 3.64 6.57             |
| Nucleotide binding |            |            |                             |                                               |                       |
| WD domain containing protein | Os01g0383700 | AT4G03020 | 2                           | 0 2                                           | 4.42 5.19             |
| Development |            |            |                             |                                               |                       |
| late embryogenesis abundant protein D-34 | Os06g0343100 | AT3G22490 | 4                           | 3 7                                           | 5.39 15.56            |
| senescence-associated gene 29 | Os02g0513100 | AT3G48740 | 2                           | 0 2                                           | 4.47 10.93            |
| Auxin regulated protein? | Os01g0851100 | AT2G37980 | 1                           | 1 2                                           | 3.86 6.41             |
| Metabolic process |            |            |                             |                                               |                       |
| fringe-related protein | Os03g0269900 | AT2G37730 | 3                           | 2 5                                           | 3.86 6.94             |
| gibberellin receptor GID1L2 | Os03g0790500 | AT5G05670 | 1                           | 1 2                                           | 4.76 6.82             |
| Anthranilate synthase alpha 2 subunit | Os03g0264400 | AT2G29690 | 2                           | 0 2                                           | 3.4 3.23              |
| starch synthase | Os06g0133000 | AT1G32900 | 1                           | 1 2                                           | 13.45 1.23            |
| Unknown function |            |            |                             |                                               |                       |
| transposon protein | Os01g0186900 | AT2G29380 | 0                           | 3 3                                           | 17.03 15.89           |
| expressed protein | Os01g0305200 | AT1G69510 | 1                           | 9 10                                          | 4.3 9.88              |
| RPGR, putative | Os03g0296200 | AT2G29380 | 1                           | 1 2                                           | 6.77 8.97             |
| expressed protein | Os02g0527200 | AT2G27830 | 2                           | 4 6                                           | 5.1 8.57              |
| expressed protein | Os02g0601000 | AT2G27830 | 4                           | 0 4                                           | 4.99 6.54             |
| expressed protein | Os06g0133000 | AT1G32900 | 4                           | 2 6                                           | 4.3 4.13              |
| expressed protein | Os01g0138500 | AT2G01260 | 0                           | 1 1                                           | 2.88 4.13             |
| DUF966 domain containing protein | Os01g0975000 | AT5G59790 | 2                           | 3 5                                           | 3.48 4.04             |
| cyclase/dehydrase family protein | Os01g0772400 | AT4G17650 | 1                           | 3 4                                           | 3.36 3.38             |
| expressed protein | Os12g0209700 | AT4G10930 | 2                           | 3 5                                           | -2.31 -2.19           |
| expressed protein | Os11g0307600 | AT4G10930 | 2                           | 0 2                                           | -7.41 -2.37           |
| Similar to pnn protein | Os12g0516700 | AT4G10930 | 1                           | 23 24                                         | -3.88 -3.67           |

^a Occurrence of GCC-box (GCCGCC or GGCGGC) in 2 kb upstream region of differentially expressed genes in bbr1 mutant compared to wild type.

^b Average values of Xoo inoculated bbr1 samples, compared to WT samples, from two independent microarray analysis. Numbers show the factor of change between wild type and mutant after Xoo inoculation or no treatment (NT); positive values represent up-regulation (e. g. 3 = 3-fold increase), negative values down-regulation (e. g. -3 = 3-fold decrease).
Table S2. Seventy-nine DRE binding domain containing differentially expressed genes in bbr1 mutant compared to wild type.

| Description | Gene Title | AGI Number | Number of DRE-box in putative promoter region$^a$ | Average fold change$^b$ | CCGAC | GTCGG | Total No. | NT | Xoo |
|-------------|------------|------------|-------------------------------------------------|--------------------------|-------|-------|-----------|----|-----|
| Transcription factor activity | Tify domain containing protein | Os10g0391400 | No | 5 | 3 | 8 | 2.98 | 85.24 |
| | transcription factor BHLH92-like | Os03g0741100 | No | 1 | 1 | 2 | 2.4 | 35.02 |
| | Heat shock transcription factor 31 | Os02g0527300 | AT2G26150 | 0 | 1 | 1 | 3.05 | 26.01 |
| | WRKY DNA -binding domain | Os01g0821300 | No | 2 | 6 | 8 | 2.14 | 25.72 |
| | Similar to MCB2 protein (Myb-type) | Os01g0863300 | AT5G04760 | 4 | 0 | 4 | 3.39 | 19.37 |
| | Chitin-inducible gibberellin-responsive protein | Os02g0682300 | No | 1 | 0 | 1 | 2.65 | 8.86 |
| | Tify domain containing protein | Os09g0439200 | No | 2 | 1 | 3 | 3.07 | 8.84 |
| | MYC/bHLH transcription factor-like | Os06g0164400 | AT5G67110 | 2 | 1 | 3 | 2.51 | 6.05 |
| | NAM protein domain containing protein | Os12g0123800 | AT5G18270 | 0 | 1 | 1 | 2.85 | 4.36 |
| | Avr9Cf-9 rapidly elicited protein 74 | Os06g0248500 | No | 4 | 1 | 5 | 3.24 | 95.27 |
| | EF-hand Ca2+-binding protein CCD1 | Os09g0434600 | AT5G14040 | 1 | 2 | 3 | 2.46 | 9.71 |
| | RPM1-INDUCED PROTEIN KINASE | Os09g0442100 | AT2G05940 | 2 | 0 | 2 | 2.56 | 13.33 |
| | expressed protein | Os01g0582600 | AT5G12010 | 2 | 1 | 3 | 2.18 | 9.38 |
| | putative beta-1,3 glucanase | Os09g0542900 | AT1G76070 | 1 | 1 | 2 | 2.23 | 9.32 |
| | heat shock protein Oshsp18.0 | Os03g0267000 | AT3G46230 | 0 | 5 | 5 | 2.54 | 4.68 |
| | Dehydrin Rab25 | Os01g0702500 | No | 2 | 1 | 3 | 4.59 | 3.68 |
| | subtilisin-chymotrypsin inhibitor-2A-like | Os12g0347800 | No | 3 | 6 | 9 | -3.81 | 1.06 |
| Response to stress | protein kinase domain containing protein | Os01g0699600 | No | 0 | 1 | 1 | 3.65 | 70.53 |
| | protein kinase domain containing protein. | Os01g0699500 | No | 2 | 0 | 2 | 3.25 | 26.42 |
| | HIGHLY ABA-INDUCED PP2C GENE 3 | Os03g0268600 | AT2G9380 | 3 | 1 | 4 | 3.24 | 22.68 |
| | Protein kinase | Os02g0165100 | No | 2 | 1 | 3 | 2.76 | 20.92 |
| | Similar to Receptor kinase-like | Os08g0374600 | No | 1 | 1 | 2 | 3.02 | 14.39 |
| | MAPKKK18 | Os01g0699400 | AT1G05100 | 2 | 0 | 2 | 2.93 | 11.11 |
| | Oryza sativa MAP kinase BIMK1 | Os03g0285800 | No | 3 | 2 | 5 | 2.61 | 10.7 |
| | protein kinase domain containing protein | Os01g0699100 | AT2G9380 | 4 | 3 | 7 | 3.61 | 6.43 |
| | diacylglycerol kinase | Os12g0224000 | No | 2 | 1 | 3 | 2.04 | 3.19 |
| | Similar to Chaperone protein dnaJ 1 | Os03g0822800 | AT5G59610 | 1 | 1 | 2 | 2.6 | -1.12 |
| Protein modification process | Similar to Viviparous-14 | Os07g0154100 | No | 0 | 1 | 1 | 2.48 | 16.14 |
| | Mog1/PsbP | Os01g0934400 | AT3G05410 | 1 | 2 | 3 | 4.89 | 8.98 |
| | GRANULE BOUND STARCH SYNTHASE 1 | Os06g0133000 | AT1G32900 | 2 | 2 | 4 | 13.45 | 1.23 |
| | similar to Cytochrome P450 | Os07g0635500 | AT2G46960 | 6 | 1 | 7 | 2.82 | 6.25 |
| Biosynthetic process | similar to Viviparous-14 | Os07g0154100 | No | 0 | 1 | 1 | 2.48 | 16.14 |
| | Mog1/PsbP | Os01g0934400 | AT3G05410 | 1 | 2 | 3 | 4.89 | 8.98 |
| | GRANULE BOUND STARCH SYNTHASE 1 | Os06g0133000 | AT1G32900 | 2 | 2 | 4 | 13.45 | 1.23 |
| | Similar to Cytochrome P450 | Os07g0635500 | AT2G46960 | 6 | 1 | 7 | 2.82 | 6.25 |
| Development | zinc finger protein ZFP15 mRNA | Os03g0820400 | No | 4 | 2 | 6 | 3.91 | 34.75 |
| | Seed maturation protein domain containing protein | Os06g0341300 | AT3G22490 | 1 | 2 | 3 | 4.37 | 19.29 |
| | CALMODULIN LIKE 39 | Os01g0955100 | AT1G76640 | 1 | 1 | 2 | 2.53 | 18.25 |
| | Late embryogenesis abundant protein | Os01g0705200 | No | 1 | 0 | 1 | 3.21 | 6.62 |
| Description                           | Gene Title                  | AGI Number | Number of DRE-box in putative promoter region | Average fold change |
|---------------------------------------|-----------------------------|------------|-----------------------------------------------|---------------------|
|                                       |                             |            | CCGAC | GTCGG | Total No. | NT | Xoo |
| Gene regulation                       |                             |            |       |       |           |    |     |
| arginine/serine-rich 12               | Os12g0516700               |            | 1     | 3     | 4         | 3.73 | 248.65 |
| Metabolic process                     |                             |            |       |       |           |    |     |
| hypothetical protein                  | Os01g0952900               | No         | 1     | 2     | 3         | 2.87 | 15.91 |
| Nuclease, Phoaphatase                 | Os01g0716800               | AT1G71710  | 2     | 0     | 2         | 2.83 | 11.52 |
| a/β hydrolase fold-3 domain containing protein | Os03g0790500               | AT5G06570  | 0     | 1     | 2         | 2.91 | 6.35  |
| putative 4-coumarate-CoA ligase       | Os01g0901600               | AT5G63380  | 1     | 1     | 2         | 2.25 | 5.04  |
| Similar to H-ATPase                   | Os03g0689300               | AT5G26270  | 1     | 1     | 1         | 3.53 | 4.88  |
| Transport                             |                             |            |       |       |           |    |     |
| peptidylprolyl isomerase ROC7         | Os06g0708300               | AT4G39220  | 2     | 0     | 2         | 2.65 | 10.09 |
| Similar to MtN3 protein precursor     | Os02g0513100               | AT5G08080  | 1     | 1     | 2         | 5.73 | 8.59  |
| putative aki 1 protein                | Os01g0851100               | AT2G37980  | 4     | 0     | 4         | 3.47 | 6.31  |
| Anthranilate synthase component I family protein | Os03g0272400               | AT4G17650  | 4     | 2     | 6         | 6.64 | 9.28  |
| Similar to GTP-binding nuclear protein Ran1B | Os06g0600301               | AT5G55190  | 5     | 2     | 7         | 2.34 | 5.79  |
| Nucleotide binding                    |                             |            |       |       |           |    |     |
| WD-40 repeat family protein           | Os01g0383700               | AT4G03020  | 4     | 2     | 6         | 3.56 | 6.49  |
| Unknown function                      |                             |            |       |       |           |    |     |
| ZIM domain containing protein         | Os03g0181100               | No         | 3     | 5     | 8         | 2.73 | 56.55 |
| hypothetical protein                  | Os06g0133500               | No         | 1     | 0     | 1         | 3.01 | 45.58 |
| hypothetical protein                  | Os02g0733900               | No         | 1     | 1     | 2         | 3.47 | 44.9  |
| DWNN domain domain containing protein | Os03g0693400               | No         | 1     | 2     | 3         | 2.91 | 19.86 |
| hypothetical protein                  | Os02g0527200               | No         | 4     | 4     | 8         | 2.55 | 17.3  |
| hypothetical protein                  | Os02g0601000               | No         | 3     | 5     | 8         | 2.47 | 13.29 |
| hypothetical protein                  | Os01g0305200               | No         | 4     | 5     | 9         | 3.23 | 13.21 |
| hypothetical protein                  | Os01g0121600               | No         | 0     | 2     | 2         | 2.51 | 10.85 |
| hypothetical protein                  | Os03g0296200               | No         | 4     | 0     | 4         | 6.64 | 9.28  |
| hypothetical protein                  | Os07g0115500               | No         | 1     | 1     | 2         | 2.58 | 9.08  |
| hypothetical protein                  | Os06g0133300               | No         | 0     | 1     | 1         | 2.34 | 7.59  |
| DUF604 family protein                 | Os03g0269900               | AT2G37730  | 1     | 0     | 1         | 3.6  | 7.49  |
| DUF966 family protein                 | Os01g0975000               | No         | 2     | 2     | 4         | 2.24 | 6.31  |
| Cyclin-like F-box domain containing protein | Os07g0561300               | No         | 5     | 2     | 7         | 3.9  | 6.17  |
| hypothetical protein                  | Os09g0445600               | AT2G31940  | 0     | 2     | 2         | 4.22 | 5.75  |
| DUF789 family protein                 | Os01g0138500               | AT2G01260  | 4     | 4     | 8         | 2.28 | 5.22  |
| TonB box domain containing protein    | Os09g0532000               | No         | 1     | 0     | 1         | 2.35 | 4.15  |
| Conserved hypothetical protein         | Os01g0121500               | No         | 1     | 0     | 1         | 2.88 | 3.07  |
| hypothetical protein                  | Os07g0516400               | No         | 1     | 2     | 3         | 4.83 | 3     |
| metallothionein-like type 2 (OsMT-2) mRNA | Os01g0492000               | No         | 1     | 3     | 4         | 2.77 | 2.78  |
| hypothetical protein                  | Os12g0209700               | No         | 4     | 2     | 6         | -2.36 | -2.14 |
| Hydroxyproline-rich glycoprotein DZ-HRGP | Os11g0307600               | No         | 3     | 0     | 3         | -4.59 | -2.89 |
| similar to GT-2 factor                | Os02g0104500               | No         | 2     | 0     | 2         | -5.62 | -2.1  |
| hypothetical protein                  | Os02g0103800               | No         | 3     | 3     | 6         | -2.08 | -14.42 |

*a Occurrence of DRE binding domain (CCGAC or GTCGG) in 2 kb region up stream of differentially expressed genes in bbr1 mutant compared to wild type.

b Average values of Xoo inoculated bbr1 samples, compared to WT samples, from two independent microarray analysis. Numbers show the factor of change between wild type and mutant after Xoo inoculation or no treatment (NT); positive values represent up-regulation (e.g. 3 = 3-fold increase), negative values down-regulation (e.g. -3 = 3-fold decrease).
Table S3. Fifty-one RAV1 binding domain containing differentially expressed genes in **bbr1** mutant compared to wild type.

| Gene Description | Gene Title | AGI Number | Number of RAV-binding site in putative promoter region | Average fold change |
|------------------|------------|------------|------------------------------------------------------|---------------------|
| **Transcription factor activity** | | | | |
| WRKY108, expressed | Os01g05821300 | AT4G11070 | 1 1 2 | 5.86 9.35 |
| HSF-type DNA-binding domain containing protein | Os06g0553100 | AT3G24520 | 1 0 1 | 2.69 4.03 |
| **Response to stress** | | | | |
| U-box protein CMPG1 | Os08g0248500 | AT5G37490 | 0 1 1 | 14.72 20.82 |
| cytochrome P450 | Os12g0150200 | AT2G27690 | 1 1 2 | 14.57 18.57 |
| Similar to ATL31 and ATL6 | Os02g0759400 | AT5G27420 | 3 0 3 | 9.25 12.55 |
| HSF-type DNA-binding domain containing protein | Os02g0527300 | AT5G03720 | 1 1 2 | 6.84 11.51 |
| Similar to RAP 2.4 | Os03g0191900 | AT1G78080 | 1 0 1 | 14.72 20.82 |
| late embryogenesis abundant protein, group 3 | Os01g0705200 | AT3G15670 | 1 0 1 | 2.87 7.34 |
| phosphate carrier protein | Os09g0445600 | AT5G19875 | 1 0 1 | 3.57 6.87 |
| Similar to RPM1-induced kinase | Os09g0442100 | AT2G05940 | 1 0 1 | 3.86 6.19 |
| Similar to PEN3 | Os01g0693000 | AT1G59870 | 1 0 1 | 3.86 6.19 |
| expressed protein | Os09g0454600 | AT3G48850 | 1 0 1 | 3.86 6.19 |
| Similar to 4CL | Os08g0442000 | AT5G27690 | 1 0 1 | 3.86 6.19 |
| glutamate decarboxylase | Os09g0454600 | AT3G48850 | 1 0 1 | 3.86 6.19 |
| Similar to PR-6 proteinase inhibitor family | Os12g0437800 | AT2G38870 | 1 0 1 | 3.86 6.19 |
| heat shock protein DnaJ | Os03g0822800 | AT5G5610 | 1 0 1 | 2.6 -1.12 |
| **Protein modification process** | | | | |
| STE_MEKK_ste11_MAP3K.7 - STE kinases | Os01g0699600 | AT2G32510 | 1 2 3 | 18.13 14.07 |
| STE_MEKK_ste11_MAP3K.6 - STE kinases | Os01g0699500 | AT5G55090 | 0 3 3 | 9.48 9 |
| receptor protein kinase CRINKLY4 precursor protein kinase | Os08g0374600 | AT3G55950 | 0 1 1 | 4.94 8.75 |
| protein phosphatase 2C | Os09g0326200 | AT5G17330 | 2 1 3 | 4.27 3.46 |
| STE_MEKK_ste11_MAP3K.4 - STE kinases | Os01g0699100 | AT1G59870 | 1 0 1 | 6.66 8.63 |
| STE_MEKK_ste11_MAP3K.5 - STE kinases | Os01g0699400 | AT5G55090 | 1 0 1 | 4.63 6.99 |
| protein phosphatase 2C | Os01g0583000 | AT1G59870 | 1 0 1 | 6.66 8.63 |
| **Nucleotide binding** | | | | |
| CCHC-type zinc finger | Os03g0659400 | AT5G47340 | 0 1 1 | 6.23 9.25 |
| WD domain | Os01g0383700 | AT4G03020 | 1 0 1 | 4.42 5.19 |
| **Development** | | | | |
| EF hand family protein | Os06g0863000 | AT2G46600 | 1 0 1 | 7.94 6.94 |
| growth regulator related protein | Os01g0851100 | AT2G37980 | 1 0 1 | 3.86 6.41 |
| glucan endo-1,3-beta-glucosidase precursor | Os03g0792800 | AT2G19440 | 1 0 1 | 3.13 5.9 |
| senescence-inducible chloroplast stay-green protein | Os09g0532000 | AT4G11910 | 0 1 1 | 2.72 3.57 |
| **Metabolic process** | | | | |
| lumenal PsbP | Os01g0934400 | AT3G05410 | 0 2 2 | 5.08 12.04 |
| expressed protein | Os06g0203600 | AT2G26310 | 1 0 1 | 9.19 11.35 |
| AMP-binding domain containing protein | Os01g0901600 | AT5G63380 | 1 0 1 | 2.69 4.2 |
| **Transport** | | | | |
| white-brown complex homolog protein | Os01g0121600 | AT2G01320 | 1 2 3 | 4.87 5.54 |
| Rer1 protein | Os06g0708300 | AT4G39220 | 1 0 1 | 4.58 5.8 |
| ras-related protein | Os06g0600300 | AT5G55190 | 0 1 1 | 2.17 2.06 |
| Gene Description                  | Gene Title    | AGI Number | Number of RAV-binding site in putative promoter region<sup>a</sup> | Average fold change<sup>b</sup> |
|----------------------------------|---------------|------------|------------------------------------------------------------------|----------------------------------|
|                                   |               |            | CAACA+ CACCTG | TGTTG+ CAGGTG | Total NO | NT | Xoo |
| Unknown function                  |               |            | CAACA+ CACCTG | TGTTG+ CAGGTG | Total NO | NT | Xoo |
| expressed protein                 | Os01g0952900  | AT5G12340  | 1               | 0            | 1        | 27.86 | 33.01 |
| transposon protein                | Os01g0186900  | AT5G12340  | 2               | 0            | 2        | 17.09 | 15.89 |
| expressed protein                 | Os06g0133500  | AT5G12340  | 1               | 0            | 1        | 10.78 | 12.64 |
| expressed protein                 | Os01g0305200  | AT1G69510  | 1               | 0            | 1        | 4.3   | 9.88  |
| RPGR, putative,                   | Os03g0296200  | AT1G69510  | 1               | 0            | 1        | 6.82  | 9     |
| expressed protein                 | Os02g0527200  | AT2G27830  | 1               | 0            | 1        | 5.1   | 8.57  |
| expressed protein                 | Os02g0601000  | AT2G27830  | 1               | 1            | 2        | 4.99  | 6.5   |
| expressed protein                 | Os07g0516400  | AT2G27830  | 1               | 0            | 1        | 2.27  | 6.34  |
| expressed protein                 | Os01g0138500  | AT2G01260  | 1               | 0            | 1        | 2.88  | 4.07  |
| expressed protein                 | Os09g0542900  | AT1G76070  | 1               | 0            | 1        | 5.26  | 3.93  |
| transposon protein                | Os01g0872900  | AT1G76070  | 1               | 0            | 1        | 5.68  | 3.64  |
| cyclase/dehydrase family protein  | Os01g0772400  | AT4G17650  | 0               | 1            | 1        | 3.36  | 3.38  |
| expressed protein                 | Os12g0209700  | AT4G10930  | 0               | 1            | 1        | -2.31 | -2.13 |

<sup>a</sup> Occurrence of RAV1 binding domain (CAACA--CACCTG or TGTTG--CAGGTG) in 2 kb region upstream of differentially expressed genes in bbr1 mutant compared to wild type.

<sup>b</sup> Average values of Xoo inoculated bbr1 samples, compared to WT samples, from two independent microarray analysis. Numbers show the factor of change between wild type and mutant after Xoo inoculation or no treatment (NT); positive values represent up-regulation (e. g. 3 = 3-fold increase), negative values down-regulation (e. g. -3 = 3-fold decrease).