Plant Mol Biol (2010) 74:549–562
DOI 10.1007/s11103-010-9695-3

Role of OsNPR1 in rice defense program as revealed by genome-wide expression analysis

Shoji Sugano · Chang-Jie Jiang · Shin-Ichi Miyazawa · Chisato Masumoto · Katsumi Yazawa · Nagao Hayashi · Masaki Shimono · Akira Nakayama · Mitsue Miyao · Hiroshi Takatsuji

Received: 23 March 2010 / Accepted: 21 September 2010 / Published online: 7 October 2010

Abstract NPR1 is a central regulator of salicylic-acid (SA)-mediated defense signaling in *Arabidopsis*. Here, we report the characterization of OsNPR1, an *Oryzae sativa* (rice) ortholog of NPR1, focusing on its role in blast disease resistance and identification of OsNPR1-regulated genes. Blast resistance tests using *OsNPR1* knockdown and over-expressing rice lines demonstrated the essential role of OsNPR1 in benzothiadiazole (BTH)-induced blast resistance. Genome-wide transcript profiling using *OsNPR1*-knockdown lines revealed that 358 genes out of 1,228 BTH-upregulated genes and 724 genes out of 1,069 BTH-downregulated genes were OsNPR1-dependent with respect to BTH responsiveness, thereby indicating that OsNPR1 plays a more vital role in gene downregulation. The OsNPR1-dependently downregulated genes included many of those involved in photosynthesis and in chloroplast translation and transcription. Reduction of photosynthetic activity after BTH treatment and its negation by *OsNPR1* knockdown were indeed reflected in the changes in Fv/Fm values in leaves. These results imply the role of OsNPR1 in the reallocation of energy and resources during defense responses. We also examined the OsNPR1-dependence of SA-mediated suppression of ABA-induced genes.

Keywords Benzothiadiazole · Blast resistance · OsNPR1 · NPR1 · Photosynthesis · Abscisic acid

Introduction

In response to attacks by a wide variety of microbial pathogens and insect herbivores, plants activate a complex series of defense responses. These induced responses are expressed not only locally but also in plant parts distant from the site of infection, thereby protecting plants from secondary infection. Systemic acquired resistance (SAR) is one of the best-studied induced defense responses in plants; it is long-lasting and effective against a broad spectrum of microbial pathogens (Ryals et al. 1996). In dicots such as *Arabidopsis* and tobacco, the onset of SAR is accompanied by an increase in endogenous levels of salicylic acid (SA) and concomitant upregulation of a large number of genes, including *pathogenesis-related* (*PR*) genes.

NPR1 is a central positive regulator of SAR in *Arabidopsis*, and transduces the SA signal to downstream *PR* gene activation (Cao et al. 1994; Delaney et al. 1995; Shah et al. 1997). Nuclear localization of NPR1 is required for

Electronic supplementary material The online version of this article (doi:10.1007/s11103-010-9695-3) contains supplementary material, which is available to authorized users.

S. Sugano · C.-J. Jiang · K. Yazawa · N. Hayashi · M. Shimono · A. Nakayama · H. Takatsuji (✉)
Plant Disease Resistance Research Unit, Division of Plant Sciences, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan
e-mail: takatsuh@affrc.go.jp

S.-I. Miyazawa · C. Masumoto · M. Miyao
Photobiology and Photosynthesis Research Unit, Division of Plant Sciences, National Institute of Agrobiological Sciences, Kannondai 2-1-2, Tsukuba, Ibaraki 305-8602, Japan

Present Address:
M. Shimono
Department of Plant Pathology, Michigan State University, 107 Center for Integrated Plant Systems, East Lansing, MI 48824-1311, USA

Present Address:
A. Nakayama
Maebashi Institute of Technology, Maebashi, Gunma 371-0816, Japan

the upregulation of *PR* genes (Kinkema et al. 2000); its nucleocytoplasmic localization is regulated by changes in cellular redox potentials (Mou et al. 2003). Under uninduced conditions, NPR1 forms an oligomer and is localized in the cytoplasm. After SA accumulation triggered by pathogen infection, cellular redox potential changes, resulting in the translocation of NPR1 to the nucleus as an active monomeric form and the interaction with TGA family members of basic domain/Leu zipper (bZIP) transcription factors (TFs) (Mou et al. 2003; Zhang et al. 1999). These interactions stimulate the binding of TGA factors to SA-responsive elements in the promoter of *PR* genes and regulate the expression of defense-related genes (Despres et al. 2003; Fan and Dong 2002; Johnson et al. 2003). Recently, proteasome-mediated turnover of NPR1 was found to play an important role in modulating transcription of its target genes (Spoel et al. 2009). Role of NPR1 in antagonistic interaction of SA signaling on abscisic acid signaling has also been reported (Yasuda et al. 2008).

Rice (*Oryza sativa*) is one of the most important food crops and is a model in monocots for investigating the molecular mechanisms behind defense responses. Unlike dicots, in which basal levels of SA are low and the levels increase substantially after a pathogen attack, rice plants accumulate high levels of SA under uninfected conditions, and those levels are apparently insensitive to pathogen infection (Silverman et al. 1995); this implies that the rice defense mechanism is different from those in dicots. However, several studies have demonstrated that rice also has an SA-mediated defense-signaling pathway. SA-deficient transgenic plants overexpressing the *nahG* gene, encoding a salicylate hydroxylase, exhibit a reduced resistance to blast disease, accompanied by an increased susceptibility to oxidative damages incurred by biotic and abiotic stresses (Yang et al. 2004). Overexpression of *Arabidopsis* NPR1 in rice confers an enhanced resistance to bacterial blight disease and fungal pathogens (Chern et al. 2001; Fitzgerald et al. 2004; Quilis et al. 2008). Moreover, the overexpression of *OsNPR1/NH1*, a rice ortholog of NPR1, confers an enhanced resistance to bacterial blight disease (Chern et al. 2005; Yuan et al. 2007), while *OsNPR1* knockdown renders rice plants more susceptible to this disease (Yuan et al. 2007). Yuan et al. (2007) suggested that the intracellular localization of OsNPR1 is regulated by changes in cellular redox potential as *Arabidopsis* NPR1. These observations suggest the presence of a defense-signaling pathway in rice similar to the SA/NPR1-mediated pathway in *Arabidopsis*, and that NPR1 function is at least in part conserved between dicots and monocots.

Benzothiadiazole (BTH) is a so-called plant activator that protects plants from infectious diseases. BTH is a functional analog of SA, and when applied exogenously, it induces *PR* gene expression and disease resistance in plants (Gorlach et al. 1996; Lawton et al. 1996). BTH induces plant resistance by priming the SA-signaling pathway mediated by NPR1 in *Arabidopsis* (Conrath et al. 2002; Lawton et al. 1996). According to Wang et al. (2006), the expression of 99% of BTH-responsive genes depends on NPR1. Several WRKY TFs act as both positive and negative regulators of SAR, downstream of NPR1 (Wang et al. 2006). BTH enhances resistance to various diseases in rice, including blast and leaf-blight diseases. We recently reported that a BTH-upregulated WRKY TF in rice, WRKY45, plays an essential role in BTH-induced blast resistance (Shimono et al. 2007). Interestingly, rice WRKY45 acts in the SA-signaling pathway, independent of OsNPR1, rather than acting downstream of OsNPR1; this suggests that the SA-signaling pathways of rice and *Arabidopsis* are evolutionally divergent (Shimono et al. 2007).

In this study, we characterized the role of OsNPR1 in the regulation of BTH-induced defense responses in rice. We showed that OsNPR1 is essential for BTH-inducible blast resistance and that an overexpression of *OsNPR1* cDNA confers blast resistance to rice. A genome-wide analysis of BTH-responsive genes in rice and of their OsNPR1-dependence revealed that OsNPR1 plays a role in the coordination of BTH-induced downregulation of genes, such as those involved in photosynthesis, chloroplastic transcription and translation, and abscisic acid (ABA)-responsive genes. Taken together with the results of the determination of photosynthetic activities, we discuss the role of *OsNPR1* in BTH-induced disease resistance.

Results

OsNPR1 is essential for BTH-inducible blast resistance

To investigate the role of OsNPR1 in BTH-inducible disease resistance, we generated *OsNPR1*-kd rice plants by expressing an inverted-repeat sequence of the 3′ region of *OsNPR1* cDNA. As shown in Fig. 1a, the basal expression of *OsNPR1* was reduced in the transformants, in comparison to wild-type (WT) plants, whereas two homologs of *OsNPR1* (*OsNPR2* and *OsNPR3*) remained unaffected, demonstrating the specificity of gene-silencing in those *OsNPR1*-kd lines. In addition, expression of *OsNPR1* after BTH treatment was also reduced in transformant lines (Fig. 1b). These transformant lines were then tested for resistance to a compatible race of blast fungus (*Magnaporthe grisea*; race 007) after BTH application, to examine the role of OsNPR1 in BTH-inducible blast resistance. As shown in Fig. 1c, d, BTH application drastically reduced the number of susceptible-type lesions in WT plants. In

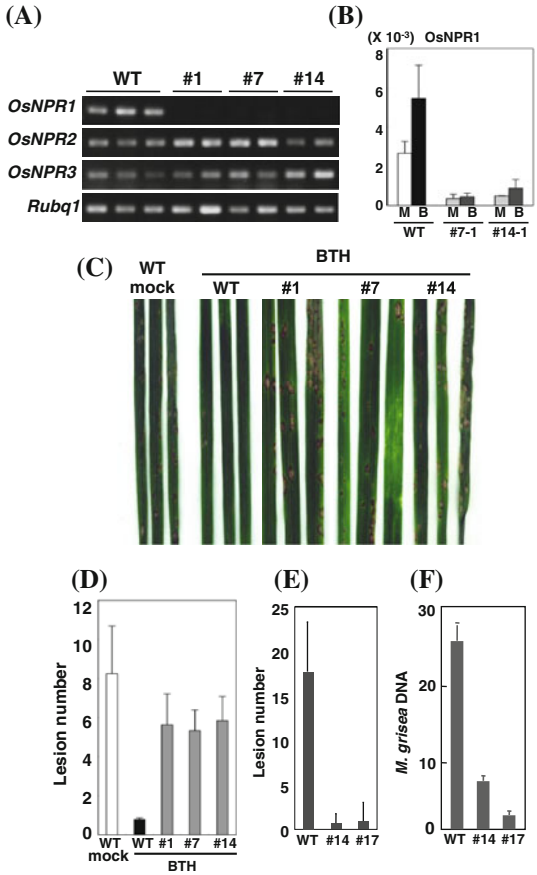


Fig. 1 OsNPR1 is essential for rice blast resistance. **a** Expression of *OsNPR1* and 2 homologous genes in *OsNPR1*-kd transgenic rice plants. *OsNPR1* expression levels in wild-type (WT) rice and 3 T3 *OsNPR1*-kd transgenic lines (lines #1, #7, and #14) were analyzed by semiquantitative RT–PCR. Transcript levels of *OsNPR2* and *OsNPR3* were also examined. The expression of *Rubq1* was used as an internal control. **b** qRT-PCR analysis of *OsNPR1* expression after mock (M) or BTH (B) treatment in wild-type (WT) rice and T3 *OsNPR1*-kd transgenic lines (lines #7 and #14). *Rubq1* was used as an internal control. Mean ± standard error of mean (SEM) values from 3 independent experiments are shown. **c** Disease symptoms on the fourth leaves of mock-treated WT, BTH-treated WT, and BTH-treated *OsNPR1*-kd plants. **d** Numbers of susceptible-type lesions in the central 10-cm region of the fourth leaves of T3 *OsNPR1*-kd and WT rice plants. Mean and SEM were calculated from 3 independent experiments. **e** Blast resistance of *OsNPR1*-ox rice plants. Average blast lesion numbers of 20–25 plants counted as in **d** are shown with SD. **f** In a separate blast-infection experiment, ratios of *M. grisea* 28S rDNA versus rice *Ubi-1* DNA were determined by qRT-PCR. DNA was extracted from each of 20 pooled plants. Averages of triplicate determinations are shown with SD

contrast, BTH application did not cause a major reduction in the number of blast lesions in *OsNPR1*-kd lines (Fig. 1d). These results demonstrate that OsNPR1 is essential for BTH-inducible blast resistance.

We also examined whether OsNPR1 is involved in basal resistance to blast fungus in the absence of BTH treatment. However, we did not observe any significant difference in disease symptoms between WT and *OsNPR1*-kd rice plants (data not shown) after inoculation with three races of blast fungus, race 007, Ken54-04, and 5203-R-21. We also generated transformant rice plants overexpressing *OsNPR1* (*OsNPR1*-ox) and tested them for blast resistance. The results showed that the 2 lines of *OsNPR1*-ox plants showed strong resistance against a compatible blast fungus (race 007, Fig. 1e, f), further supporting the positive role of OsNPR1 in blast resistance. Yuan et al. (2007) reported the absence of blast resistance in *OsNPR1*-ox plants. Differences in experimental conditions—such as expression levels of transgene, growth conditions, plant ages, and the blast fungal races used—may have influenced the blast-resistance phenotype.

Identification of OsNPR1-dependent, BTH-responsive genes using a rice whole-genome oligo microarray

The fact that OsNPR1 is essential for BTH-induced disease resistance prompted us to investigate the details of the defense reactions that OsNPR1 mediates in BTH-treated rice plants. To this end, we analyzed global gene-expression profiles in response to BTH in WT and *OsNPR1*-kd rice plants, using an Agilent rice oligo microarray comprising 29,923 rice genes. A statistical analysis of the results of 4 biological replicate experiments revealed that a total of 8,567 genes were differentially expressed between mock- and BTH-treated WT rice plants with a q-value of <0.05, after applying a false-discovery rate (FDR) multiple-testing correction, according to the method of Benjamini and Hochberg (1995). We selected 2,297 genes that showed >2-fold change and defined them as being BTH-responsive, of which 1,228 and 1,069 genes were upregulated and downregulated, respectively. Thus, the numbers of BTH-upregulated and BTH-downregulated genes identified herein were roughly comparable to those previously reported for *Arabidopsis*: Of the approximate 24,000, 1,147 and 1,133 genes were upregulated and downregulated, respectively, after BTH treatments (Wang et al. 2006). We performed two-way analysis of variance (ANOVA; WT vs. *OsNPR1*-kd, mock vs. BTH) for the microarray results with WT and 2 lines (#7 and #14) of *OsNPR1*-kd plants after mock and BTH treatments, to identify OsNPR1-dependently BTH-responsive genes (Nettleton 2006; Wang et al. 2006). We then selected the genes that either showed a genotype-treatment interaction or were affected by genotype and treatment in both *OsNPR1*-kd lines after applying an FDR multiple testing correction with a criteria of $q < 0.1$ (Benjamini and Hochberg 1995). The genes that passed these filters represented those whose BTH-responsiveness was at least partially negated by *OsNPR1*-knockdown, and therefore defined as OsNPR1-dependent BTH-responsive genes. Consequently, the BTH-

responsiveness of 358 genes out of the 1,228 BTH-upregulated genes was found to be OsNPR1-dependent (29%); meanwhile, that of 724 genes out of the 1,069 BTH-downregulated genes was OsNPR1-dependent (68%).

Detailed lists of BTH-responsive and OsNPR1-dependent genes are available in the supplemental data (Tables S1 and S2). Quantitative real-time polymerase chain reaction (qRT–PCR) analysis for several arbitrarily chosen representative genes completely reproduced the expression patterns in the microarray data with respect to both BTH-responsiveness and OsNPR1-dependence, thus validating the reliability of the microarray data (Fig. 2).

Functional categorization of BTH-responsive and OsNPR1-dependent genes

To deduce the effects of BTH application on various biological processes from the (predicted) functions of BTH-regulated genes, we analyzed the BTH-responsive genes using Gene Ontology terms (Gene-Ontology-Consortium 2006; Harris et al. 2004). This analysis showed that BTH regulated a broad range of genes with diverse functions classified into several categories (Fig. 3a). Among the BTH-responsive genes, upregulated genes outnumbered the downregulated genes, in nine out of 13 categories; downregulated genes dominated only two categories, i.e., "photosynthesis" and "transcription." The genes in each category were further analyzed with respect to the OsNPR1-dependence of their BTH-responsiveness (Fig. 3b, c). In contrast to all the BTH-responsive genes, OsNPR1-dependent genes were dominated by downregulated genes in many categories: Upregulated and downregulated genes dominated three and eight categories, respectively (Fig. 3, 3c).

NPR1-dependent genes in *Arabidopsis* (Wang et al. 2006) were analyzed in a similar manner for comparison (Fig. 3d). Results showed that the genes categorized as photosynthesis and translation-related genes were dominated by downregulated genes, as is the case with OsNPR1-dependent genes in rice; thus, this is a common feature of (Os)NPR1-dependent genes in rice and *Arabidopsis*. Among *Arabidopsis* NPR1-dependent genes in many categories, the proportions of upregulated genes were greater than or similar to those of downregulated genes, unlike the rice OsNPR1-dependent genes. Another evident difference was noted in the genes categorized under "defense response." While all of the BTH-upregulated genes categorized under "defense response" were NPR1-dependent in *Arabidopsis*, only 5.9% of those were OsNPR1-dependent in rice.

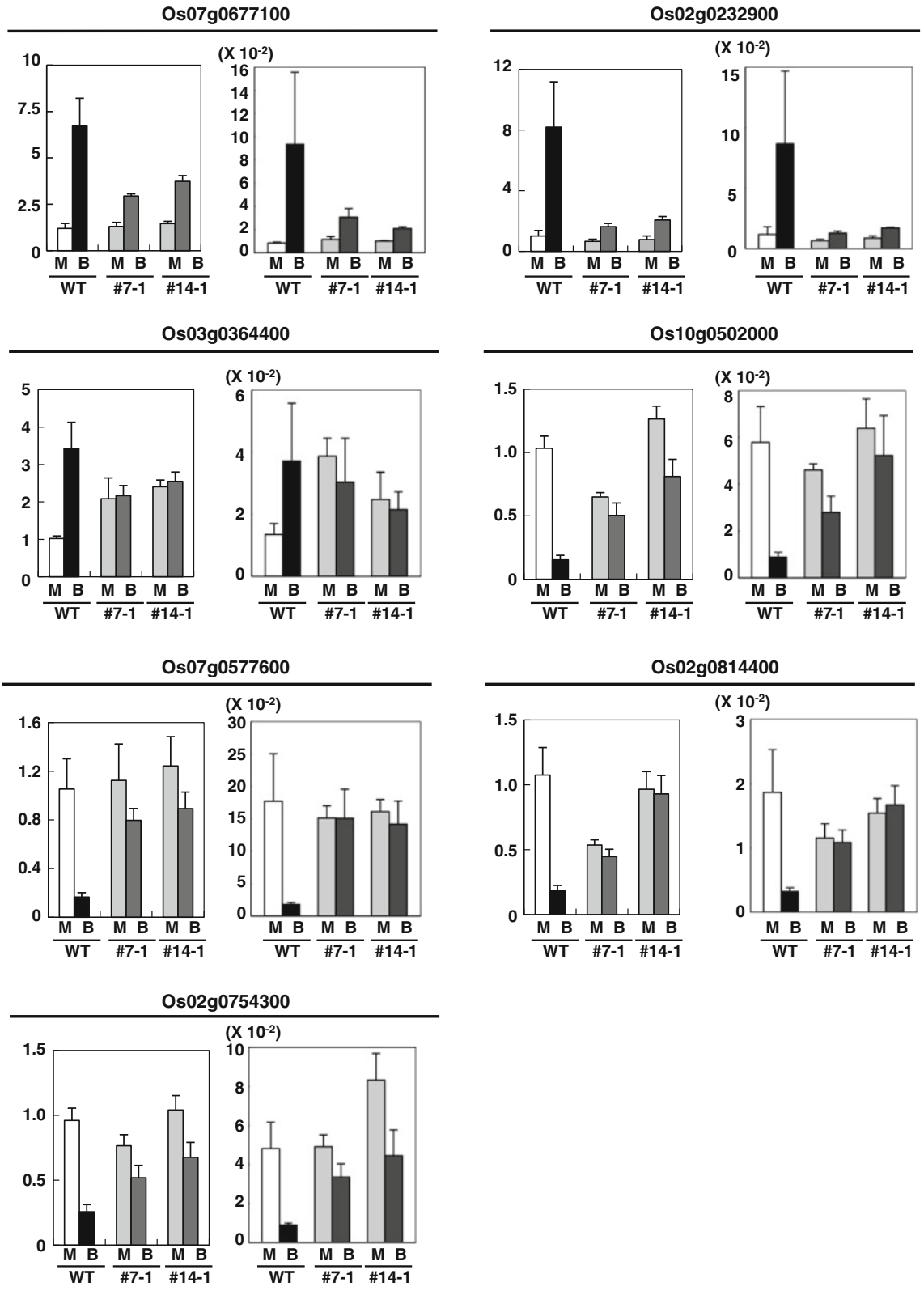
We further categorized (Os)NPR1-dependently BTH-responsive genes both in rice and *Arabidopsis* using BioMaps (Hayano-Kanashiro et al. 2009). As shown in Tables S5 and S6, genes involved in various photosynthetic processes were significantly enriched in OsNPR1-dependently BTH-downregulated genes in rice. On the other hand, genes including those involved in translation, amino acid and polysaccharide metabolic processes, and those encoding proteins with chitinase activity and glutathione transferase activity were enriched in OsNPR1-independently BTH-upregulated genes (Table S7). In *Arabidopsis*, genes involved in defense response and protein phosphorylation were significantly enriched in NPR1-upregulated genes (Table S8), whereas genes involved in response to abiotic stimulus, lipid metabolic process, carbohydrate metabolic process and photosynthesis were enriched in NPR1-downregulated genes (Table S9). These results reveal some differences in the categories of genes that are regulated by (Os)NPR1 in rice and *Arabidopsis*.

Fig. 2 Validation of microarray data by qRT-PCR. Expression data of arbitrarily chosen representative genes from the microarray (*left*) and qRT-PCR (*right*) experiments are shown in a pairwise manner. T3 homozygous *OsNPR1*-kd (lines #7-1 and #14-1) and wild-type rice plants were treated with either DMSO (mock, M) or 10 μM BTH (B) in DMSO. The third and fourth leaves were harvested 24 h after BTH treatment. RNAs extracted from these samples, which were also analyzed by microarray, were analyzed by qRT-PCR for some arbitrary chosen representative genes to validate the BTH-responsiveness and OsNPR1-dependence of expression in microarray analysis. Expression of endogenous *OsNPR1* was also examined. Mean ± SEM values from 3 independent experiments are shown. *Rubq1* expression was used as an internal control. Putative functions of analyzed genes are as follows: Os07g0677100, peroxidase; Os02g0232900, aquaporin NIP1-1 (OsNIP1;1); Os03g0364400, protein kinase domain containing protein; Os10g0502000, thylakoid lumenal 17.4 kDa protein, chloroplast precursor (P17.4); Os07g0577600, Lhca2 protein; Os02g0814400, cytochrome c domain containing protein; Os02g0754300, 50S ribosomal protein L29, chloroplast precursor. Experiments were repeated three times with similar results

OsNPR1 mediates BTH-induced downregulation of photosynthesis and chloroplast-related genes

We further inspected the functions of BTH-responsive and OsNPR1-dependent genes in reference to known metabolic pathways by using KEGG pathway maps (Kanehisa et al. 2008; Kanehisa et al. 2004). This analysis revealed that the genes involved in various photosynthesis processes were coordinately downregulated by BTH in an OsNPR1-dependent manner (Table 1). These included several genes that encoded components of photosystems I and II, as well as the light-harvesting chlorophyll complex (Table 1, Figs. S2 and S3). These genes also included those involved in the Calvin cycle and in the biosynthesis of porphyrin and chlorophyll (Table 1; Fig. S1).

A number of genes involved in chloroplastic translation and transcription were found to be downregulated by BTH in an OsNPR1-dependent manner. These included the genes for



the components of 50S and 30S subunits of chloroplastic ribosome; their BTH responses were exclusively OsNPR1-dependent (Table 2). The genes for chloroplastic ribosomes were significantly enriched in OsNPR1-dependently BTH-downregulated genes (*P*-value: 9.33E-32); chloroplastic ribosomal genes were also enriched in NPR1-dependently

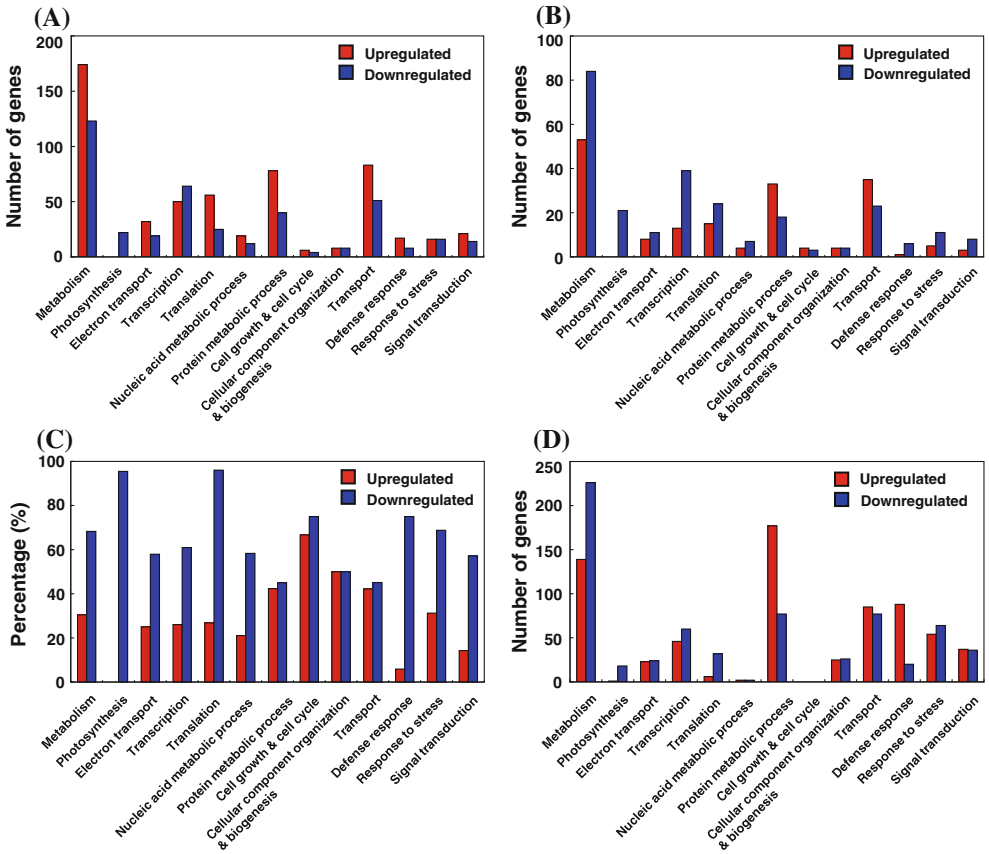


Fig. 3 Functional classification of OsNPR1-dependent and BTH-responsive genes. **a** Total number of BTH-upregulated and BTH-downregulated rice genes in each functional category. The Y-axis shows the number of genes that exhibited >2-fold or <0.5-fold differential expression, after BTH treatments. Genes in different functional categories according to the Gene Ontology database were analyzed. **b** Total number of OsNPR1-dependent rice genes among BTH-responsive genes. The Y-axis shows the number of genes whose expression is OsNPR1-dependent. **c** Percentage of OsNPR1-dependent rice genes among the BTH-upregulated and BTH-downregulated genes in each functional category. **d** Total number of BTH-upregulated and -downregulated *Arabidopsis* genes in each functional category. Y-axis shows the number of genes that exhibit >2- or <0.5-fold differential expression, after BTH treatments. Functional categories are based on the Gene Ontology database in the Arabidopsis Information Resource (http://www.arabidopsis.org/)

BTH-downregulated genes in *Arabidopsis* (*P*-value: 6.95E-12). Nuclear-encoded sigma factors are known to regulate organelle transcription (Ishizaki et al. 2005; Kanamaru et al. 2001); the genes for 3 rice sigma factors (Kasai et al. 2004) were downregulated by BTH in an OsNPR1-dependent manner (Table 2). Thus, OsNPR1 mediates the downregulation of the genes for chloroplastic translation and transcription. Of note, several genes for the 60S and 40S subunits of cytoplasmic ribosome were upregulated by BTH (Table 2)—some of them (26%) OsNPR1-dependently. Collectively, OsNPR1 plays a role in the suppression of chloroplastic translation and transcription while activating cytoplasmic translation, thereby coordinating overall cellular activity upon SA-pathway-mediated defense responses.

To examine whether the changes in gene expression of photosynthetic genes and those involved in chloroplastic translation and transcription were reflected in photosynthetic activities, we determined the changes in leaf maximum PSII quantum yield (Fv/Fm), which is the maximum quantum yield of photosystem II, after BTH treatment in WT and *OsNPR1* knockdown rice plants (Fig. 4). Before the treatments, there was no significant difference in Fv/Fm values between WT and the 2 *OsNPR1*-kd lines (0.80–0.82). Some decreases in Fv/Fm were observed even in control mock-treated WT plants after control treatment, presumably reflecting suboptimal growth conditions. Notably, the decreases in Fv/Fm values were markedly faster in BTH-treated than in the control plants, indicating that the BTH treatment reduced photosynthetic activity. In addition, in *OsNPR1*-knockdown lines, the BTH-induced decreases in Fv/Fm values were negated; the values were even higher than those in the control plants. These results are essentially consistent with the downregulation of photosynthetic (and chloroplastic translational and transcriptional) genes by BTH and its negation by *OsNPR1* knockdown.

Table 1 List of OsNPR1-dependently BTH-downregulated genes involved in photosynthesis

Locus ID	Fold change (BTH/mock in WT)	Fold change (BTH/mock in *OsNPR1*-kd #14)	Fold change [WT (BTH)/ *OsNPR1*-kd #14 (BTH)]	*q*-value (OsNPR1-dependen-cy)	Description
Os04g0457000	0.14	0.91	0.22	8.80E-02	Chlorophyll a/b-binding protein CP24
Os07g0558400	0.21	0.78	0.36	9.62E-02	Chlorophyll a/b-binding protein CP29 precursor
Os02g0197600	0.35	0.65	0.37	3.28E-03	Chlorophyll a/b-binding protein type III
Os06g0320500	0.30	0.75	0.41	9.14E-02	Chlorophyll a/b-binding protein
Os11g0242800	0.42	0.87	0.30	6.20E-03	Chlorophyll a/b-binding protein family protein
Os09g0439500	0.12	0.79	0.35	5.07E-02	Chlorophyll a/b-binding protein family protein
Os07g0562700	0.46	0.72	0.43	2.49E-03	Chlorophyll a/b-binding protein type III
Os07g0577600	0.17	0.75	0.21	6.78E-02	Lhca2 protein
Os08g0435900	0.16	0.74	0.20	9.01E-02	Lhca4 protein, light-harvesting complex protein of photosystem I precursor
Os03g0563300	0.39	0.91	0.47	8.65E-04	Magnesium-chelatase subunit chlI, chloroplast precursor (Mg-protoporphyrin IX chelatase)
Os07g0544800	0.27	0.75	0.31	5.67E-02	Oxygen-evolving enhancer protein 3-2, chloroplast precursor (OEE3)
Os08g0560900	0.28	0.71	0.34	9.32E-04	Photosystem I reaction center subunit II, chloroplast precursor
Os03g0778100	0.28	0.75	0.35	8.30E-04	Photosystem I reaction center subunit III, chloroplast precursor
Os07g0435300	0.29	0.79	0.32	8.41E-02	Photosystem I reaction center subunit IV, chloroplast precursor (PSI- E)
Os07g0148900	0.24	0.83	0.26	5.27E-03	Photosystem I reaction center subunit psaK, chloroplast precursor
Os09g0481200	0.22	0.95	0.23	5.28E-03	Photosystem I reaction center subunit V (PSI-G)
Os05g0560000	0.19	0.61	0.26	8.99E-02	Photosystem I reaction center subunit VI, chloroplast precursor (PSI- H)
Os12g0420400	0.35	0.75	0.40	9.99E-02	Photosystem I reaction center subunit XI, chloroplast precursor (PSI- L)
Os07g0105600	0.26	0.74	0.38	4.46E-04	Photosystem II oxygen evolving complex protein PsbQ family protein
Os02g0578400	0.14	0.56	0.43	6.65E-02	Photosystem II oxygen evolving complex protein PsbQ family protein
Os03g0343900	0.15	0.93	0.22	4.47E-02	Photosystem II protein PsbX family protein.
Os12g0291400	0.079	1.19	0.41	3.18E-02	Ribulose 1,5-bisphosphate carboxylase small subunit
Os12g0292400	0.19	1.18	0.48	9.01E-02	Ribulose 1,5-bisphosphate carboxylase small subunit
Os12g0291100	0.23	1.11	0.32	7.22E-02	Ribulose 1,5-bisphosphate carboxylase, small subunit
Os03g0781400	0.28	0.76	0.59	7.33E-02	Ribose 5-phosphate isomerase family protein.

The reason for the higher Fv/Fm in *OsNPR1*-kd lines than in WT is unclear. It could be an indirect effect of the BTH-induced activation of the WRKY45-dependent pathway, which is basically independent of OsNPR1 (Shimono et al. 2007).

OsNPR1 regulates BTH-downregulation of ABA-responsive genes

We also noted that OsNPR1-dependently BTH-downregulated genes include those bearing annotations suggestive of ABA-responsiveness (Table S2). Of these, Os01g0348900 (*SalT*) and Os01g0705200 (*OsWSI18*) have been reported to be indeed ABA-responsive (Shobbar et al. 2008). Os06g0324400 encodes late embryogenesis-abundant (LEA) protein. We then examined the ABA responses of these genes in leaf-disc assays by using qRT–PCR, demonstrating that all these genes were indeed ABA-inducible (Fig. 5). Moreover, ABA-induced upregulation of these genes was negated by the simultaneous addition of SA (Fig. 5). However, the suppression of their ABA-induced upregulation by SA was not affected in *OsNPR1*-kd plants.

Table 2 List of BTH-responsive and OsNPR1-dependent genes involved in translation and transcription

Locus ID	Fold change (BTH/mock in WT)	Fold change (BTH/mock in *OsNPR1*-kd #14)	Fold change [WT (BTH)/ *OsNPR1*-kd #14 (BTH)]	*q*-value (OsNPR1-dependency)	Description
Os07g0207400	2.80	1.60	1.80	3.84E-03	40S ribosomal protein S2
Os02g0287000	2.36	1.54	1.66	3.26E-02	40S ribosomal protein S3a
Os02g0105900	2.66	1.47	1.53	5.89E-03	40S ribosomal protein S4
Os02g0162500	2.01	1.25	1.43	2.21E-02	40S ribosomal protein S14
Os07g0616600	2.47	1.49	1.68	9.16E-04	40S ribosomal protein SA
Os04g0598200	2.13	1.33	1.44	9.56E-02	60S ribosomal protein L12
Os12g0150100	2.26	1.41	1.56	1.15E-02	60S ribosomal protein L26A
Os08g0156800	2.23	1.49	1.66	1.88E-03	60S ribosomal protein L34
Os08g0436800	2.09	1.31	1.72	2.07E-03	60S ribosomal protein L34
Os09g0568400	2.25	1.43	1.58	2.42E-03	60S ribosomal protein L40
Os07g0450000	2.09	1.34	1.55	1.38E-02	60S ribosomal protein L44
Os07g0224000	2.06	1.44	1.58	6.85E-02	Ribosomal protein L24E family protein
Os08g0542100	2.13	1.23	1.53	9.11E-02	Ribosomal protein L30 family protein
Os02g0111700	2.12	1.36	1.53	3.41E-02	Ribosomal protein L37e family protein
Os11g0482000	2.02	1.45	1.43	7.56E-03	Ribosomal protein S7 family protein
Os03g0452300	0.32	0.71	0.61	8.08E-02	30S ribosomal protein S5
Os03g0843400	0.30	0.80	0.51	7.53E-02	30S ribosomal protein S6, chloroplast precursor
Os03g0704000	0.29	0.66	0.47	8.11E-02	30S ribosomal protein S13, chloroplast precursor
Os02g0137200	0.47	0.83	0.71	5.10E-03	50S ribosomal protein L3-1, chloroplast precursor
Os03g0265400	0.29	0.63	0.53	1.27E-02	50S ribosomal protein L4, chloroplast precursor
Os03g0125000	0.31	0.78	0.52	7.22E-02	50S ribosomal protein L5, chloroplast precursor
Os02g0822600	0.31	0.76	0.59	5.25E-03	50S ribosomal protein L9, chloroplast precursor
Os03g0122200	0.47	0.85	0.60	4.32E-04	50S ribosomal protein L11
Os01g0749200	0.39	0.75	0.50	1.69E-03	50S ribosomal protein L13
Os03g0219900	0.33	0.72	0.51	8.38E-02	50S ribosomal protein L15, chloroplast precursor
Os03g0828100	0.32	0.70	0.63	1.03E-03	50S ribosomal protein L18
Os02g0259600	0.35	0.72	0.59	9.07E-02	50S ribosomal protein L21, chloroplast precursor
Os02g0754300	0.24	0.68	0.49	7.00E-02	50S ribosomal protein L29, chloroplast precursor
Os01g0805000	0.22	0.71	0.38	7.43E-02	50S ribosomal protein L34
Os03g0196800	0.48	0.74	0.58	8.81E-02	Cyanelle 30S ribosomal protein S10
Os11g0216900	0.31	0.82	0.73	7.41E-02	IDI2
Os03g0284400	0.29	0.75	0.44	6.48E-02	Ribosomal protein L10-like
Os03g0815400	0.31	0.90	0.55	8.54E-02	Ribosomal protein L17 family protein
Os03g0356300	0.30	0.79	0.54	6.48E-02	Ribosomal protein L6 family protein
Os01g0678600	0.28	0.72	0.42	7.31E-02	Ribosomal protein S20 family protein
Os07g0570700	0.43	0.70	0.60	2.64E-03	Ribosome recycling factor, chloroplast precursor
Os03g0271100	0.29	0.71	0.39	4.69E-02	Sigma factor SIG2B.
Os08g0242800	0.44	0.76	0.44	9.28E-03	Sigma factor SIG6
Os11g0448400	0.49	0.84	0.72	2.79E-02	Sigma factor SIG2A

Collectively, these results suggest that SA-induced down-regulation of these ABA-responsive genes from their basal expression level is OsNPR1-dependent, but antagonistic effect of SA on their ABA-induced upregulation is through OsNPR1-independent mechanism.

BTH-responsiveness and OsNPR1-dependence of TF genes

The BTH-responsive genes included the genes for a number of TFs, some of which were OsNPR1-dependent.

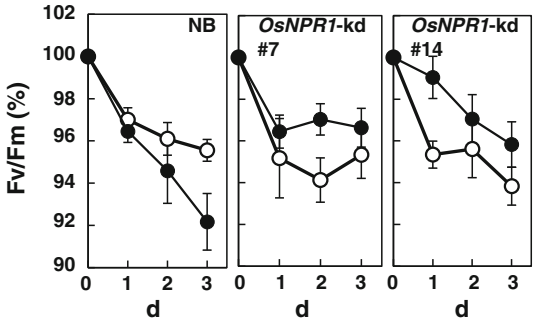


Fig. 4 Effects of BTH and *OsNPR1* knockdown on leaf maximum PSII quantum yield (Fv/Fm). The Fv/Fm values after BTH (*closed circles*) and mock (*open circles*) treatments were expressed as relative to those measured before the treatments. Fv/Fm values on day 0 (i.e., before treatments) were 0.810 ± 0.004 (mean ± S.E., $n = 4$) and 0.810 ± 0.005 for mock and BTH-treated wild type, respectively. The values were 0.812 ± 0.007 (control) and 0.818 ± 0.003 (BTH) in *OsNPR1*-kd #7, and 0.810 ± 0.004 (mock) and 0.799 ± 0.006 (BTH) in *OsNPR1*-kd #14

Particular families of TFs are often associated with specific biological processes; we inspected the BTH-responsiveness and OsNPR1-dependence of the TF genes in different classes with this view (Table 3).

A total of 8 *AUX/IAA*-type TFs were downregulated by BTH—5 of them in an OsNPR1-dependent manner (Table 3). Two *ARF* genes were also downregulated by BTH in an OsNPR1-dependent manner. Both AUX/IAAs and ARFs are associated with auxin-related regulation. The downregulation of several *AUX/IAA* and *ARF* genes has also been reported in *Arabidopsis*; it was presumably indirectly NPR1-dependent (Wang et al. 2007). Therefore, (Os)NPR1 suppresses the auxin-related regulatory pathway in response to BTH in rice and *Arabidopsis*, in a similar manner.

BTH-responsiveness and OsNPR1-dependence of other genes

We also inspected the BTH-responsiveness and OsNPR1-dependence of the genes involved in some metabolic pathways. The genes involved in the TCA cycle, and the shikimate, aromatic-acid biosynthetic, and phenylpropanoid pathways were upregulated by BTH. The upregulation of these genes should lead to the biosynthesis of phenylpropanoid compounds, such as lignins and phytoalexins, which may play a role in plant defense. Interestingly, unlike in *Arabidopsis*, only a few of them were OsNPR1-dependent.

Discussion

In previous studies, it has been reported that rice shares a defense-signaling pathway similar to the NPR1-mediated

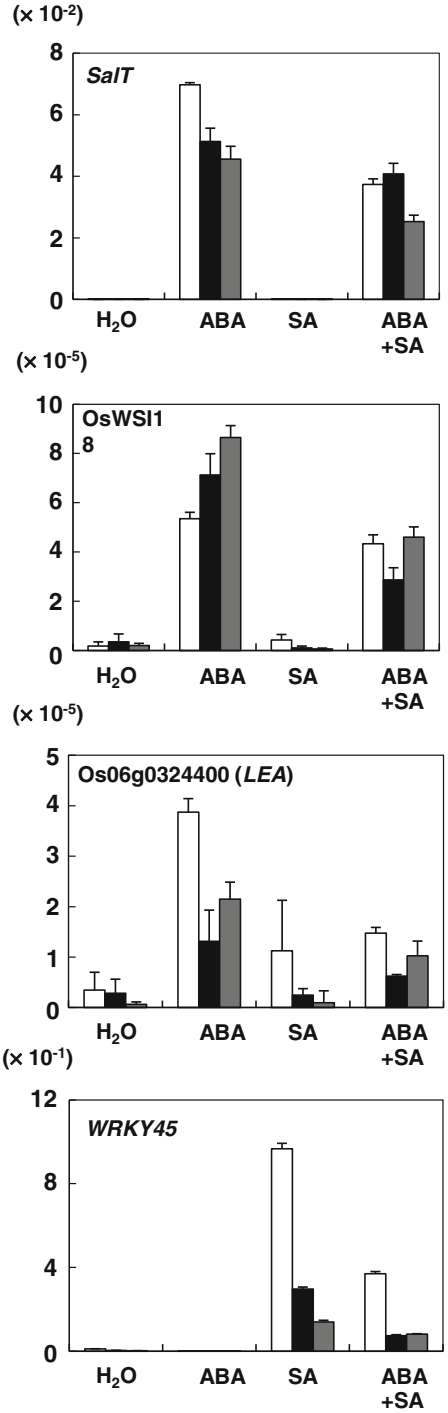


Fig. 5 ABA-responsive expression of OsNPR1-regulated genes, and their suppression by SA. Expression of genes in leaf discs from wild type (*white bar*), *OsNPR1*-kd lines #7 (*black bar*), and #14 (*gray bar*) at 4 h after treatments with SA, ABA, SA, and ABA or control H_2O. The concentrations of applied SA and ABA solutions were 1 mM and 50 μM, respectively. Responses of *WRKY45* were examined as control of treatments. Expression levels in qRT-PCR relative to those of *Rubq1* as an internal control are shown. Averages of 3 determinations are shown, along with SD. The primer pairs used are listed in Table S4

Table 3 List of BTH-responsive and OsNPR1-dependent genes for transcription factors

Locus ID	Fold change (BTH/mock in WT)	Fold change (BTH/mock in *OsNPR1*-kd #14)	Fold change [WT (BTH)/ *OsNPR1*-kd #14 (BTH)]	*q*-value (OsNPR1-dependen-cy)	Description
Os09g0501600	2.14	1.20	1.46	4.67E-02	Basic helix-loop-helix dimerisation region
Os06g0622700	2.22	1.56	1.24	5.07E-02	Basic-leucine zipper transcription factor
Os03g0723000	3.42	1.86	2.43	8.32E-02	GRAS transcription factor domain
Os11g0705800	2.41	1.26	1.34	2.00E-02	Scarecrow-like 9
Os04g0517100	2.77	1.44	1.96	8.98E-04	Myb protein
Os01g0816100	3.04	1.12	1.58	8.23E-02	NAC-domain containing protein 2
Os08g0562200	3.25	1.92	1.48	1.41E-02	No apical meristem (NAM) protein domain
Os04g0550200	4.52	1.74	1.49	7.03E-02	Pathogenesis-related transcription factor and ERF domain
Os01g0868000	3.44	1.41	1.54	4.45E-03	Pathogenesis-related transcription factor and ERF domain
Os05g0230700	0.45	0.60	0.59	4.83E-02	Auxin-responsive protein IAA2
Os01g0231000	0.46	0.67	0.60	1.58E-02	Auxin-responsive protein (Aux/IAA)
Os01g0178500	0.39	0.32	0.73	4.92E-03	AUX/IAA protein family protein
Os02g0805100	0.41	0.55	0.37	1.94E-02	AUX/IAA protein family protein
Os05g0230700	0.45	0.60	0.59	4.83E-02	Auxin-responsive protein IAA2
Os05g0563400	0.32	0.63	0.49	6.31E-04	Auxin response factor 5
Os01g0236300	0.38	0.54	0.55	1.36E-04	Auxin response factor 18
Os01g0900800	0.47	0.57	0.65	5.40E-03	Basic helix-loop-helix dimerisation region
Os07g0143200	0.49	0.76	0.63	1.25E-02	Basic helix-loop-helix dimerisation region
Os06g0172000	0.36	0.68	0.49	5.89E-04	Basic helix-loop-helix dimerisation region
Os02g0128200	0.48	0.88	0.54	6.50E-02	Basic-leucine zipper transcription factor
Os01g0808100	0.29	0.64	0.38	1.05E-03	Transcription factor HBP-1b(c1)
Os02g0148000	0.16	0.83	0.35	7.07E-02	Zinc finger protein
Os02g0731700	0.35	0.71	0.40	7.67E-02	CONSTANS-like 1 protein
Os08g0499300	0.29	0.63	0.41	2.42E-02	WRKY transcription factor 30
Os04g0683900	0.50	0.74	0.40	2.98E-04	HMG-I and HMG-Y
Os12g0160500	0.35	0.64	0.47	5.02E-02	Homeobox domain containing protein
Os08g0416000	0.47	0.72	0.59	9.21E-02	Homeodomain leucine zipper protein
Os03g0198600	0.03	0.67	0.33	5.20E-02	Homeodomain leucine zipper protein CPHB-7
Os05g0579300	0.39	0.81	0.71	9.82E-02	ZF-HD homeobox protein, Cys/His-rich
Os05g0195700	0.47	0.92	0.84	5.63E-02	Transcription factor MYBS2
Os09g0401000	0.42	1.11	0.41	2.73E-02	Myb-related protein Pp2
Os08g0549000	0.29	0.80	0.30	9.85E-02	Myb, DNA-binding domain
Os11g0700500	0.19	0.77	0.35	9.45E-02	Myb, DNA-binding domain
Os02g0618400	0.21	0.68	0.43	7.49E-02	MYB8 protein
Os05g0589400	0.28	0.68	0.48	6.88E-02	I-box binding factor
Os03g0113500	0.38	0.68	0.61	4.89E-02	GT-2 factor
Os04g0541100	0.27	0.83	0.75	4.69E-02	GT-2
Os02g0810900	0.28	1.06	0.61	4.67E-02	NAC-domain containing protein 21/22
Os02g0555300	0.10	0.60	0.40	6.50E-02	No apical meristem (NAM) protein domain
Os10g0477600	0.14	0.65	0.51	7.22E-02	OsNAC7 protein
Os11g0448400	0.49	0.84	0.72	2.79E-02	RNA polymerase, sigma subunit family
Os08g0242800	0.44	0.76	0.44	9.28E-03	Sigma factor SIG6
Os03g0271100	0.29	0.71	0.39	4.69E-02	Sigma factor SIG2B
Os08g0112700	0.47	0.59	0.68	4.15E-02	TAGL12 transcription factor
Os01g0871200	0.49	0.72	0.67	2.34E-04	Zn-finger, C2H2 type domain
Os06g0571800	0.44	0.85	0.67	9.97E-02	Zn-finger, GATA type domain

pathway in dicots, and that components of this pathway largely share functions common to both rice and dicots (Chern et al. 2005; Yuan et al. 2007). In addition to the role of OsNPR1 in bacterial leaf-blight resistance (Chern et al. 2005; Yuan et al. 2007), in this study, we demonstrated that OsNPR1 plays an essential role in BTH-induced resistance against fungal blast disease by showing that BTH-inducible blast resistance was compromised in *OsNPR1*-kd rice plants and that an overexpression of *OsNPR1* conferred blast resistance to rice (Fig. 1). These results indicate that the role of OsNPR1 in the (chemical-induced) resistance to biotrophic and hemibiotrophic pathogens is consistent with rice sharing a SA-signaling pathway similar to those observed in dicots. We showed in our previous study that the SA-signaling pathway of rice branches into 2 subpathways downstream of SA—that is, the OsNPR1-dependent and WRKY45-dependent ones (Shimono et al. 2007). The WRKY45-dependent pathway is also essential for BTH-induced resistance to blast disease (Shimono et al. 2007). The question then arises as to how OsNPR1 and WRKY45 share their functions in the SA pathway in rice. In *Arabidopsis*, nearly all the BTH-responsive genes are NPR1-dependent (Wang et al. 2006), although larger proportion of genes is NPR-independent in early response to SA (Blanco et al. 2009). In contrast, we showed in this study that only a subset of BTH-responsive genes were OsNPR1-dependent in rice. The genes upregulated by BTH were less OsNPR1-dependent than those downregulated by BTH (Fig. 3c). These results imply that the role of OsNPR1 in the SA-signaling pathway of rice is rather limited as compared to that of NPR1 in *Arabidopsis*, and thus reflects the branched SA pathway in rice (Shimono et al. 2007). We cannot completely rule out the possibility that the partialness of the OsNPR1-dependence is (partly) due to incomplete suppression of *OsNPR1* and/or the possibility that our analysis of the microarray data is somehow biased toward BTH-downregulated genes due to statistical factors (Fig. 2). Nevertheless, the extensively compromised blast resistance phenotype in *OsNPR1*-knockdown rice suggests that OsNPR1 function is substantially impaired in these plants and hence the validity of the microarray results seems to be supported by this observation (Fig. 1c, d).

Many studies have shown that biotrophic pathogens, such as rusts, mildews, and powdery mildews, cause a reduction in the rate of photosynthesis and a loss of chlorophylls from infected leaves as a whole (Schloles 1992). It has also been recognized that reductions in photosynthesis transcripts/proteins are common in defense responses (Somssich and Hahlbrock 1998). In rice, too, a coordinated repression of transcripts for chloroplast-related genes, including photosynthetic genes, has been observed after infection with the rice dwarf virus (Shimizu et al. 2007). The biological significance of the suppression of photosynthetic activities *vis-à-vis* pathogen infection has been discussed in relation to the reallocation of resources from plant growth to defense reactions as part of defense responses (Lerdau 1992). This is relevant to fitness cost that defense reactions impose on plant growth and development. Production of defensive proteins during SAR requires large amounts of resources and consequently reduces plant fitness, in particular under nutrition-limited conditions (Heidel et al. 2004; Heidel and Dong 2006). Contribution of NPR1 in reducing the fitness cost of SAR has been reported in *Arabidopsis* (Heidel et al. 2004; Heidel and Dong 2006; van Hulten et al. 2006). We found in this study that photosynthesis-related genes, as well as chloroplastic ribosomal genes, were transcriptionally repressed OsNPR1-dependently when the SA-signaling pathway was activated (Tables 1, 2), which led to a reduction of photosynthetic activities (Fig. 4). *Arabidopsis* NPR1 was also found to have similar functions (Fig. 3d). Because photosynthetic proteins account for high proportion of cellular proteins in plants, this regulation presumably saves substantial amounts of amino acids to be used for production of defensive proteins. Thus, the allocation of resources from household activities to defensive activities during SAR seems to be one of common functions of (Os)NPR1 in rice and *Arabidopsis*.

In *Arabidopsis*, the NPR1-mediated suppression of ABA-responsive gene expression has been reported (Yasuda et al. 2008). Expression of some ABA-responsive genes is suppressed by BTH/SA also in rice (Jiang et al. 2010). However, our data shows that this regulation is independent of OsNPR1, unlike in *Arabidopsis*. Thus, the SA pathway acts antagonistically upon the ABA-signaling pathway in the two plant species in common, but the antagonistic interactions in them differ in their (Os)NPR1-dependence. We previously proposed that the SA pathway in rice is branched into WRKY45-dependent and OsNPR1-dependent pathways downstream of SA (Shimono et al. 2007), and ABA has negative impacts on both pathways (Jiang et al. 2010). The branched nature of the SA pathway may account for the difference in (Os)NPR1 dependency in the SA-ABA interaction between rice and in *Arabidopsis*.

In summary, our results demonstrate that OsNPR1 plays a crucial role in SA-mediated defense signaling, which is also effective against blast disease. Our data shed new light on the role of OsNPR1 including its suppression of chloroplast activities and photosynthesis. The information should help advance an understanding of the SA/BTH-signaling pathway in rice, a major staple food worldwide.

Materials and methods

Plant materials, chemicals, and pathogen treatments

All experiments were carried out with rice (*Oryza sativa*) cv. Nipponbare. Plants were grown in a growth chamber as described previously (Shimono et al. 2007). For BTH treatments, rice seedlings at the four-leaf stage were excised about 5 mm above the soil surface and held for 24 h in a solution containing 10 μM BTH in 0.01% dimethyl sulfoxide (DMSO) or the solvent only (mock treatment). For *M. grisea* inoculations, spores were suspended in 0.02% Tween 20 at a density of 10^5/mL and sprayed onto rice plants as described previously (Shimono et al. 2007). Disease symptoms were evaluated by lesion numbers and fungal DNA contents. Fungal DNA contents were determined by RT–PCR-based quantification of *M. grisea* 28S rDNA in rice leaves (Qi and Yang 2002). Fungal growth was evaluated by the ratio of *M. grisea* 28S rDNA versus rice *Ubi-1* DNA. Mann–Whitney U test was used to determine if the effects of the *OsNPR1* mutants on disease symptoms were statistically significant.

Plasmid construction and plant transformation

To construct a plasmid for *OsNPR1* RNAi, part of the *OsNPR1* cDNA was amplified by PCR, using the primers NL0RiF2 (5′-CGAGGACAAGGAGGAGAATG-3′) and NL0RiR2 (5′-ACGAGCAAACCACTGGAAAT-3′); it was then inserted into the pANDA vector, as described previously (Miki et al. 2005; Miki and Shimamoto 2004; Shimono et al. 2007). Rice was transformed by an *Agrobacterium tumefaciens* (strain EHA105)-mediated procedure, as described previously (Toki et al. 2006).

Microarray analysis

Total RNA was isolated from the fourth leaves of BTH- or mock-treated rice plants and labeled with Cy3, as described previously (Shimono et al. 2007). Cy3-labeled probes (1 mg each) were hybridized to an Agilent Rice Oligo Microarray (44 K, custom-made; Agilent Technologies). Four biological replicate sample sets from BTH- and mock-treated leaves were analyzed. All microarray experiments, including data analysis, were carried out as described previously (Shimono et al. 2007; Wang et al. 2006). Briefly, BTH-responsive genes in WT were identified on the basis of statistical significance with an ANOVA q-value <0.05, after applying an FDR multiple-testing correction (Benjamini and Hochberg 1995) and fold change (>2). These genes were filtered through a two-way ANOVA, with consideration for both genotype (WT vs. *OsNPR1*-kd) and treatment (mock vs. BTH) effects. We then selected the genes that either showed a genotype-treatment interaction or were affected by genotype and treatment in both *OsNPR1*-kd lines #7 and #14 with $q < 0.1$ (Benjamini and Hochberg 1995). For the functional assignment of BTH-responsive genes, Gene Ontology terms (http://www.geneontology.org/) were used. We obtained Gene Ontology and annotations of rice (*Oryza sativa*) cv. Nipponbare and *Arabidopsis thaliana* genomes from RAP-DB (http://rapdb.dna.affrc.go.jp/index.html) and TAIR (http://www.arabidopsis.org/), respectively. For the analysis of microarray data in conjunction with KEGG pathway maps (http://www.genome.jp/kegg/pathway.html), the KeggArray tool (0.5.3 beta; http://www.genome.jp/kegg/kegg4.html) was used. For functional classification of differentially expressed transcripts, the microarray data was analyzed using BioMaps at the Virtual-Plant site (http://virtualplant.bio.nyu.edu). Statistic significance of differential expression of the genes for functional category of ribosome was examined by a Chi-squared test based on the Gene Ontology terms obtained from the Virtual-Plant site.

RT-PCR analysis

RT-PCR and qRT-PCR was performed as described previously (Shimono et al. 2007). *Rice ubiquitin 1* (*Rubq1*; AK121590) was used as an internal standard. PCR primers used in this study are listed in Table S4; the primer sets were tested by dissociation curve analysis and verified for the absence of nonspecific amplification.

Determination of chlorophyll fluorescence

Rice plants at the seventh-leaf emergence stage were transferred from a greenhouse to a growth chamber and grown during the taking of chlorophyll fluorescence measurements. The condition in the growth chamber were 16/8 h of day/night cycle, day/night temperatures of 28/23°C and a light intensity at plant height of 300 μmol m^{-2} s^{-1}. WT and *OsNPR1*-kd rice plants were supplied with 50 μM BTH in 0.05% (v/v) DMSO or solvent only (mock) from the roots by bathing the roots with sufficient solution. Maximum PSII quantum yield (Fv/Fm) was measured on the fifth leaves, after 30 min-2 h dark adaptation (PAM-2100; Walz, Germany). Four plants were used for each treatment.

ABA and SA treatments of leaf discs

Stock solutions of ABA (10 mM, in ethanol) and SA (1 M, aqueous) were diluted with distilled water containing 0.01% (v/v) Silwet L-77 (NIPPON Genetics, Tokyo, Japan) to prepare the test solutions. Rice leaves were cut to 5-mm lengths and soaked in test solutions containing 0.5% (v/v) ethanol in a 12-well microtiter plate. The samples were

incubated for 4 h at room temperature, weighed, and stored at −80°C.

Acknowledgments This work was supported by grants from the Ministry of Agriculture, Forestry, and Fisheries of Japan (Green Technology Project, IP-4006, and Genomics for Agricultural Innovation, PMI-0008). We are grateful for the excellent technical support provided by Ms. M. Ishikawa and Ms. T. Yasuhara. We thank Prof. K. Shimamoto for providing the RNAi vector pANDA; Dr. S. Toki and Ms. K. Ono, for technical advice; and Mr. T. Numa, for advice on microarray data analysis. We also thank the Rice Genome Resource Center at NIAS for the use of the rice microarray analysis system, as well as Dr. Y. Nagamura and Ms. R. Motoyama for their technical support.

References

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Statistical Soc B57:289–300

Blanco F, Salinas P, Cecchini N, Jordana X, Van Hummelen P, Alvarez M, Holuigue L (2009) Early genomic responses to salicylic acid in *Arabidopsis*. Plant Mol Biol 70:79–102

Cao H, Bowling SA, Gordon AS, Dong X (1994) Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6:1583–1592

Chern MS, Fitzgerald HA, Yadav RC, Canlas PE, Dong X, Ronald PC (2001) Evidence for a disease-resistance pathway in rice similar to the NPR1-mediated signaling pathway in *Arabidopsis*. Plant J 27:101–113

Chern M, Fitzgerald HA, Canlas PE, Navarre DA, Ronald PC (2005) Overexpression of a rice NPR1 homolog leads to constitutive activation of defense response and hypersensitivity to light. Mol Plant-Microbe Interact 18:511–520

Conrath U, Pieterse CM, Mauch-Mani B (2002) Priming in plant-pathogen interactions. Trends Plant Sci 7:210–216

Delaney TP, Friedrich L, Ryals JA (1995) *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. Proc Natl Acad Sci USA 92: 6602–6606

Despres C, Chubak C, Rochon A, Clark R, Bethune T, Desveaux D, Fobert PR (2003) The *Arabidopsis* NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. Plant Cell 15:2181–2191

Fan W, Dong X (2002) In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in *Arabidopsis*. Plant Cell 14:1377–1389

Fitzgerald HA, Chern MS, Navarre R, Ronald PC (2004) Overexpression of (At)NPR1 in rice leads to a BTH- and environment-induced lesion-mimic/cell death phenotype. Mol Plant-Microbe Interact 17:140–151

Gene-Ontology-Consortium (2006) The Gene Ontology (GO) project in 2006. Nucleic Acids Res 34:D322–D326

Gorlach J, Volrath S, Knauf-Beiter G, Hengy G, Beckhove U, Kogel KH, Oostendorp M, Staub T, Ward E, Kessmann H, Ryals J (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. Plant Cell 8:629–643

Harris MA, Clark J, Ireland A, Lomax J, Ashburner M, Foulger R, Eilbeck K, Lewis S, Marshall B, Mungall C, Richter J, Rubin GM, Blake JA, Bult C, Dolan M, Drabkin H, Eppig JT, Hill DP, Ni L, Ringwald M, Balakrishnan R, Cherry JM, Christie KR, Costanzo MC, Dwight SS, Engel S, Fisk DG, Hirschman JE, Hong EL, Nash RS, Sethuraman A, Theesfeld CL, Botstein D, Dolinski K, Feierbach B, Berardini T, Mundodi S, Rhee SY, Apweiler R, Barrell D, Camon E, Dimmer E, Lee V, Chisholm R, Gaudet P, Kibbe W, Kishore R, Schwarz EM, Sternberg P, Gwinn M, Hannick L, Wortman J, Berriman M, Wood V, De la Cruz N, Tonellato P, Jaiswal P, Seigfried T, White R (2004) The Gene Ontology (GO) database and informatics resource. Nucleic Acids Res 32:D258–D261

Heidel AJ, Dong X (2006) Fitness benefits of systemic acquired resistance during Hyaloperonospora parasitica infection in *Arabidopsis thaliana*. Genetics 173:1621–1628

Heidel AJ, Clarke JD, Antonovics J, Dong X (2004) Fitness costs of mutations affecting the systemic acquired resistance pathway in *Arabidopsis thaliana*. Genetics 168:2197–2206

Ishizaki Y, Tsunoyama Y, Hatano K, Ando K, Kato K, Shinmyo A, Kobori M, Takeba G, Nakahira Y, Shiina T (2005) A nuclear-encoded sigma factor, *Arabidopsis* SIG6, recognizes sigma-70 type chloroplast promoters and regulates early chloroplast development in cotyledons. Plant J 42:133–144

Jiang CJ, Shimono M, Sugano S, Kojima M, Yazawa K, Yoshida R, Inoue H, Hayashi N, Sakakibara H, Takatsuji H (2010) Abscisic acid interacts antagonistically with salicylic acid signaling pathway in rice-Magnaporthe grisea interaction. Mol Plant-Microbe Interact 23:791–798

Johnson C, Boden E, Arias J (2003) Salicylic acid and NPR1 induce the recruitment of trans-activating TGA factors to a defense gene promoter in *Arabidopsis*. Plant Cell 15:1846–1858

Kanamaru K, Nagashima A, Fujiwara M, Shimada H, Shirano Y, Nakabayashi K, Shibata D, Tanaka K, Takahashi H (2001) An *Arabidopsis* sigma factor (SIG2)-dependent expression of plastid-encoded tRNAs in chloroplasts. Plant Cell Physiol 42: 1034–1043

Kanehisa M, Goto S, Kawashima S, Okuno Y, Hattori M (2004) The KEGG resource for deciphering the genome. Nucleic Acids Res 32:D277–D280

Kanehisa M, Araki M, Goto S, Hattori M, Hirakawa M, Itoh M, Katayama T, Kawashima S, Okuda S, Tokimatsu T, Yamanishi Y (2008) KEGG for linking genomes to life and the environment. Nucleic Acids Res 36:D480–D484

Kasai K, Kawagishi-Kobayashi M, Teraishi M, Ito Y, Ochi K, Wakasa K, Tozawa Y (2004) Differential expression of three plastidial sigma factors, OsSIG1, OsSIG2A, and OsSIG2B, during leaf development in rice. Biosci Biotechnol Biochem 68:973–977

Kinkema M, Fan W, Dong X (2000) Nuclear localization of NPR1 is required for activation of *PR* gene expression. Plant Cell 12:2339–2350

Lawton KA, Friedrich L, Hunt M, Weymann K, Delaney T, Kessmann H, Staub T, Ryals J (1996) Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. Plant J 10:71–82

Lerdau M (1992) Future discounts and resource-allocation in plants. Funct Ecology 6:371–375

Miki D, Shimamoto K (2004) Simple RNAi vectors for stable and transient suppression of gene function in rice. Plant Cell Physiol 45:490–495

Miki D, Itoh R, Shimamoto K (2005) RNA silencing of single and multiple members in a gene family of rice. Plant Physiol 138:1903–1913

Mou Z, Fan W, Dong X (2003) Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell 113:935–944

Nettleton D (2006) A discussion of statistical methods for design and analysis of microarray experiments for plant scientists. Plant Cell 18:2112–2121

Qi M, Yang Y (2002) Quantification of *Magnaporthe grisea* during infection of rice plants using real-time polymerase chain reaction and northern blot/phosphoimaging analyses. Phytopathology 92:870–876

Quilis J, Penas G, Messeguer J, Brugidou C, San Segundo B (2008) The Arabidopsis AtNPR1 inversely modulates defense responses against fungal, bacterial, or viral pathogens while conferring hypersensitivity to abiotic stresses in transgenic rice. Mol Plant-Microbe Interact 21:1215–1231

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner HY, Hunt MD (1996) Systemic acquired resistance. Plant Cell 8:1809–1819

Schloles J (1992) Photosynthesis: cellular and tissue aspects in diseased leaves. In: Ayres P (ed) Pests and pathogens. Bios Scientific Publishers, Oxford, pp 85–106

Shah J, Tsui F, Klessig DF (1997) Characterization of a salicylic acid-insensitive mutant (*sai1*) of *Arabidopsis thaliana*, identified in a selective screen utilizing the SA-inducible expression of the *tms2* gene. Mol Plant-Microbe Interact 10:69–78

Shimizu T, Satoh K, Kikuchi S, Omura T (2007) The repression of cell wall- and plastid-related genes and the induction of defense-related genes in rice plants infected with Rice dwarf virus. Mol Plant-Microbe Interact 20:247–254

Shimono M, Sugano S, Nakayama A, Jiang CJ, Ono K, Toki S, Takatsuji H (2007) Rice WRKY45 plays a crucial role in benzothiadiazole-inducible blast resistance. Plant Cell 19: 2064–2076

Shobbar ZS, Oane R, Gamuyao R, De Palma J, Malboobi MA, Karimzadeh G, Javaran MJ, Bennett J (2008) Abscisic acid regulates gene expression in cortical fiber cells and silica cells of rice shoots. New Phytol 178:68–79

Silverman P, Seskar M, Kanter D, Schweizer P, Metraux JP, Raskin I (1995) Salicylic acid in rice: biosynthesis, conjugation, and possible role. Plant Physiol 108:633–639

Somssich IE, Hahlbrock K (1998) Pathogen defence in plants—a paradigm of biological complexity. Trends Plant Sci 3:86–90

Spoel SH, Mou Z, Tada Y, Spivey NW, Genschik P, Dong X (2009) Proteasome-mediated turnover of the transcription coactivator NPR1 plays dual roles in regulating plant immunity. Cell 137:860–872

Toki S, Hara N, Ono K, Onodera H, Tagiri A, Oka S, Tanaka H (2006) Early infection of scutellum tissue with *Agrobacterium* allows high speed transformation of rice. Plant J 47:969–976

van Hulten M, Pelser M, van Loon LC, Pieterse CM, Ton J (2006) Costs and benefits of priming for defense in Arabidopsis. Proc Natl Acad Sci USA 103:5602–5607

Wang D, Amornsiripanitch N, Dong X (2006) A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. PLoS Pathog 2:e123

Wang D, Pajerowska-Mukhtar K, Culler AH, Dong X (2007) Salicylic acid inhibits pathogen growth in plants through repression of the auxin signaling pathway. Curr Biol 17:1784–1790

Yang Y, Qi M, Mei C (2004) Endogenous salicylic acid protects rice plants from oxidative damage caused by aging as well as biotic and abiotic stress. Plant J 40:909–919

Yasuda M, Ishikawa A, Jikumaru Y, Seki M, Umezawa T, Asami T, Maruyama-Nakashita A, Kudo T, Shinozaki K, Yoshida S, Nakashita H (2008) Antagonistic interaction between systemic acquired resistance and the abscisic acid-mediated abiotic stress response in *Arabidopsis*. Plant Cell 20:1678–1692

Yuan Y, Zhong S, Li Q, Zhu Z, Lou Y, Wang L, Wang J, Wang M, Li Q, Yang D, He Z (2007) Functional analysis of rice NPR1-like genes reveals that OsNPR1/NH1 is the rice orthologue conferring disease resistance with enhanced herbivore susceptibility. Plant Biotechnol J 5:313–324

Zhang Y, Fan W, Kinkema M, Li X, Dong X (1999) Interaction of NPR1 with basic leucine zipper protein transcription factors that bind sequences required for salicylic acid induction of the *PR-1* gene. Proc Natl Acad Sci USA 96:6523–6528