Salicylic acid-dependent immunity contributes to resistance against *Rhizoctonia solani*, a necrotrophic fungal agent of sheath blight, in rice and *Brachypodium distachyon*

Yusuke Kouzai1,2, Mamiko Kimura1, Megumi Watanabe1, Kazuki Kusunoki1, Daiki Osaka1, Tomoko Suzuki1, Hidenori Matsui1, Mikihiro Yamamoto1, Yuki Ichinose1, Kazuhiro Toyoda1, Takakazu Matsuura4, Izumi C. Mori4, Takashi Hirayama4, Eiichi Minami5, Yoko Nishizawa5, Komaki Inoue2, Yoshihiko Onda2, Keiichi Mochida2,4,6 and Yoshiteru Noutoshi1

1Graduate School of Environmental and Life Science, Okayama University, Kita-ku, Okayama 700-8530, Japan; 2Cellulose Production Research Team, Biomass Engineering Research Division, RIKEN Center for Sustainable Resource Science, Tsurumi, Yokohama 230-0045, Japan; 3Department of Science, Japan Women’s University, Mejirodai, Bunkyo-ku, Tokyo 112-8681, Japan; 4Institute of Plant Science and Resources (IPSR), Okayama University, Kurashiki 710-0046, Japan; 5Division of Plant and Microbial Sciences, Institute of Agrobiological Sciences, National Agriculture and Food Research Organization (NARO), Tsukuba 305-8602, Japan; 6Kihara Institute for Biological Research, Yokohama City University, 641-12 Maioka-cho, Totsuka-ku, Yokohama 244-0813, Japan

Author for correspondence:
Yoshiteru Noutoshi
Tel: +81 86 251 8311
Email: noutoshi@okayama-u.ac.jp

Received: 9 May 2017
Accepted: 13 September 2017

New Phytologist (2018) 217: 771–783
doi: 10.1111/nph.14849

Key words: biotroph, *Brachypodium distachyon*, disease resistance, necrotroph, *Rhizoctonia solani*, rice, salicylic acid (SA), sheath blight.

Summary

- *Rhizoctonia solani* is a soil-borne fungus causing sheath blight. In consistent with its necrotrophic life style, no rice cultivars fully resistant to *R. solani* are known, and agrochemical plant defense activators used for rice blast, which upregulate a phytohormonal salicylic acid (SA)-dependent pathway, are ineffective towards this pathogen. As a result of the unavailability of genetics, the infection process of *R. solani* remains unclear.
- We used the model monocotyledonous plants *Brachypodium distachyon* and rice, and evaluated the effects of phytohormone-induced resistance to *R. solani* by pharmacological, genetic and microscopic approaches to understand fungal pathogenicity.
- Pretreatment with SA, but not with plant defense activators used in agriculture, can unexpectedly induce sheath blight resistance in plants. SA treatment inhibits the advancement of *R. solani* to the point in the infection process in which fungal biomass shows remarkable expansion and specific infection machinery is developed. The involvement of SA in *R. solani* resistance is demonstrated by SA-deficient NahG transgenic rice and the sheath blight-resistant *B. distachyon* accessions, Bd3-1 and Gaz-4, which activate SA-dependent signaling on inoculation.
- Our findings suggest a semi-biotrophic nature of *R. solani*, which can be targeted by SA-dependent plant immunity. Furthermore, *B. distachyon* provides a genetic resource that can confer disease resistance against *R. solani* to plants.

Introduction

Sheath blight, caused by the soil-borne fungal phytopathogen *Rhizoctonia solani* Kühn, is a major disease of cultivated rice (*Oryza sativa*). It causes severe agricultural and economic losses, especially in East Asia and southern USA (Lee & Rush, 1983). In the paddy field, the infection process of *R. solani* is initiated by the attachment of sclerotia, masses of compacted mycelium that can survive under unfavorable environmental conditions, to the leaf sheath of rice. Oval necrotic lesions are formed on the sheath near the water line, and the infection extends to the upper parts of the plant. The aerial hyphae from the affected plants infect adjacent plants in the field, and growth defects, lodging and death occur, leading to yield losses. In c. 1350 rice cultivars, none was found to be completely resistant to sheath blight, although the level of disease symptoms varied (Hashiba, 1984). Thus, at present, sheath blight disease is managed using fungicides in the field. However, quinone outside inhibitor (QoI) fungicides were unable to control *R. solani* in the USA in 2011 owing to the development of resistance isolates (Olaya et al., 2012). In addition, as warm temperature and high humidity are favorable for the growth of *R. solani*, global warming could further increase the future risk of agricultural damage by this pathogen.

Plant pathogens are divided into biotrophs and necrotrophs on the basis of their nutrient feeding strategies (Glazebrook, 2005). Obligate biotrophs infect specific plant species and draw...
Necrotrophs kill host cells, often by producing phytotoxins, and feed on damaged tissues. Pathogens for which the infection process involves both biotrophic and necrotrophic phases are called hemi-biotrophs. Most such pathogens initially infect hosts biotrophically and then shift to the necrotrophic stage. The transition time depends on the pathogen type. Based on the disease symptoms with severe necrotic lesions and its broad host range, *R. solani* is considered to be a simple necrotrophic parasite. Indeed, *R. solani* is also known to infect > 200 crop species and induces seed decay, seedling damping-off, stem canker, black scour and root rot.

Plants use a phytos hormone, salicylic acid (SA), to induce defense responses against biotrophic pathogens. The SA-dependent signaling pathway leads to the production of antimicrobial molecules, such as pathogenesis-related proteins and phytoalexins, as well as the induction of hypersensitive responses (HR), often associated with programmed cell death in the infected area, which restricts pathogen colonization. By contrast, plants use the phytohormones jasmonic acid (JA) and/or ethylene (ET) for defense against necrotrphs, and their responses are similar to those of wounding. Exogenously applied JA or ET suppressed the disease symptoms caused by infection with the necrotrophic fungal pathogens *Botrytis cinerea* and *Alternaria brassicicola* in Arabidopsis thaliana (Thomma et al., 1999). The application of SA to *A. thaliana* decreased resistance to *A. brassicicola*, probably as a result of its antagonistic effect to JA (Spoel et al., 2007). By contrast, SA increased *A. thaliana* resistance to *B. cinerea*, and the importance of endogenous SA, probably through phenylalanine ammonia lyase, in the resistance to *B. cinerea* has been demonstrated (Ferrari et al., 2003). The effectiveness of phytohormone application on induced resistance is dependent on the pathogen’s infection strategy. For the control of rice blast caused by a hemi-biotrophic pathogen, *Pyricularia oryzae*, agrochemical immune potentiators, called plant defense activators, have been used successfully. Agrochemicals such as probenazole, tiadinil and isothianil boost the SA-related defense response in plants, thereby conferring resistance, even to virulent pathogens. As such chemicals target host plants, no drug-resistant pathogens have yet been identified. Therefore, they provide durable and sustainable crop protection. However, the plant defense activators used in agriculture have no crop protection effects against *R. solani*. The increase in plant resistance to sheath blight disease has been investigated using genetic engineering approaches to fortify plant responses to JA or ET. Transgenic rice with enhanced production of pathogen-induced ET was found to be more resistant to *R. solani* (Helliwell et al., 2013). The overexpression of the *OsWRKY30* gene in rice induced an elevated level of JA accumulation after infection and increased resistance to *R. solani* (Peng et al., 2012).

The entire genome sequence of *R. solani* has been revealed and several secreted proteins have been identified as potential agents for necrosis in *Arabidopsis* (Zheng et al., 2013). However, the virulence mechanism of *R. solani* remains unclear, as genetic approaches cannot be applied owing to its multinucleate and heterokaryotic nature. The pathogen’s pathogenicity and the plant’s defense system at the molecular level can be determined using an appropriate model pathosystem. Brachypodiaceae belongs to a tribe which configures the core Pooidae with Littledaleae, Bromae and Triticeae (Soreng et al., 2015), and a small grass, *B. distachyon*, is used as an experimental model plant for economically important cereals such as wheat, barley and rice because of its short life cycle, self-fertility and small diploid genome (Kellogg, 2015). The complete genome sequence and cDNA database of the standard accession Bd21 and a number of accessions are available (Vogel et al., 2006, 2009, 2010; Mochida et al., 2013). Various major crop pathogens can infect *B. distachyon* (Sandoya & Buana-fina, 2014; Fitzgerald et al., 2015). *Brachypodium distachyon* has been used as a model for the investigation of wheat root rot disease caused by *R. solani* AG-8 (Schneebeli et al., 2016).

In this study, we used *B. distachyon* and rice as hosts to investigate sheath blight disease and, unexpectedly, found that exogenously applied SA can induce sheath blight resistance in plants. The decreased level of endogenous SA induced by the transformed bacterial salicylate hydroxylase gene makes rice more susceptible to *R. solani*. We also identified two *R. solani*-resistant accessions of *B. distachyon*, in which SA signaling was rapidly activated in response to fungal inoculation, indicating the existence of disease resistance genes for *R. solani*. The protective effect of plants against sheath blight disease by chemically and genetically induced SA-dependent immunity suggests a two-phase infection process of *R. solani*, with a preceding biotrophic stage and a subsequent necrotrophic stage as a novel aspect of its infection strategy. Our study demonstrates the possibility of the application of durable and sustainable crop protection methods, such as disease resistance genes or chemical defense activators, to *Rhizoctonia* diseases.

**Materials and Methods**

**Plant and fungal materials**

The *B. distachyon* accessions Bd21, Bd3-1 and Gaz-4 were obtained from the National Plant Germplasm System of the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) (Vogel et al., 2006, 2009). Dry seeds were germinated on moist filter paper in a plastic Petri dish. After 7 d, the seedlings were transferred to wells of 24-well microplates filled with soil (Sakata Supermix-A; Sakata Seed, Kanagawa, Japan) and grown for 3–4 wk in a growth chamber with LED lights (Nippon Medical & Chemical Instruments, Osaka, Japan) at 23°C under a 24 h: 4 h, light : dark photoperiod. The 12 *R. solani* isolates and *B. cinerea* (MAFF23796) were provided by Genebank of the National Agricultural Research Organization (NARO) in Japan, and *P. oryzae* Guy11 was kindly provided by Dr Yukio Tosa and cultured on potato dextrose agar (PDA; BD, Franklin Lakes, NJ, USA) plates at 23°C for 3–5 d. Rice plants (*Oryza sativa L. japonica*) carrying the blast resistance genes *Pia* and *Pish* (cv Nipponbare (Pia)) were used as the wild-type. Transgenic rice plants expressing *NahG* which had the ‘Nipponbare (Pia)’ background were kindly provided by Dr Chang-Jie Jiang. Rice plants were grown hydroponically in nutrient solution under a photoperiod of 14 h of light (28°C) and 10 h of darkness (25°C).
Polyphosphin hormones and chemical treatments

Sodium salicylate (Wako, Osaka, Japan), methyl jasmonate (Wako) and ethephon (Sigma-Aldrich, St Louis, MO, USA), an ET generator, were used as phytohormones. Acetylsalicylic acid (Ac-SA) (Nacalai Tesque, Kyoto, Japan), 3,5-dichloroanthranilic acid (DCA) (Tokyo Chemical Industry, Tokyo, Japan), 2,6-dichlorisonicotianic acid (INA) (Wako) and acibenzolar-S-methyl (benzothiadiazole, BTH) (Wako) were used as structural or functional analogs of SA. All phytohormones and chemicals were diluted with water or dimethyl sulfoxide (DMSO). The B. distachyon seedlings or detached leaves were sprayed, soaked or soil-drenched with a chemical solution containing 0.04% (v/v) Tween 20 and incubated for 24 or 48 h at 23°C. Droplets of chemical solution adhering to the leaf surface were wiped off before the following experiments.

Inoculation tests

Detached shoots of B. distachyon were used for the screening of pathogenic R. solani strains, and detached leaves of B. distachyon and rice were used for other inoculation tests. Detached plants were placed on moist filter paper in a Petri dish for B. distachyon or a square plate for rice. The detached site was covered with moist Kimwipes (Nippon Paper Crecia, Tokyo, Japan). After 1 d of wound acclimation, cubic mycelial plugs (2–3 mm) cut from the edge of R. solani mycelia growing on PDA plates were placed on the leaf sheath or in the middle of detached leaves as inoculum. The lid of the plate was closed and sealed immediately with surgical tape to avoid drying. The plates were incubated at 23°C and 25°C for B. distachyon and rice, respectively, under continuous light. For the inoculation of P. oryzae and B. cinerea, conidial suspensions were spotted onto leaves. To quantify disease severity, the fungal biomass in the inoculated leaves was measured by qPCR according to previous reports (Saylor & Yang, 2007; Budge et al., 2009). Surface fungal mycelia of the inoculated leaves were removed by wet-wipes with 70% ethanol and adhesive tape, and DNA was extracted using a Nucleospin Plant II Kit (Takara Bio, Shiga, Japan). PCR for R. solani DNAs (28S rDNA for MAFF305230, a tubulin gene for MAFF305256) was performed using a KAPA SYBR Fast qPCR Kit (Kapa Biosystems, Woburn, MA, USA) with a GVP-9600 instrument (Shimadzu, Kyoto, Japan) or SYBR Premix Ex Taq II (Takara Bio) with an Applied Biosystems 7500 System (Thermo Fisher Scientific, Waltham, MA, USA). The B. distachyon BdFIM gene was used for normalization (Zhu et al., 2014). Primers are listed in Supporting Information Table S1.

Microscopic observations

Rhizoctonia solani hyphae were stained with trypan blue (Wako) dissolved in 40 ml of 1 : 1 : 1 : 1 phenol/lactic acid/glycerol/water. The inoculated leaves were collected in 2-ml tubes and boiled for 5 min in 1 : 1 ethanol/staining solution, followed by incubation for 30 min at room temperature. The samples were then washed with water three times and stored in 30% glycerol before microscopic observation.

Gene expression analysis

Total RNAs were extracted from the inoculated leaves using an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) with on-column DNase treatment. cDNAs were synthesized using an Omniscript RT Kit (Qiagen). Gene expression analysis was performed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) using SYBR Premix Ex Taq II with an Applied Biosystems 7500 System, and the data were normalized with the BdUbi4 gene (Bradi3g04730). Primers are listed in Table S1.

Transcriptome analysis

Total RNAs were extracted from B. distachyon leaves after 24 h of treatment with 0.5 mM SA, 0.5 mM BTH or 0.5% (v/v) DMSO using an RNeasy Plant Mini Kit (QIAGEN). The quality and quantity of total RNAs were checked busing a NanoDrop spectrophotometer (Thermo Fisher Scientific) and a 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). Libraries for RNA-seq were constructed for three biological replicates using a TrueSeq RNA library preparation kit (Illumina, San Diego, CA, USA) according to the manufacturer’s instructions, and the libraries were sequenced using a Hiseq4000 sequencer (Illumina). The dataset of the Illumina reads has been submitted to the DNA Data Bank of Japan (DDBJ) Sequence Read Archive under the accession number DRA005681. The Illumina reads were mapped to the B. distachyon reference genome Bdistachyon_314 retrieved from Phytome using TopHat v2.1.1 (Kim et al., 2013) with Bowtie v2.2.6 as its mapping tool (Langmead & Salzberg, 2012). The number of mapped reads of each gene was counted using FeatureCounts and normalized by reads per million (RPKM). Genes with a mean RPKM in three biological replicates in the same treatment of ≥1 were defined as expressed genes. Differentially expressed genes (DEGs) in SA and BTH treatments compared with the DMSO treatment as the control condition were determined based on the read counts of expressed genes using edgeR (McCarthy et al., 2012). The genes with a false discovery rate (FDR) from Fisher’s exact test of < 0.01 and a log2 transformed fold change of > 1 or < −1 were identified as DEGs for each of the SA and BTH treatments. The distribution of DEGs in the three treatments was depicted using an R package: VennDiagram.

Gene ontology enrichment analysis

Gene ontology (GO) enrichment analysis for the DEGs was performed to infer functional properties in the transcriptome responding to each of the treatments. For the GO enrichment analysis, GO terms were assigned to Brachypodium genes based on the GO annotations of their putative homologs of Arabidopsis which showed highest similarities in a blastp search with a threshold e-value of < 1e-5. Significantly over- or less-represented GO terms were identified using the agriGO web service (http://bioinf
Quantification of phytohormones

Approximately 50 mg of leaf blades were used for each extraction. The content of multiple phytohormones was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS), as reported previously (Mikami et al., 2016).

Statistical analysis

Significance between populations was determined using unpaired Student’s t-tests. Graphs throughout the article show the mean value and error bars show the standard error (SE).

Results

Surveillance of infectivity of R. solani isolates on B. distachyon

To analyze the molecular basis for both pathogen virulence and plant resistance in sheath blight disease, we used a model plant B. distachyon. We evaluated the infectivity of R. solani on B. distachyon standard accession Bd21 using 12 Japanese field isolates from various anastomosis groups (AGs) (Table 1), which were determined by hyphal fusion and were related to pathogenicity and host range (Anderson, 1982; Ogoshi, 1987). Mycelial plugs prepared from nutrient agar were inoculated on the detached shoots of B. distachyon. Eight fungal isolates formed visible lesions, and the leaf blades became etiolated by 2–3 d post-inoculation (dpi) (Fig. 1). The AG-1 IA isolate (MAFF305230), sampled from rice sheath blight which showed the most severe symptoms, was used for further analysis.

Phytohormone-induced resistance to R. solani in plants

To test whether B. distachyon has the potential to induce resistance to R. solani, we treated Bd21 seedlings with SA, JA or ET, and inoculated seedlings with the pathogen. Contrary to our expectation, spray treatment of SA on detached leaf blades suppressed lesion formation and fungal biomass growth in R. solani-inoculated leaves (Fig. 2a,b). By contrast, JA enhanced the susceptibility and ET had no effect on the symptoms. A concentration-dependent analysis confirmed the effects of SA and JA on R. solani resistance (Fig. 2c,d). To further examine the effect of SA on R. solani resistance, we treated Bd21 intact plants with SA and inoculated them with the pathogen. Both the disease symptoms and R. solani foliar biomass were also suppressed by SA treatment in undetached B. distachyon (Fig. 3a,b). The phytohormones themselves did not inhibit fungal growth on agar medium (Fig. S1). To determine whether our experimental system could appropriately evaluate phytohormone-induced plant defense, we inoculated a hemi-biotrophic pathogen, P. oryzae strain Guy 11, and a necrotrophic pathogen, Botrytis cinerea, on B. distachyon (Govrin & Levine, 2002; Ebbole, 2007).

In accord with the well-known infection strategies of these fungi, treatments with SA and ET induced resistance to P. oryzae and B. cinerea, respectively (Fig. S2). SA application to B. distachyon also enhanced resistance against a different isolate of R. solani AG-5 (MAFF305256) (Fig. 2e,f), indicating that SA-induced immunity in B. distachyon is independent of, at least, these two fungal AGs.

To determine whether these unexpected results were specific to B. distachyon, we conducted the same experiment using rice. Suppression of R. solani infection was also observed in SA-treated rice, as evidenced by decreases in lesion formation and foliar fungal biomass (Fig. 4a,b).

Contribution of SA to R. solani resistance in rice

To test whether innate SA signaling contributes to R. solani resistance in plants, we used transgenic rice plants expressing a bacterial SA hydroxylase (the NahG gene); this gene causes SA deficiency, leading to the loss of resistance to biotrophic pathogens (Yang et al., 2004). Rice NahG plants inoculated with R. solani were more susceptible than wild-type plants (Fig. 4c,d). These results indicate that SA signaling plays a positive role in plant resistance to R. solani.

Infection process of R. solani on B. distachyon

The infection behavior of R. solani on B. distachyon was monitored by both microscopic observations and fungal biomass quantification. Mycelia randomly expanded on the leaf surfaces...
at 20 h post-inoculation (hpi) (Fig. 5a). At 40 hpi, the mycelia were very dense, and the specialized infection structures, termed infection cushions, began to appear (Marshall & Rush, 1980a,b; Matsuura, 1986). Many infection cushions were evident at 60 hpi. Fungal hyphae inside the inoculated leaves were detected at 20 hpi (Fig. 5b), but not at 5 hpi (Fig. S3). The number of fungal hyphae within inoculated leaves was slightly higher at 30 hpi, and then increased significantly, accompanied by the formation of infection cushions. Our findings indicate that *R. solani* invaded *B. distachyon* leaves within 20 hpi before the development of infection cushions. Previous studies have shown that *R. solani* can also penetrate the epidermis from the lobate appressorium and mycelial tip (Marshall & Rush, 1980a,b; Singh et al., 2003). Thus, the infection process of *R. solani* appeared to be divided into two phases. First, *R. solani* intrudes into the host by suppressing various layers of immunity using enzymes, chemicals and effectors. Next, infection cushions are formed using nutrients absorbed from the host, enabling the pathogen to penetrate large numbers of infection hyphae into the epidermal cells. After or during this second phase, toxins may be produced that shift the infection behavior of *R. solani* to the necrotrophic phase (Vidyasekaran et al., 1997). Indeed, necrotic lesions and chlorosis were observed 2–3 d (48–72 h) after inoculation (Figs 1, 2a,e).

**Effect of SA-induced resistance on *R. solani* development in *B. distachyon***

In SA-treated plants, the mycelial density increased with time; however, interestingly, infection cushions were hardly found at the time points investigated (Fig. 5a). Further, massive increments in fungal biomass were not detected at 40 and 60 hpi; the fungal biomass was suppressed to a significantly lower level than that in the control (Fig. 5b). We repeated these experiments at least twice with the same results. Given that the fungal biomass in SA-treated leaves was comparable with that in the control at 20 hpi (enlarged box in Fig. 5b), we speculated that SA treatment did not prevent the primary invasion of *R. solani*. Conversely, the unsuccessful formation of infection cushions suggested that the transition from the first to the second phase was inhibited by the SA-induced resistance in *B. distachyon*.

**Effects of plant defense activators on *R. solani* resistance***

Our finding that SA can confer disease resistance to *R. solani* is inconsistent with the fact that no commercial plant defense activators for rice blast are effective in controlling sheath blight, although all potentiate SA signaling (Schreiber & Desveaux, 2008; Noutoshi et al., 2012). To validate this point, we investigated the effects of probenazole and tiadinil on *R. solani* resistance in *B. distachyon*, and found that none conferred resistance (Fig. 6a,b). As these compounds used in agriculture are potentia- tors, but not strong inducers, of defense responses, we hypothesized that an already elevated or considerably rapid induction of SA signaling, or a particular set of SA-specific transcripts, is required for *R. solani* resistance. To test this hypothesis, we further evaluated the effect of the following structurally or functionally related SA analogs: Ac-SA, DCA, INA and BTH. These compounds induced the transcription of *BdWRKY45L1* (*Bradi2 g30695*), a marker gene for SA response, after 24 h of treatment in *B. distachyon* (Fig. 6c) (Kouzai et al., 2016). The *B. distachyon* leaves were then treated with these analogs for 24 h, followed by inoculation with *R. solani*. We found that Ac-SA,
Fig. 2 Salicylic acid (SA) induces Rhizoctonia solani resistance in Brachypodium distachyon. (a, b) Lesion formation (a) and relative biomass (linear scale) (b) of R. solani AG-1 on B. distachyon leaves treated with water (Mock) or SA, jasmonic acid (JA) or ethylene (ET) (1 mM each) at 3 d post-inoculation (dpi). Bars, 1 cm. Data are represented as means ± SEM, n = 4; *, P < 0.05; **, P < 0.01 using Student’s t-tests. (c, d) Relative biomass of R. solani AG-1 in B. distachyon leaves treated with 0, 1, 10, 100 and 1000 μM of SA (c) or JA (d) at 3 dpi. Data are represented as means ± SEM, n = 3; *, P < 0.05 using Student’s t-tests. (e, f) Lesion formation (e) and relative biomass (linear scale) (f) of R. solani AG-5 in B. distachyon leaves treated with water (Mock) or 1 mM SA at 3 dpi. Bars, 1 cm. Data are represented as means ± SEM, n = 4; *, P < 0.05 using Student’s t-tests. All experiments were repeated three times with similar results.

Fig. 3 Salicylic acid (SA) induces Rhizoctonia solani resistance in soil-grown Brachypodium distachyon intact plants. (a, b) Disease symptoms (a) and fungal biomass (linear scale) in leaves (b) of R. solani AG-1 in B. distachyon intact plants grown on soil treated with water (Mock) or 1 mM SA. B. distachyon intact plants grown on soil for 3 wk were sprayed with water (Mock) or 1 mM SA for 48 h and inoculated with R. solani (MAFF3055230) by a mycelial agar plug held on the stem with tape. Photographs were taken at 7 d post-inoculation (dpi). Data are represented as means ± SEM, n = 4; **, P < 0.01 using Student’s t-tests. Similar results were obtained in three independent experiments.
DCA and INA suppressed fungal growth compared with that in the control treatment at 3 dpi; however, the resistance provided by DCA and INA was impaired at 6 dpi (Fig. 6d,e). Surprisingly, BTH did not induce resistance at either 3 or 6 dpi (Fig. 6d,e), although it could induce resistance in rice against blast disease and bacterial leaf blight (Shimono et al., 2007, 2012). These results support the idea that a previously induced defense by SA confers resistance to R. solani; however, the activity and durability of R. solani resistance varied across the compounds tested.

Transcriptome analysis of B. distachyon treated with SA or BTH

To understand the reason why SA, but not BTH, induced resistance in B. distachyon to R. solani, we compared the datasets of the transcriptome of Bd21 leaves after SA treatment with those after BTH treatment, and identified the genes that were differentially expressed in each treatment. Through our RNA-seq analysis of three biological replicates with SA, BTH and Mock (DMSO), 94.7% of the sequencing reads were mapped to the reference genome of three biological replicates with SA, BTH and Mock (DMSO), self-assembly expressed in each treatment. Through our RNA-seq analysis after BTH treatment, and identified the genes that were differentially expressed compared with DMSO (Mock) or 1 mM SA. Photographs were taken at 3 d post-inoculation (dpi). The experiments were repeated at least twice with similar results.

**Fig. 4** Salicylic acid (SA) induces post-invasion resistance to Rhizoctonia solani resistance in rice. (a, b) Lesion formation (a) and fungal biomass (linear scale) (b) of R. solani AG-1 in rice leaves treated with water (Mock) or 1 mM SA. Photographs were taken at 3 d post-inoculation (dpi). The graph shows the relative R. solani biomass in the inoculated leaves at 3 dpi. Bars, 1 cm. Data are represented as means ± SEM of values relative to the mock treatment, n = 12. ***, P < 0.01 using Student’s t-test. (c, d) Lesion formation (c) and relative biomass (linear scale) (d) of R. solani AG-1 in the leaves of wild-type and NahG-overexpressing transgenic rice at 3 dpi. Bars, 1 cm. Data are represented as means ± SEM, n = 12. ***, P < 0.001 using Student’s t-tests. All experiments were repeated three times with similar results.

Fig. 5 Salicylic acid (SA) induces post-invasion resistance to Rhizoctonia solani in Brachypodium distachyon. (a) Hyphal growth of R. solani AG-1 on B. distachyon leaf surfaces treated with dimethyl sulfoxide (DMSO; Mock) or 1 mM SA. The inoculated leaves were collected at the indicated time points and R. solani hyphae were stained with trypan blue. Infection cushions were recognized as aggregates of convoluted hyphae. Bars, 100 μm. (b) Relative biomass (linear scale) of R. solani AG-1 in B. distachyon leaves treated with DMSO (Mock) or 1 mM SA at the indicated time points. Data are represented as means ± SEM, n = 6. *, P < 0.05; ***, P < 0.005 using Student’s t-tests relative to the values of mock at 20 h post-inoculation (hpi). The experiments were repeated at least twice with similar results.

These DEGs in both up- and down-regulated genes are depicted in Fig. 7a; 82.0% of the DEGs in the SA treatment were shared with those in the BTH treatment, indicating that BTH acts as a functional SA analog in B. distachyon, as in Arabidopsis and rice (Fig. 7a). However, we found that 89 genes were induced by SA only (Fig. 7a). The GO enrichment analysis demonstrated that GO terms related to the biosynthesis of the secondary cell wall (SCW) were clearly over-represented in the given gene set (Fig. 7b,c). We found that at least 17 genes (20.7%) showed homology to Arabidopsis genes with a GO term (GO:0042546) related to cell wall biogenesis (Table S5). In B. distachyon, BdCESA6, 7 and 8 encode cellulose synthase A (CESA) proteins and the loss-of-function mutants for BdCESA4 and 7 showed a reduced amount of crystalline cellulose in the SCW (Handakumbara et al., 2013). BdLAC5, 6 and 10 are members of the laccase family and mutation in BdLAC5 leads to a reduction in the culm lignin level (Wang et al., 2015). In our RNA-seq analysis, BdLAC5 was induced by both SA and BTH, but BdLAC6 and 10 were found in the SA-specific DEGs. The expression profiles of BdCESA4, 7 and 8, and BdLAC6 and 10, were evaluated by qRT-PCR analysis, and were shown to be induced by SA, but not BTH, thus confirming the transcriptome data (Fig. 7d).
These results imply that SCW synthesis and reinforcement are induced by SA, which may act as a critical cellular response in SA-induced resistance against *R. solani* infection in *B. distachyon*. The number of DEGs for BTH is 2.8 times greater than that for SA, and >1300 genes were specifically regulated by BTH at an equal concentration to the SA treatment (Fig. 7a); therefore, we cannot exclude the possibility that these BTH-specific genes compromise the defense responses to *R. solani*.

Sheath blight resistance accession of *B. distachyon* against *R. solani*

For host resistance often found in a cultivar-specific trait, plants recognize pathogen-derived effector proteins using resistance (R) proteins and activate robust immune responses termed effector-triggered immunity (ETI) (Jones & Dangl, 2006), in which SA recognize pathogen-derived effector proteins using resistance (R) proteins and activate robust immune responses termed effector-triggered immunity (ETI) (Jones & Dangl, 2006), in which SA recognize pathogen-derived effector proteins using resistance (R) proteins and activate robust immune responses termed effector-triggered immunity (ETI) (Jones & Dangl, 2006). Our results confirmed the recent finding that the Bd3-1 accession is resistant to *R. solani* RhBC 2-1-35; however, the AG of this isolate has not been reported (Sandoya & Buanafrica, 2014). The lesions and accumulated fungal biomasses in leaves of Bd3-1 and Gaz-4, caused by infection with *R. solani* AG-1, were significantly less than those in Bd21 (Fig. 8a,b). During the infection of *R. solani*, the SA marker genes *BdWRKY45L1* and *BdWRKY45L2* were transcribed within 5 and 24 hpi in Bd3-1 and Gaz-4, respectively, but not in Bd21 (Fig. 8c) (Kouzai *et al.*, 2016), suggesting the activation of ETI in these *R. solani*-resistant accessions. By contrast, the JA marker *BdAOS* was clearly induced only in Bd21 (Kouzai *et al.*, 2016). The quantitative measurement of endogenous phytohormones during infection showed that SA levels were not altered by fungal inoculation in either accession, but JA and JA-Ile significantly accumulated only in Bd21 (Fig. S4). The levels of other phytohormones, such as auxin, abscisic acid and cytokinins, did not change in any accession (Fig. S4). The elevated JA levels and JA marker gene expression were well correlated in the susceptible Bd21. These findings could be a result of the suppression of SA signaling by the pathogen and the necrotic lesions recognized as wounding.

**Discussion**

In this study, we found that foliar pretreatments with SA in *B. distachyon* and rice prevented symptom development caused by *R. solani* (Figs 2a,b, 4a,b). The fungal biomass of *R. solani* in the infected leaves was maintained at a lower level by SA pretreatment compared with that of non-treated leaves, indicating that SA can induce resistance, at least in these plant species, against *R. solani* (Fig. 5). However, *NahG* transgenic rice plants which showed decreased SA levels became more susceptible to *R. solani* (Fig. 4c,d). These results suggest that *R. solani* probably uses the biotrophic stage in a short window of time for early infection; SA-induced defense responses could attenuate the infection at
this point to combat the disease. This result is consistent with the recent finding that *R. solani* expresses secreted proteinaceous effectors during infection as potential virulence factors (Zheng et al., 2013; Anderson et al., 2016). The JA-induced susceptibility of *B. distachyon* to *R. solani* might be attributed to the antagonistic interaction between SA and JA, based on observations mainly in Arabidopsis (Kunkel & Brooks, 2002). However, JA did not decrease resistance to *P. oryzae* (Fig. S2). As SA and JA share a certain range of defense-related genes, designated as the common defense system in rice, unlike in dicots (Tamaoki et al., 2013 [Correction added after online publication 19 October 2017: the citation Tamaoki et al. (2013) has been corrected here and in the References list]; De Vleesschauwer et al., 2014; Kouzai et al., 2016), the results might depend on the degree of contribution of this common system to resistance against *R. solani* and *P. oryzae*.

*Rhizoctonia solani* might possess complicated virulence mechanisms that include not only chemical or enzymatic toxins, but also effector proteins, to suppress host immunity. *Rhizoctonia solani* has been isolated from many infested field crops showing various necrotic symptoms, but the host range of each isolate seems to be limited and is dependent on the virulence related to the AGs (Anderson, 1982; Ogoshi, 1987). For example, *A. thaliana* is susceptible to *R. solani* AG-2-1 and AG-4 HG-I, but not to AG-8 (Sharon et al., 2011; Foley et al., 2013), and the different types of AGs of *R. solani* exhibit varying infectivity to cauliflower (Pannecouque & Hofte, 2009). The host specificity of *R. solani*, considering the effector proteins in addition to toxins, should provide new insights into the infection strategy of this pathogen, although the sensitivity to pathogen-produced toxins also contributes strongly to symptom development in rice (Brooks, 2007).

The formation of infection cushions of *R. solani* was clearly suppressed by SA pretreatment in *B. distachyon* (Fig. 5). The relationships between infection cushions or lobate appressoria and disease severity have been investigated using various rice cultivars with varying susceptibility levels (Marshall & Rush, 1980b). The resistance level and infection structure numbers were correlated and, interestingly, infection cushions, but not lobate appressoria, were not observed in resistant rice cultivars. Further, a relationship is known to exist between the endogenous SA level and blast resistance in rice cultivars (Silverman et al., 1995). From the data demonstrated in these two previous reports, we noted that the

![Fig. 7](image_url) Salicylic acid (SA)-specific transcripts include secondary cell wall-related genes in *Brachypodium distachyon*. (a) Proportional Venn diagrams showing the overlap of the up-regulated (left) and down-regulated (right) gene sets 24 h after treatment with SA or benzothiadiazole (BTH). (b, c) Functional classification of the genes specifically upregulated by SA with gene ontology (GO) categories related to biological process (b) and cellular component (c) using a REVIGO scatterplot. Circles denote significantly enriched GO terms with false discovery rate (FDR) < 0.05. The circle size represents the \(-\log_{10} \text{transformed} \) FDR in REVIGO analysis. (d) Expression levels of secondary cell wall-related genes after 24 h of treatment with SA or BTH. Data are represented as means ± SEM relative to those of the dimethyl sulfoxide (DMSO) treatment, n = 3; *, P < 0.05 using Student’s t-tests. All experiments were repeated at least twice with similar results.
R. solani-resistant cultivar Tetep containing high levels of SA was resistant to rice blast, whereas the R. solani very susceptible cultivar Labelle containing low SA levels was susceptible (Marshall & Rush, 1980b; Silverman et al., 1995). The endogenous SA content could also be related to the sheath blight susceptibility in rice cultivars.

Rhizoctonia solani-resistant rice cultivars, but not susceptible ones, deposit cuticular wax on the outer sheath surface, and the removal of wax in resistant cultivars reduced the resistance level (Marshall & Rush, 1980a). Wax production may be regulated by SA signaling. It has also been demonstrated that the exogenous application of glucose or 3-o-methylglucose (MEG) confers disease resistance in rice and cotton against R. solani (Weinhold & Bowman, 1974; Marshall & Rush, 1980a). As some types of rare sugar have also been found to induce disease resistance to blast and bacterial blight in rice, such sugar-induced defense responses might also be effective against sheath blight disease (Kano et al., 2010, 2011).

We have shown that SA, Ac-SA and INA, but not probenazole, tiadinil and BTH, confer resistance to R. solani in B. distachyon (Fig. 6). This variation might depend on certain chemical properties, such as the affinity to receptors and rates of incorporation and metabolism in plants. For instance, the DCA-induced transcriptional profile is different from that of SA in Arabidopsis (Knoth et al., 2009). SA is known to induce thermogenesis in voodoo lily, and only two of the 33 SA analogs can induce this response (Vlot et al., 2009). The immunity contributing to R. solani resistance might be attributed to a specific set of defense responses or the appropriate induction timing of SA signaling. The difference in these synthetic compounds from SA provides an opportunity to identify which type of plant defense response can function for the inhibition of R. solani infection. Transcriptome analysis after treatment with SA and BTH in B. distachyon leaves revealed a difference between these two compounds (Fig. 7a). In Arabidopsis, 89% and 99% of the genes transcriptionally induced by SA and BTH, respectively, were dependent on NPR1, indicating the high overlap between them (Wang et al., 2006; Blanco et al., 2009). However, in B. distachyon, BTH regulated 82% of the SA-related DEGs, but corresponded to only 29.2% of BTH-related DEGs. BTH could affect various developmental and physiological phenomena in B. distachyon compared with those in A. thaliana. 18.0% of SA-related DEGs were independent of BTH and 89 genes (17.5% of SA-induced genes) were specifically upregulated by SA. The GO enrichment analysis showed the over-representation of SCW-related GO terms in this gene set (Fig. 7b,c; Table S5). SCW is known to play a central role as a physical barrier against pathogens, and SCW reinforcement, such as lignification, is induced in response to pathogen infection as a defense response (Miedes et al., 2014). Recently, the resistance gene Xa4, which encodes a cell wall-associated kinase, has been reported to confer race-specific resistance to a bacterial pathogen Xanthomonas oryzae pv. oryzae (Xoo) via the promotion of SCW reinforcement in rice, and the cesa4 rice mutant, as well as the RNAi transgenic rice lines for OsCESA4, 7 and 9, compromised the Xa4-mediated race-specific resistance to Xoo (Hu et al., 2017). From the culture filtrates of vegetative and infection samples of R. solani AG-8, many proteins with predicted function relating to lignin catabolism were detected, and were thought to be involved in the modification of the host cell wall (Anderson et al., 2016). Therefore, SCW reinforcement might be a crucial event in SA-induced R. solani resistance in B. distachyon. Further studies are needed to clarify the contribution of wall strengthening to R. solani resistance.

In rice, six quantitative trait loci (QTLs) contributing to resistance to R. solani were identified using siblings between susceptible Lemont and resistant Teqing cultivars (Li et al., 1995). The mapped chromosomal regions were associated with QTLs responsible for plant height and subsequent heading. Another group identified QTLs for sheath blight resistance in the rice line derived from the resistant Tetep variety; these QTLs were also associated with culm length and heading date (Sato et al., 2004). These results suggest that R. solani resistance in rice strongly relies on...
on morphological or developmental phenotypes, especially during field evaluation. By contrast, the sheath blight resistance of *B. distachyon* accessions Bd3-1 and Gaz-4, found in this study, seemed to be associated with true resistance, as it clearly activated SA-dependent signaling after inoculation of *R. solani* (Fig. 8c). As activation was detected within 24 h, these accessions seem to recognize infection of *R. solani* at an early time point after inoculation. Interestingly, Bd3-1 was also resistant to *R. solani* AG-5 (data not shown), suggesting that Bd3-1 recognizes the common factor between the different AGs of *R. solani* isolates or possesses another resistance gene for AG-5. Further studies to identify plant genes that confer resistance or tolerance to *R. solani* might elucidate the mechanisms of how plants develop resistance to *R. solani*, as well as the molecular infection strategy of this pathogen.

In the resistant Bd3-1 accession, the SA marker genes were highly expressed, but SA levels were not upregulated (Fig. S4). This finding is similar to the situation reported in rice, in which the SA levels were not changed locally and systemically even after challenge with an incompatible pathogen, but an SA marker gene was upregulated (Jiang et al., 2010; Takatsuji, 2014). Furthermore, SA accumulation did not occur in barley in response to an incompatible powdery mildew fungus, although an HR cell death was observed at the infection sites (Huckelhoven et al., 1999). Unlike in dicotyledonous plants (Vlot et al., 2009; Noutoshi et al., 2012), activation of SA signaling might not be associated with the obvious accumulation of free SA in monocots.

The present study provides the means to produce crop protection against sheath blight disease with environmental sustainability and economy. The first strategy is to identify disease resistance inducers. Plant defense-activating compounds with SA-related functionality could be developed as an alternative method for *R. solani*. As our results suggest the importance of the activation of SCW biosynthesis for the resistance to *R. solani* (Fig. 7b,c; Table S5), the activity for the transcriptional induction of the 89 genes including SCW-related genes may be required for the potential candidate chemicals. The second strategy is the development of disease-resistant/tolerant cultivars. Recently, disease resistance genes have been explored from wild relatives or closely related species to complement the depletion or limitation of genetic resources for disease resistance in cultivated crops (Jones et al., 2014; Du et al., 2015; Kawashima et al., 2016). *Rhizoctonia solani* is also a causal agent of root rot diseases in various plant species, and wheat cultivars and *B. distachyon* accessions partially resistant to *R. solani* AG-8 have been identified (Mahoney et al., 2016; Schneebeli et al., 2016). The disease-resistant genes identified in *B. distachyon* can be used for the molecular breeding of sheath blight resistance in rice and probably to increase resistance to various *Rhizoctonia* diseases in other crops.

Acknowledgements

We thank Dr Yukio Tosa for maintaining and providing *P. oryzae* Guyl11. We thank Nihon Nohyaku Co. Ltd. and Dr Chang-Jie Jiang for providing tiadinil and rice NahG plants, respectively. We also thank Ms Nobuyo Aoyagi for support with rice cultivation. This research was supported by an ALCA Grant from the Japan Science and Technology Agency, KAKENHI Grant 25292035 from the Ministry of Education, Culture, Sports, Science and Technology of Japan to Y.N., and financial support to the Institute of Plant Science and Resources (IPSJ) by the Japan Advanced Plant Science Research Network from Japan Society for the Promotion of Science.

Author contributions

Y.K., H.M., M.Y., Y.I., K.T., E.M., Y. Nishizawa and Y. Noutoshi conceived the study and designed the experiments. Y.K., M.K., M.W., K.K. and Y. Noutoshi carried out the experiments and performed the statistical analysis, except as noted below. Y.K., K.T. and D.O. performed the experiment shown in Fig. 2(e,f). T.M., I.C.M. and T.H. carried out phytohormone measurements. K.I. and K.M. carried out the RNA-seq analysis and data deposition on the DDBJ Sequence Read Archive. Y.K., M.K., E.M. and Y. Nishizawa performed the inoculation tests using rice. Y.K. and T.S. performed the microscopic analysis. Y.K. and Y. Noutoshi drafted the manuscript. H.M., M.Y., Y.I., K.T., Y.O. and K.M. contributed to the analysis, interpretation of the data and critical revision of the manuscript.

References

Anderson J, Hane J, Stoll T, Pain N, Hastie M, Kaur P, Hoogland C, Gorman J, Singh K. 2016. Proteomic analysis of *Rhizoctonia solani* identifies infection-specific, redox associated proteins and insight into adaptation to different plant hosts. *Molecular & Cellular Proteomics* 15: 1188–1203.

Anderson N. 1982. The genetics and pathology of *Rhizoctonia solani*. *Annual Review of Phytopathology* 20: 329–347.

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 Molecular Biology* 70: 79–102.

Brooks S. 2007. Sensitivity to a phytotoxin from *Rhizoctonia solani* correlates with sheath blight susceptibility in rice. *Phytopathology* 97: 1207–1212.

Budge G, Shaw M, Colyer A, Pietravalle S, Boonham N. 2009. Molecular tools to investigate *Rhizoctonia solani* distribution in soil. *Plant Pathology* 58: 1071–1080.

De Vleesschauwer D, Xu J, Hofre M. 2014. Making sense of hormone-mediated defense networking: from rice to Arabidopsis. *Frontiers in Plant Science* 5: 611.

Du J, Verzaux E, Chaparro-Garcia A, Bijsterbosch G, Keizer LCP, Zhou J, Liebrand TWH, Xie C, Govers F, Robatzek S et al. 2015. Elicitin recognition confers enhanced resistance to *Phytophthora infestans* in potato. *Nature Plants* 1: 15034.

Ebbole D. 2007. Magnaporthe as a model for understanding host–pathogen interactions. *Annual Review of Phytopathology* 45: 437–456.

Ferrari S, Plotnikova J, De Lorenzo G, Ausubel F. 2003. Arabidopsis local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS1 and PAD2, but not SID2, EDS5 or PAD4. *Plant Journal* 35: 193–205.

Fitzgerald T, Powell J, Schneebeli K, Hsia M, Gardiner D, Bragg J, McIntyre C, Manners J, Ayliffe M, Watt M et al. 2015. *Brachypodium* as an emerging model for cereal–pathogen interactions. *Annals of Botany* 115: 717–731.

Foley R, Gleason C, Anderson J, Hamann T, Singh K. 2013. Genetic and genomic analysis of *Rhizoctonia solani* interactions with Arabidopsis: evidence of resistance mediated through NADPH oxidases. *PLoS ONE* e56814.

Glazebrook J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* 43: 205–227.
Govrin E, Levine A. 2002. Infection of Arabidopsis with a necrotrophic pathogen, Botrytis cinerea, elicits various defense responses but does not induce systemic acquired resistance (SAR). Plant Molecular Biology 48: 267–276.

Handakumbura P, Matos D, Osment K, Harrington M, Heo K, Kafle K, Kim S, Baskin T, Hazen S. 2013. Perturbation of Brachypodium distachyon CELLULOSE SYNTHASE A4 or 7 results in abnormal cell walls. BMC Plant Biology 13: 131.

Hashiba T. 1984. Estimating method of severity and yield loss by rice sheath blight disease. Bulletin of the Hokuriku National Agricultural Experiment Station 26: 115–164.

Hellwell E, Wang Q, Yang Y. 2013. Transgenic rice with inducible ethylene production exhibits broad-spectrum disease resistance to the fungal pathogens Magnaporthe oryzae and Rhizobiotica solani. Plant Biotechnology Journal 11: 33–42.

Hu K, Cao J, Zhang J, Xia F, Ke Y, Zhang H, Xie W, Liu H, Cui Y, Gao Y et al. 2017. Improvement of multiple agronomic traits by a disease resistance gene via cell wall reinforcement. Nature Plants 3: 17009.

Huckelhoven R, Fodor J, Preis C, Kogel K. 1999. Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. Plant Physiology 119: 1251–1260.

Jin Be, 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. Molecular Plant–Microbe Interactions 23: 791–798.

Jones J, Dangl J. 2006. The plant immune system. Nature 444: 323–329.

Jones J, Wittek K, Verweij W, Jupe F, Cooke D, Dorling S, Tomlinson L, Smoker M, Perkins S, Foster S. 2014. Elevating crop disease resistance with cloned genes. Philosophical Transactions of the Royal Society B: Biological Sciences 369: 20130087.

Kano A, Gomi K, Yamasaki-Kokudo Y, Sato M, Fukimoto T, Ohtani K, Tajima S, Izumori K, Tanaka A, Ishida Y et al. 2010. A rare sugar, D-Allose, confers resistance to rice bacterial blight with upregulation of defense-related genes in Oriza sativa. Phytopathology 100: 85–90.

Kano A, Hosotani K, Gomi K, Yamasaki-Kokudo Y, Shirakawa C, Fukimoto T, Ohtani K, Tajima S, Izumori K, Tanaka A, Ishida Y et al. 2011. D-Psicose induces upregulation of defense-related genes and resistance in rice against bacterial blight. Journal of Plant Physiology 168: 1852–1857.

Kawahama C, Guimares G, Nogueira S, MacLean D, Cook D, Steuernagel B, Baek J, Boyoukoskos C, Melo B, Tristao G et al. 2016. A pigeonpea gene confers resistance to Asian soybean rust in soybean. Nature Biotechnology 34: 661–665.

Kellogg E. 2015. Brachypodium distachyon as a genetic model system. Annual Review of Genetics 49: 1–20.

Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, Salzberg S. 2013. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biology 14: R36.

Knoth C, Salus M, Girké T, Eulgem T. 2009. The synthetic elicitor 3,5-dichloroantranilic acid induces NPR1-dependent and NPR1-independent mechanisms of disease resistance in Arabidopsis. Plant Physiology 150: 333–347.

Kouzai Y, Kimura M, Yamanaka Y, Watanabe M, Matsui H, Yamamoto M, Ichinose Y, Toyoda K, Onda Y, Mochida K et al. 2016. Expression profiling of marker genes responsive to the defence-associated phytohormones salicylic acid, jasmonic acid and ethylene in Brachypodium distachyon. BMC Plant Biology 16: 59.

Kunkel B, Brooks D. 2002. Cross talk between signaling pathways in pathogen defense. Current Opinion in Plant Biology 5: 325–331.

Langmead B, Salzberg S. 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9: 357–359.

Lee F, Rush M. 1983. Rice sheath blight: a major rice disease. Plant Disease 67: 829–832.

Li Z, Pinson S, Marchetti M, Stansel J, Park W. 1995. Characterization of quantitative trait loci (QTLs) in cultivated rice contributing to field resistance to sheath blight (Rhizobiotica solani). Theoretical and Applied Genetics 91: 382–388.

Mahoney A, Babiker E, Paulitz T, See D, Okubara P, Hulbert S. 2016. Characterizing and mapping resistance in synthetic-derived wheat to Rhizobiotica root rot in a green bridge environment. Phytopathology 106: 1170–1176.

Marshall D, Rush M. 1980a. Infection cushion formation on rice sheaths by Rhizobiotica solani. Phytopathology 70: 947–956.

Marshall D, Rush M. 1980b. Relation between infection by Rhizobiotica solani and R. oryzae and disease severity in rice. Phytopathology 70: 941–946.

Matsushita K. 1996. Scanning electron microscopy of the infection process of Rhizobiotica solani in leaf sheaths of rice plants. Phytopathology 76: 811–814.

McCarthy D, Chen Y, Smyth G. 2012. Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Research 40: 4288–4297.

Miedes E, Vanholme R, Boerjan W, Molina A. 2014. The role of the secondary cell wall in plant resistance to pathogens. Frontiers in Plant Science 5: 358.

Mikami K, Mori IC, Matsusura T, Ikeda Y, Kojima M, Sakakibara H, Hirayama T. 2016. Comprehensive quantification and genome survey reveal the presence of novel phytohormone action modes in red seaweeds. Journal of Applied Phycol 28: 2539–2548.

Mochida K, Uehara-Yamaguchi Y, Takahashi F, Yoshida T, Sakurai T, Shirasaka K. 2013. Large-scale collection and analysis of full-length cDNAs from Brachypodium distachyon and integration with Pooideae sequence resources. PLAs ONE 7: e75265.

Noutoshi Y, Okazaki M, Kida T, Nishina Y, Morishita Y, Ogawa T, Suzuki H, Shibata D, Jikumaru Y, Hanada A et al. 2012. Novel plant immune-promoting compounds identified via high-throughput chemical screening target salicylic acid glucosyltransferases in Arabidopsis. Plant Cell 24: 3795–3804.

Ogoshi A. 1987. Ecology and pathogenicity of anamostosis and intraspecific groups of Rhizobiotica solani Kühn. Annual Review of Phytopathology 25: 125–143.

Olaya G, Buitrago C, Pearsaud D, Sierotzki H, Tally A. 2012. Detection of resistance to Qol fungicides in Rhizobiotica solani isolates from rice. Phytopathology 102: S4.88 (Abstr).

Panneconque J, Hofre M. 2009. Interactions between cauliflower and Rhizobiotica anamostosis groups with different levels of aggressiveness. BMC Plant Biology 9: 35.

Peng X, Hu Y, Tang X, Zhou P, Deng X, Wang H, Guo Z. 2012. Constitutive expression of rice WRKY30 gene increases the endogenous jasmonic acid accumulation, PR gene expression and resistance to fungal pathogens in rice. Planta 236: 1485–1498.

Sanduya G, Buanafina M. 2014. Differential responses of Brachypodium distachyon genotypes to insect and fungal pathogens. Physiological and Molecular Plant Pathology 85: 53–64.

Sato H, Ideta O, Ando I, Kunihiro Y, Hirabayashi H, Iwano M, Miyasaka A, Nemoto H, Imbe T. 2004. Mapping QTLs for sheath blight resistance in the rice line WSS2. Breeding Science 54: 265–271.

Sayer R, Yang Y. 2007. Detection and quantification of Rhizobiotica solani AG-1 IA, the rice sheath blight pathogen, in rice using real-time PCR. Plant Disease 91: 1663–1668.

Schneebech K, Mathiessus U, Zwart A, Bragg J, Vogel J, Watt M. 2016. Brachypodium distachyon genotypes vary in resistance to Rhizobiotica solani AG8. Functional Plant Biology 43: 189–198.

Schreiber K, Desveaux D. 2008. Message in a bottle: chemical biology of induced disease resistance in plants. Plant Pathology Journal 24: 245–268.

Sharon M, Freeman S, Sneh B. 2011. Assessment of resistance pathways induced in Arabidopsis thaliana by hypervirulent Rhizobiotica spp. isolates. Phytopathology 101: 828–838.

Shimono M, Koga H, Akagi A, Hayashi N, Goto S, Sawada M, Kuribara T, Matsushita A, Sugano S, Jiang C et al. 2012. Rice WRKY45 plays important roles in fungal and bacterial disease resistance. Molecular Plant Pathology 13: 83–94.

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

Silverman P, Seskar M, Kanter D, Schweizer P, Metraux J, Raskin L. 1995. Salicylic acid in rice – biosynthesis, conjugation, and possible role. Plant Physiology 108: 633–639.
Singh A, Rohlín R, Savary S, Willocquet L, Singh US. 2003. Infection process in sheath blight of rice caused by *Rhizoctonia solani*. *Indian Phytopathology* 56: 434–438.

Soreng R, Peterson P, Romaschenko K, Davidese G, Zuloaga F, Judziewicz E, Filgueiras T, Davis J, Morrone O. 2015. A worldwide phylogenetic classification of the Poaceae (Gramineae). *Journal of Systematics and Evolution* 53: 117–137.

Spoel SH, Johnson JS, Dong X. 2007. Regulation of tradeoffs between plant defenses against pathogens with different lifestyles. *Proceedings of the National Academy of Sciences, USA* 104: 18842–18847.

Takatsuji H. 2014. Development of disease-resistant rice using regulatory components of induced disease resistance. *Frontiers in Plant Science* 5: 630.

Tamaki D, Seo S, Yamada S, Kano A, Miyamoto A, Shishido H, Miyoshi S, Taniguchi S, Akimitsu K, Gomi K. 2013. Jasmonic acid and salicylic acid activate a common defense system in rice. *Plant Signaling & Behavior* 8: e24260.

Thomma BP, Eggemont K, Tiersens KF, Broekaert WF. 1999. Requirement of functional ethylene-insensitive 2 gene for efficient resistance of Arabidopsis to infection by *Botrytis cinerea*. *Plant Physiology* 121: 1093–1102.

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

Vlot A, Demeule K, Dessel D. 2009. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology* 47: 177–206.

Vogel J, Garvin D, Leong O, Hayden D. 2006. Agrobacterium-mediated transformation and inbred line development in the model grass *Brachypodium distachyon*. *Plant Cell Tissue and Organ Culture* 84: 199–211.

Vogel J, Garvin D, Mockler T, Schmutz J, Rokhsar D, Bevan M, Barry K, Lucas S, Harmon-Smith M, Lail K et al. 2010. Genome sequencing and analysis of the model grass *Brachypodium distachyon*. *Nature* 463: 763–768.

Vogel J, Tuna M, Budak H, Huo N, Gu Y, Steinwand M. 2009. Development of SSR markers and analysis of diversity in Turkish populations of *Brachypodium distachyon*. *BMC Plant Biology* 9: 88.

Wang D, Amornsiripanich N, Dong X. 2006. A genomic approach to identify regulatory nodes in the transcriptional network of systemic acquired resistance in plants. *PLoS Pathogen* 2: 1042–1050.

Wang Y, Bouchabke-Coussa O, Lebris P, Antelme S, Soulhat C, Gineau E, Dalmair M, Bendahmane A, Morin H, Mouille G et al. 2015. LACCASE5 is required for lignification of the *Brachypodium distachyon* culm. *Plant Physiology* 168: 192–204.

Weinhold A, Bowman T. 1974. Repression of virulence in *Rhizoctonia solani* by glucose and 3-O-methyl glucose. *Phytopathology* 64: 985–990.

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 Journal* 40: 909–919.

Zheng A, Lin R, Zhang D, Qin P, Xu L, Ai P, Ding L, Wang Y, Chen Y, Liu Y et al. 2013. The evolution and pathogenic mechanisms of the rice sheath blight pathogen. *Nature Communications* 4: 1424.

Zhu H, Wen F, Li P, Liu X, Cao J, Jiang M, Ming F, Chu Z. 2014. Validation of a reference gene (*BdFIM*) for quantifying transgene copy numbers in *Brachypodium distachyon* by real-time PCR. *Applied Biochemistry and Biotechnology* 172: 3163–3175.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Fig. S1** Hyphal growth of *Rhizoctonia solani* on nutrient agar medium containing phytohormones.

**Fig. S2** Effects of phytohormones on *Brachypodium distachyon* disease resistance to *Pyricularia oryzae* and *Botrytis cinerea*.

**Fig. S3** *Rhizoctonia solani* biomass in *Brachypodium distachyon* leaves at the initial infection stage.

**Fig. S4** Endogenous levels of phytohormones in *Brachypodium distachyon* accessions Bd21 and Bd3-1 after inoculation with *Rhizoctonia solani*.

**Table S1** Primers used in this study

**Table S2** Summary of the sequence reads from RNA-seq analysis mapped to the *Brachypodium distachyon* Bd21 genome

**Table S3** Differentially expressed genes (DEGs) of *Brachypodium distachyon* in salicylic acid treatment

**Table S4** Differentially expressed genes (DEGs) of *Brachypodium distachyon* in benzothiadiazole treatment

**Table S5** Cell wall biogenesis (GO:0042546)-related genes in the differentially expressed genes (DEGs) specifically induced by salicylic acid

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.