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Title: The transcriptional response to salicylic acid plays a role in *Fusarium* yellows resistance in *Brassica rapa* L.

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Abstract

Key message

Fusarium yellows resistant and susceptible lines in *Brassica rapa* showed different salicylic acid responses; the resistant line showed a similar response to previous reports, but the susceptible line differed.

Abstract

Fusarium yellows caused by *Fusarium oxysporum* f. sp. *conglutinans* (*Foc*) is an important disease. Previous studies showed that genes related to salicylic acid (SA) response were more highly induced following *Foc* infection in *Brassica rapa* Fusarium yellows resistant lines than susceptible lines. However, SA-induced genes have not been identified at the whole genome level and it was unclear whether they were up-regulated by *Foc* inoculation. Transcriptome analysis with and without SA treatment in the *B. rapa* Fusarium yellows susceptible line ‘Misugi’ and the resistant line ‘Nanane’ was performed to obtain insights into the relationship between SA sensitivity/response and Fusarium yellows resistance. ‘Nanane’s up-regulated genes were related to SA response and down-regulated genes were related to jasmonic acid (JA) or ethylene (ET) response, but differentially expressed genes in ‘Misugi’ were not. This result suggests that Fusarium yellows resistant and susceptible lines have a different SA response, and that an antagonistic transcription between SA and JA/ET responses was found only in a Fusarium yellows resistant line. SA-responsive genes were induced by *Foc* inoculation in Fusarium yellows resistant (RJKB-T23) and susceptible lines (RJKB-T24). By contrast, 39 SA-induced genes specific to RJKB-T23 might function in the defense response to *Foc*. In this study, SA-induced genes were identified at the whole genome level, and the possibility, the defense response to *Foc* observed in a resistant line could be mediated by SA-induced genes, is suggested. These results will be useful for future research concerning the SA importance in *Foc* or other diseases resistance in *B. rapa*.

Keywords

Salicylic acid; Fusarium yellows; *Brassica rapa*; Transcriptome; Systemic acquired resistance

Introduction

Brassica rapa L. is in the Brassicaceae family and includes important vegetables and crops including leafy vegetables such as komatsuna (var. *perviridis*), Chinese cabbage (var. *pekinensis*), and pak choi (var. *chinensis*), root vegetables such as turnip (var. *rapa*), and oilseed (var. *oleifera*). There are various pathogens that cause problems of quality and yield losses in *B. rapa* vegetables, and the use of disease resistant cultivars is the most effective method to manage damage by pathogens (Lv et al. 2020; Mehraj et al. 2020). Thus, breeding disease resistant cultivars is one of the important aims in developing new cultivars.

Plants have evolved their immunity to pathogens via two mechanisms (Jones and Dangl 2006; Dodds and Rathjen 2010). Plants have pattern recognition receptors (PRRs) located in the cell membrane, and these PRRs recognize pathogen-associated molecular patterns (PAMPs) such as flagellin and chitin. PAMP recognition by PRRs activates PAMP-triggered immunity (PTI), which restricts pathogen development. Most pathogens secrete effectors (avirulence (Avr) proteins) into plant cells to suppress PTI. On the other hand, plants have various *resistance* (*R*) genes, which mainly encode Toll/Interleukin-1 receptor (TIR) or coiled-coil (CC), nucleotide-binding (NB), and leucine-rich repeat (LRR) domains, to detect effectors. Recognition of effectors by *R* genes induces effector-triggered immunity (ETI). *R* genes recognize specific effectors, termed ‘gene-for-gene resistance’ (Jones and Dangl 2006). Following attempted local infection, a long-lasting, broad-spectrum immune response is induced throughout the entire plant, which is called systemic acquired resistance (SAR) (Spoel and Dong 2012). In *B. rapa* vegetables, several *R* genes conferring resistance to disease such as clubroot or Fusarium yellows have been identified and DNA marker-assisted breeding systems based on them have been developed (Lv et al. 2020; Mehraj et al. 2020). DNA marker-associated selection based on PTI-related genes has not progressed.

In PTI, ETI, and SAR, salicylic acid (SA) has important roles. SA is a plant hormone, and regulates many stress responses and developmental processes including the plant immune response, thermogenesis, senescence, and abiotic stress responses (Yan and Dong 2014). The role of SA in the plant immune response is well studied in *Arabidopsis thaliana*. SA positively regulates resistance to biotrophic pathogens, which derive nutrients from living host cells, and negatively regulates resistance to

necrotrophic pathogens, which derive nutrients from dead host cells (Bari and Jones 2009; Klessig et al. 2018). For pathogen-induced SA accumulation and immunity, *ENHANCED DISEASE SUSCEPTIBILITY 1* (*EDS1*) and *PHYTOALEXIN-DEFICIENT 4* (*PAD4*), which encode lipase-like proteins, and *NONSPECIFIC DISEASE RESISTANCE 1* (*NDRI*), which encodes the integrin-like protein, work upstream of SA biosynthesis (Vlot et al. 2009; Knepper et al. 2011; Fu and Dong 2013; Klessig et al. 2018). SA is biosynthesized from chorismate by two distinct pathways, the isochorismate (IC) pathway and the phenylalanine ammonia-lyase (PAL) pathway in plastids, of which the IC pathway is the main pathway for pathogen-induced SA synthesis in *A. thaliana* (Kumar 2014; Klessig et al. 2018; Rekhter et al. 2019). SA is converted to methyl salicylate (MeSA) catalyzed by BA/SA CARBOXYL METHYLTRANSFERASE 1 (*BSMT1*), MeSA spreads into the cytoplasm, and then MeSA is converted back to SA by MeSA esterase. In the cytoplasm, SA induces redox changes and monomerization of NONEXPRESSOR OF PR GENES 1 (*NPR1*). Monomeric *NPR1* is transferred to the nucleus and interacts with TGA transcription factors to activate the expression of SA-responsive genes including *PATHOGENESIS-RELATED* (*PR*) genes (Kinkema et al. 2000; Kumar 2014). In *B. rapa*, several genes encoding steps in the SA pathway have been identified and include some genes homologous to *A. thaliana* pathway genes (Lee and Cho 2003; Park et al. 2003; Abe et al. 2011), however, SA-responsive genes at the whole genome level have not been identified.

SA accumulation is required for SAR (Spoel and Dong 2012). SAR is induced by SA treatment in living plants and harvested crops. Exogenous SA treatment protected plants from disease in tomato and cucumber (Narusaka et al. 1999; Mandal et al. 2009). Pre-harvest SA treatment induced post-harvest disease resistance in some fruits (Yao and Tian 2005; Cao et al. 2006; Zeng et al. 2006). Post-harvest SA treatment also enhanced resistance to pathogens in some fruits (Yao and Tian 2005; Cao et al. 2006).

Fusarium yellows caused by the hemibiotrophic pathogen *Fusarium oxysporum* f. sp. *conglutinans* (*Foc*) is an important disease in *B. rapa* and *Brassica oleracea* (Lv et al. 2014; Shimizu et al. 2014; 2015; Pu et al. 2016). When plants are infected by this pathogen, they show symptoms such as leaf yellowing, wilting, defoliation, stunted growth, and death (Walker 1930; Daly and Tomkins 1995). Resistance genes to *Foc*, Bra012688 and Bra012689, have been identified and DNA markers for these

genes have been developed in *B. rapa* (Pu et al. 2012; Shimizu et al. 2014; Kawamura et al. 2016). Transcriptome analysis following *Foc* inoculation in *B. rapa* Fusarium yellows resistant and susceptible lines showed that genes categorized into the GO term ‘response to salicylic acid stimulus’ or ‘systemic acquired resistance’ were up-regulated at 24 hours after inoculation in a resistant line but not in a susceptible line (Miyaji et al. 2017). The induction level of genes related to the SA response was predicted to be higher in resistant lines compared with susceptible lines following *Foc* inoculation. Genes categorized into the ‘response to salicylic acid stimulus’ or ‘systemic acquired resistance’ were predicted from the database, however whether they are induced by SA in *B. rapa* has not been analyzed. In this study, we examined the differences in SA sensitivity/response between Fusarium yellows resistant and susceptible lines. We used two *B. rapa* cultivars, ‘Misugi’ and ‘Nanane’, which are Fusarium yellows susceptible and resistant lines, respectively, to analyze a transcriptome following SA treatment, and compared their response to SA.

Materials and methods

Plant materials, growth condition and SA treatment

Two commercial komatsuna (*Brassica rapa* var. *perviridis*) cultivars ‘Misugi’ (Sakata Seed Corporation, Japan) and ‘Nanane’ (Takii & Co., Ltd., Japan) were used as plant material. ‘Misugi’ and ‘Nanane’ were confirmed as Fusarium yellows susceptible and resistant lines, respectively, by inoculation tests (data not shown) and DNA marker analysis. Seeds were surface sterilized and sown in plastic pots containing Murashige and Skoog (MS) agar medium supplemented with 1.0 % sucrose (pH 5.7). Plants were grown in growth chambers under a 16-h/8-h light/dark cycle at 22 °C for seven days. Seedlings were transplanted into plastic pots containing MS agar medium with 0.010, 0.10, and 1.0 mM salicylic acid (SA) at seven days after sowing. Before transplanting (0 h), and at 3, 6, 12, 24, 48, and 72 h after SA treatment (HAT), cotyledons were harvested and frozen with liquid nitrogen for RNA extraction. At seven days after sowing, seedlings have cotyledons and no true leaves. Each sample contains cotyledons from one individual plant.

To detect the induction level of SA-induced genes in *B. rapa* cultivars, three commercial Chinese cabbage (var. *pekinensis*) cultivars ‘Akimeki’ (Nippon Norin Seed Co., Japan),

‘Matsushima-jun-2-go’ (WATANABE SEED CO., LTD., Japan), and ‘Minebuki505’ (Sakata Seed Corporation), two komatsuna cultivars, ‘Natsu-no-koshien’ (TOKITA SEED CO., LTD., Japan) and ‘Zaoh’ (WATANABE SEED CO., LTD.), and a turnip (var. *rapa*) cultivar ‘CR-shirowarabe’ (Takii & Co., Ltd.) were used as plant material as well as ‘Misugi’ and ‘Nanane’. Seeds were surface sterilized and sown in plastic pots containing MS agar medium supplemented with 1.0 % sucrose (pH 5.7). Plants were grown in growth chambers under a 16-h/8-h light/dark cycle at 22 °C for seven days. Seedlings were transplanted into plastic pots containing MS agar medium with 1.0 mM SA at seven days after sowing. Before transplanting and at 72 HAT, cotyledons were harvested and frozen with liquid nitrogen for RNA extraction.

Prediction of Fusarium yellows resistance by DNA markers

Genomic DNA was isolated from leaves by the CTAB method (Murray and Thompson 1980). To predict the Fusarium yellows resistance, dominant markers, Bra012688m and Bra012689m, were used (Kawamura et al. 2016). PCR was performed using QuickTaq[®]HS DyeMix (TOYOBO Co., Ltd., Japan). The reaction mixture was incubated in the thermal cycler at 94 °C for 2 min following by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 68 °C for 1 min. PCR products were detected by electrophoresis using 1.0 % agarose gel. The primer sets of the DNA markers are listed in Table S1.

RNA extraction and BGISEQ sequence

Total RNAs from cotyledons were extracted by SV Total RNA Isolation System (Promega Co., USA). Eight sequence libraries were prepared for RNA sequencing (RNA-seq), (1) Misugi_0h, samples before transplanting of ‘Misugi’ (two biological replicates); (2) Misugi_72h, samples at 72 h after transplanting to MS with SA of ‘Misugi’ (two biological replicates); (3) Nanane_0h, samples before transplanting of ‘Nanane’ (two biological replicates); (4) Nanane_72h, samples at 72h after transplanting to MS with SA of ‘Nanane’ (two biological replicates). The mRNAs were purified from total RNA by oligo(dT) selection and first-strand cDNA was generated using random N6 primers. The second-strand cDNA synthesis, end-repair, 3’ adenylation, and adaptor ligation were performed to produce double stranded PCR products. PCR products were heat denatured and single stranded circular DNAs were used

for sequence library construction. RNA-seq was performed at 0 and 72 HAT in ‘Misugi’ and ‘Nanane’ using BGISEQ-500 (pair-end, 100 bp).

Differentially expressed genes (DEGs) detection and gene ontology and pathway enrichment analyses

Sequenced reads were quality checked by FastQC version 0.11.8 and trimmed by quality using Trimmomatic (version 0.36) (Bolger et al. 2014). Trimmed reads were aligned to the *B. rapa* reference genome version 1.5 (<http://brassicadb.org/brad/>) by HISAT2 version 2.1.0 (Kim et al. 2015). Gene expression levels were scored by fragments per kilo-base per million (FPKM) using cuffdiff v2.2.1 (Trapnell et al. 2013). Differentially expressed genes (DEGs) before and after SA treatments were identified using three criteria, two-fold difference ($\log_2$ ratio ≥ 1), p value < 0.01 , and q value < 0.01 . The gene ontology (GO) tool, agriGO (Du et al. 2010), was used for enrichment analysis of gene functional ontology term following the methods described by Shimizu et al. 2014. The Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper (Kanehisa and Sato 2020) was used for pathway enrichment analysis. *A. thaliana* ortholog genes of *B. rapa* DEGs were used for KEGG analysis. Pathways, which include 10 or more genes into at least one category, were selected.

Gene expression analysis

cDNA was synthesized from 500 ng total RNA using ReverTra Ace qPCR RT Master Mix with gDNA Remover (TOYOBO Co., Ltd.). The specificity of the primer set of each gene was first tested by electrophoresis of RT-PCR amplified products using QuickTaq[®]HS DyeMix on 1.5 % agarose gel in which single products were observed. RT-PCR conditions were 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 68 °C for 30 s. The absence of genomic DNA contamination was confirmed by the PCR of no RT control.

Real-time RT-PCR (qPCR) was performed using a LightCycler 96 (Roche Molecular Systems, Inc., USA). cDNA was amplified using FastStart Essential DNA Green Master (Roche). qPCR conditions were 95 °C for 10 min followed by 40 cycles of 95 °C for 10 s, 60 °C for 10 s, and 72 °C for 10 s, and Melting program (65-97 °C at 0.1 °C/s). After amplification cycles, each reaction was subjected to melt

temperature analysis to confirm the presence of single amplified products. The relative expression level of each gene relative to *ACTIN* (*Bractin*) was automatically calculated using automatic CQ calling according to the manufacture's instructions (Roche) (Fujimoto et al. 2006). Data presented are the average and standard error of three biological and experimental replicates and statistically analyzed using Dunnett's test or Student's *t* test, *p* value < 0.05 or 0.01. The primer sets are listed in Table S1.

Results

Determination of the SA concentration and time point for identification of SA-responsive genes

The *Brassica rapa* Fusarium yellows resistant line 'Nanane' and susceptible line 'Misugi' were used (Table S2). To determine the SA concentration to induce expression, we assayed 0.010, 0.10, and 1.0 mM SA against seven-day seedlings of 'Nanane'. It has been shown that the expression of *BrPRI* was induced by SA treatment in *B. rapa* (Abe et al. 2011). In this study, the expression level of *BrPRI* at 72 h was highest after 1.0 mM SA treatment (Fig. S1a). We also confirmed the induction of *BrPRI* at 72 h after 1.0 mM SA treatment (HAT) in 'Misugi' (Fig. S1b). These results indicate that 1.0 mM is suitable for detecting the transcriptional change by SA treatment in both lines.

Next, we examined the expression pattern of SA-induced genes at seven time points, at 0, 3, 6, 12, 24, 48, and 72 HAT. The expression levels of *BrPRI* and two more genes, *BETA-1,3-GLUCANASE 3* (*BrBG3*) and *GLUTATHIONE S-TRANSFERASE 25* (*BrGST25*), which have been shown to be induced by SA treatment in *B. rapa* and *A. thaliana* (Blanco et al. 2009; Abe et al. 2011), were examined by qPCR (Fig. 1a). The expression level of *BrPRI* gradually increased until 24 HAT in both lines, and further increased at 48 and 72 HAT in 'Nanane', but in 'Misugi', the expression levels did not change from 12 to 72 HAT (Fig. 1a). The expression level of *BrBG3* increased at 3 HAT and was maintained until 48 HAT; the expression was further increased at 72 HAT in 'Nanane'. In 'Misugi', expression levels gradually increased from 3 to 12 HAT, and then expression levels decreased after 12 HAT (Fig. 1a). The expression of *BrGST25* was slightly induced at 3 HAT, but it did not change after 3 HAT in 'Misugi' (Fig. 1a). The expression level of *BrGST25* increased from 3 to 6 HAT and then decreased from 12 to 72 HAT in 'Nanane' (Fig. 1a). Because expression levels of *BrPRI* and *BrBG3* were highest at 72 HAT in 'Nanane', and *BrPRI* and *BrGST25* showed higher expression level at 72 HAT than before treatments in 'Misugi',

RNA sequencing (RNA-seq) was performed at 72 HAT to compare the SA response at the whole genome level in ‘Misugi’ and ‘Nanane’.

Identification of differentially expressed genes by SA treatment

RNA-seq was performed before and 72 hours after SA treatment in ‘Misugi’ and ‘Nanane’ using BGISEQ-500 (pair-end, 100 bp). Sequenced reads were quality checked and trimmed. Trimmed reads were mapped to the *B. rapa* reference genome version 1.5 by HISAT2 (Kim et al. 2015). 16.2 M to 16.9 M reads were mapped to the reference genome, and 13.2 M to 13.8 M reads (about 80 % of total reads) were uniquely mapped to the reference genome (Table S3). The expression level of each gene was calculated by cuffdiff (Trapnell et al. 2013). We identified differentially expressed genes (DEGs) using the following criteria, two-fold difference ($\log_2$ ratio ≥ 1), p value < 0.01 , and q value < 0.01 ; 3,423 and 3,780 DEGs were identified, including 1,841 and 1,818 up-regulated and 1,582 and 1,962 down-regulated genes following SA treatment in ‘Misugi’ and ‘Nanane’, respectively (Table S4, S5, S6). 1,061 up- and 994 down-regulated genes overlapped between ‘Misugi’ and ‘Nanane’, and overlapped up- and down-regulated genes were defined as *B. rapa* SA-induced genes (BrSAIGs) and *B. rapa* SA-suppressed genes (BrSASGs), respectively (Fig. 2a, b, Table S4, S7).

PR genes are induced in response to infection by pathogens, and have important roles in pathogen response (Kitajima and Sato 1999). There are five *PR* genes, *AtPR1*, *AtPR2*, *AtPR3*, *AtPR4*, and *AtPR5*, in *A. thaliana*. There is no orthologous gene of *AtPR5* in *B. rapa*, and two, nine, five, and three genes in *B. rapa* are orthologs of *AtPR1*, *AtPR2*, *AtPR3*, and *AtPR4*, respectively. *BrPR* genes except *BrPR3* tended to be up-regulated by SA treatment in both lines (Fig. 3). In ‘Misugi’, *BrPR1* (Bra013123), *BrPR2* (Bra014635), and *BrPR4* (Bra001121 and Bra001122) showed up-regulation, and *BrPR3* (Bra001453) showed down-regulation (Fig. 3). In ‘Nanane’, *BrPR1* (Bra013123), *BrPR2* (Bra007315, Bra014635, and Bra014636), and *BrPR4* (Bra001121, Bra001122, and Bra001123) showed up-regulation, and *BrPR3* (Bra001453 and Bra001454) showed down-regulation (Fig. 3).

Transcription factors (TFs) were collected from the Plant Transcription Factor Database (Pérez-Rodríguez et al. 2010). 2,192 genes are classified as TFs in *A. thaliana*, and 3,794 genes were identified as orthologous genes of 1,903 of the 2,192 *A. thaliana* TFs in *B. rapa*. About 300-400 of 3,794

TF genes (about 10 %) showed a change of expression level at 72 HAT (Fig. S2a). The number of up-regulated TF genes was more in ‘Misugi’ than in ‘Nanane’, while the number of down-regulated genes was more in ‘Nanane’ than in ‘Misugi’ (Fig. S2a, Table S8, S9). The class of TFs with up- and down-regulated gene expression levels differed except for MYB and Orphans TFs (Fig. S2b). In up-regulated DEGs, genes categorized into NAC and WRKY TFs tended to be induced in both lines (Fig. S2b, Table S9). In down-regulated DEGs, the top three categories except for bHLH and bZIP TFs were different between lines (Fig. S2b, Table S9). In ‘Misugi’, G2-like and Orphans TFs tended to be down-regulated. While in ‘Nanane’, AP2-EREBP, MYB, and Tify TFs tended to be down-regulated (Fig. S2b, Table S9).

WRKY TFs tended to be up-regulated in both lines (Fig. S2b). *WRKY* TFs have been reported to be involved in defense signaling, and they positively and negatively regulate disease resistance (Eulgem and Somssich 2007; Pandey and Somssich 2009). In *A. thaliana*, 72 *WRKY* TFs have been identified (Eulgem et al. 2000; Pérez-Rodríguez et al. 2010), and in total, 151 genes in *B. rapa* are orthologs of *AtWRKYs*. In ‘Misugi’, 14 genes (*BrWRKY1*, *BrWRKY2*, *BrWRKY6*, *BrWRKY18*, *BrWRKY26*, *BrWRKY33*, *BrWRKY38*, *BrWRKY46*, *BrWRKY50*, *BrWRKY54*, *BrWRKY70*, and *BrWRKY75*) showed up-regulation and two genes (*BrWRKY7* and *BrWRKY74*) showed down-regulation. (Table S10). In ‘Nanane’, 19 (*BrWRKY8*, *BrWRKY18*, *BrWRKY26*, *BrWRKY28*, *BrWRKY38*, *BrWRKY46*, *BrWRKY47*, *BrWRKY51*, *BrWRKY54*, *BrWRKY65*, *BrWRKY67*, *BrWRKY70*, *BrWRKY72*, and *BrWRKY75*) and eight genes (*BrWRKY3*, *BrWRKY7*, *BrWRKY22*, *BrWRKY33*, *BrWRKY40*, and *BrWRKY74*) showed up- and down-regulation, respectively (Table S10).

MYB TFs showed up- and down-regulation in ‘Nanane’ (Fig. S2b). Some *MYB* TFs have been also reported as regulators of disease resistance (Dubos et al. 2010). In *A. thaliana*, 147 genes are categorized as *MYB* TFs (Pérez-Rodríguez et al. 2010), and in total, 276 genes in *B. rapa* are orthologs of *AtMYBs*. In ‘Misugi’, 12 *MYB* TFs (*BrMYB28*, *BrMYB34*, *BrMYB51*, *BrMYB57*, *BrMYB59*, *BrMYB60*, *BrMYB95*, Bra018772 and Bra009134) showed up-regulation by SA treatment, and four genes (*BrMYB16*, *BrMYB34*, Bra034853 and Bra002610) showed down-regulation (Table S11). In ‘Nanane’, 12 (*BrMYB2*, *BrMYB4*, *BrMYB7*, *BrMYB28*, *BrMYB32*, *BrMYB38*, *BrMYB43*, *BrMYB59*, *BrMYB116*, *BrMYB122* and Bra009134) and 17 genes (*BrMYB13*, *BrMYB21*, *BrMYB30*, *BrMYB34*, *BrMYB44*, *BrMYB47*, *BrMYB73*,

BrMYB77, *BrMYB88*, *BrMYB95*, *BrMYB96*, *BrMYB108* and *Bra005818*) showed up- and down-regulation by SA treatment, respectively (Table S11).

Genes related to hormone response and systemic acquired resistance induced by SA treatment

Jasmonic acid (JA) and ethylene (ET) are also important plant hormones for disease resistance. JA and ET positively regulate resistance to necrotrophic pathogens, and JA also regulates the defense response to insect feeding and wounding (Glazebrook 2005; Pieterse et al. 2012). Generally the SA pathway acts antagonistically to the JA/ET pathways, but synergetic interactions between these pathways are also reported (Bari and Jones 2009; Pieterse et al. 2012; Caarls et al. 2015; Shigenaga and Argueso 2016; Shigenaga et al. 2017; Klessig et al. 2018; Zhang et al. 2018). In *A. thaliana*, SA treatment induces SAR (Edgar et al. 2006). We collected all genes categorized into GO terms related to SA, JA, ET, gibberellin, and SAR from The Arabidopsis Information Resource (TAIR) (<https://www.arabidopsis.org/>). In total, 106, 110, 100, 68, and 52 genes were categorized into ‘response to salicylic acid stimulus’, ‘response to jasmonic acid stimulus’, ‘response to ethylene stimulus’, ‘response to gibberellin stimulus’, and ‘systemic acquired resistance’, respectively, in *A. thaliana*. In *B. rapa*, their orthologous 213, 217, 198, 115, and 92 genes were categorized into these categories, respectively. The number of up-regulated genes categorized into ‘response to salicylic acid stimulus’ were similar between ‘Misugi’ and ‘Nanane’, while the number of down-regulated genes were about doubled in ‘Nanane’ relative to ‘Misugi’ (Fig. 4, Table S8, S12). About 20 % of genes categorized into ‘response to salicylic acid stimulus’ were identified as DEGs following SA treatment (Fig. 4). The number of up-regulated genes categorized into ‘response to jasmonic acid stimulus’ was more in ‘Misugi’ than in ‘Nanane’, while the number of down-regulated genes was more in ‘Nanane’ than in ‘Misugi’ (Fig. 4, Table S8, S12). The number of up-regulated genes categorized into ‘response to ethylene stimulus’ was similar between ‘Misugi’ and ‘Nanane’, while the number of down-regulated genes was more in ‘Nanane’ than in ‘Misugi’ (Fig. 4, Table S8, S12). The number of genes categorized into ‘response to gibberellin stimulus’ was much lower than the number of genes categorized into other hormone stimulus responses, while the percentage of DEGs categorized into ‘response to gibberellin stimulus’ (11.3 % and 17.4 % genes as DEGs in ‘Misugi’ and ‘Nanane’, respectively) was similar to that categorized into ‘response to ethylene stimulus’ (13.1 % and 15.2 %

genes as DEGs in ‘Misugi’ and ‘Nanane’, respectively) (Fig. 4, Table S8, S12). The number of genes categorized into ‘systemic acquired resistance’ was similar between ‘Misugi’ and ‘Nanane’ (Fig. 4, Table S8, S12). About 35 % of genes categorized into ‘systemic acquired resistance’ were identified as DEGs following SA treatment in ‘Misugi’ and ‘Nanane’.

Validation of gene expression by qPCR

We compared the expression levels of nine genes, *BrPR1*, *BrBG3*, *BrGST25*, *ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 15 (BrERF15)*, *MYB DOMAIN PROTEIN 59 (BrMYB59)*, *BrPR2*, *BrPR4*, *UDP-GLUCOSE TRANSFERASE 1 (BrUGT1)*, and *WRKY DNA-BINDING PROTEIN 70 (BrWRKY70)*, with and without SA treatment (0 and 72 HAT) between qPCR and RNA-seq data for validation (Fig. S3). The expression pattern determined by RNA-seq corresponded to the qPCR patterns in all genes of both lines with the exception of *BrPR1* (Fig. S3).

The expression patterns of six genes (*BrERF15*, *BrMYB59*, *BrPR2*, *BrPR4*, *BrUGT1*, and *BrWRKY70*) at seven time points were examined in ‘Misugi’ and ‘Nanane’ (Fig. 1b). The expression pattern of *BrMYB59* was similar between lines, and the expression level increased at 24 HAT and was stable or increased more after 24 HAT in ‘Misugi’ and ‘Nanane’, respectively (Fig. 1b). The expression patterns of *BrERF15*, *BrPR2*, *BrPR4*, *BrUGT1*, and *BrWRKY70* were different between lines. The plateauing of expression level in *BrERF15* was faster in ‘Misugi’ than in ‘Nanane’, while the saturation of expression levels in *BrUGT1* was faster in ‘Nanane’ than in ‘Misugi’ (Fig. 1b). The expression level of *BrPR2* and *BrPR4* increased from 6 and 48 HAT in ‘Nanane’, respectively, and these genes were still expressed at a higher level at later times (Fig. 1b). The expression level of *BrPR2* and *BrPR4* was stable following SA treatment in ‘Misugi’ (Fig. 1b). The expression level of *BrWRKY70* was highest at 6 HAT and was stable after 12 HAT in ‘Misugi’, while it was stable following SA treatment in ‘Nanane’ (Fig. 1b).

Gene ontology analysis

We identified DEGs in response to SA treatment with more stringent parameters using the following criteria, four-fold difference ($\log_2$ ratio ≥ 2), p value < 0.001 , and q value < 0.001 for Gene

ontology (GO) analysis; in 'Misugi', 550 and 389 genes were up- and down-regulated, respectively, (Table S4, S5), and in 'Nanane', 597 and 655 genes were up- and down-regulated, respectively (Table S4, S6). There were 265 up- and 225 down-regulated genes overlapped between 'Misugi' and 'Nanane', and they were named BrSAIGs-stringent and BrSASGs-stringent, respectively (Fig. 2c, d, Table S4, S7).

Up- or down-regulated DEGs in 'Misugi' or 'Nanane' were categorized into GO cellular component (CC), GO molecular function (MF), and GO biological process (BP). 48 and 80 categories were overrepresented in up-regulated DEGs in 'Misugi' and 'Nanane', respectively ($p < 0.001$, FDR < 0.05) (Table S13, S14). 73 and 147 categories were overrepresented in down-regulated DEGs in 'Misugi' and 'Nanane', respectively ($p < 0.001$, FDR < 0.05) (Table S15, S16). Three categories overlapped between lines in up-regulated DEGs, and included 'external encapsulating structure', 'cell wall', and 'plasma membrane' in CC (Fig. S4, Table S13, S14). 52 categories overlapped between lines in down-regulated DEGs (Fig. S4, Table S15, S16).

In up-regulated DEGs in 'Misugi', genes categorized into 'glutathione transferase activity', 'glucosyltransferase activity', and 'organic acid transmembrane transporter activity' in MF were overrepresented (group A) (Fig. 5, Table S13). Whereas, genes categorized into 'toxin metabolic process', 'systemic acquired resistance', 'lignin biosynthetic process' in BP were overrepresented in up-regulated DEGs in 'Nanane' (group C) (Fig. 5, Table S14). In down-regulated DEGs, genes categorized into 'xyloglucan:xyloglucosyl transferase activity' in MF and genes categorized into 'plant-type cell wall organization or biogenesis', 'regulation of cell size', 'response to gibberellin stimulus', and 'response to hormone stimulus' in BP were overrepresented in both lines (group B) (Fig. 5, Table S15, S16). Genes categorized into 'defense response to fungus', 'response to salicylic acid stimulus', 'defense response to bacterium', 'response to jasmonic acid stimulus', 'response to wounding', and 'response to ethylene stimulus' in BP were overrepresented in the down-regulated DEGs and sometimes in the up-regulated DEGs in 'Nanane', which were specific in 'Nanane' (group D) (Fig. 5, Table S14, S16).

265 BrSAIGs-stringent and 225 BrSASGs-stringent were also categorized into CC, MF, and BP. In 265 BrSAIGs-stringent, genes categorized into 'glucosyltransferase activity' in MF, and genes categorized into 'response to salicylic acid stimulus' and 'defense response to bacterium' in BP were overrepresented (Fig. 5, Table S17). Genes categorized into 'xyloglucan:xyloglucosyl transferase activity'

in MF, and ‘plant-type cell wall organization or biogenesis’, ‘regulation of cell size’ in BP were overrepresented in 225 BrSASGs-stringent (Fig. 5, Table S18).

Pathway enrichment analysis

Up-/down-regulated DEGs in ‘Misugi’ or ‘Nanane’, BrSAIG-stringent and BrSASG-stringent were categorized by Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper for pathway enrichment analysis. Pathways, which classified 10 or more genes into at least one category, were selected. Ten categories including ‘Biosynthesis of secondary metabolites’, ‘Metabolic pathway’, ‘Plant hormone signal transduction’ and ‘Plant-pathogen interaction’ were identified (Fig. S5).

Comparison of SA-induced genes between *B. rapa* and *A. thaliana*

In *A. thaliana*, a transcriptome analysis of Columbia-0 seedlings treated for 2.5 h SA has been performed using microarrays, and 217 genes were identified as AtSAIGs (Blanco et al. 2009). 194 of the 217 AtSAIGs have orthologous genes in *B. rapa*. In total, 422 genes in *B. rapa* are orthologs of 194 AtSAIGs. 85 and 94 of the 422 genes were identified as up-regulated DEGs in ‘Misugi’ and ‘Nanane’, respectively, and 59 genes (about 5.6% of total BrSAIGs) overlapped between species, which indicates that most SAIGs are different between species (Fig. 2a). The 59 genes included some *WRKY* genes (*WRKY18*, *WRKY38*, *WRKY46*, *WRKY54*, and *WRKY75*), *GST25*, and *SIGMA FACTOR BINDING PROTEIN 1 (SIB1)* (Fig. 6).

Comparison between SA-induced genes and genes differentially expressed in response to *Fusarium oxysporum* f. sp. *conglutinans* inoculation

We have shown that genes categorized into the GO term ‘response to salicylic acid stimulus’ or ‘systemic acquired resistance’ were up-regulated at 24 hours after *Foc* inoculation (HAI) in a *Fusarium* yellows resistant line (RJKB-T23) but not in a susceptible line (RJKB-T24) (Miyaji et al. 2017). In this study, overrepresentation of ‘response to salicylic acid stimulus’ or ‘systemic acquired resistance’ were found in the up-regulated genes following SA treatment in the *Fusarium* yellows resistant line ‘Nanane’, but not in the susceptible line ‘Misugi’ (Fig. 5). Thirty-eight genes were common to BrSAIGs and

up-regulated genes following *Foc* inoculation in the RJKB-T23 and RJKB-T24 (Fig. S6), and these genes might have functions in recognition or response to *Foc*. The 38 genes include *BrBG3*, *CHITINASE (BrCHI)*, *BrGSTF3*, *BrGSTF9*, *BrPR4*, and *ISOCHORISMATE SYNTHASE 1 (BrICS1)* (Fig. S6, S7a). Thirty-nine genes overlapped between SA-induced genes in the Fusarium yellows resistant line ‘Nanane’ and up-regulated genes in the Fusarium resistant line (RJKB-T23) but did not overlap in the susceptible line (RJKB-T24) (Fig. S6). These genes might function in the defense response to *Foc*. The 39 genes include *BrPR1*, *BrPR2*, *BrGSTF10*, *BrGSTU11*, *CALMODULIN BINDING PROTEIN 60-LIKE.G (BrCBP60g)*, *DOWNY MILDEW RESISTANT 6 (BrDMR6)*, *BrWRKY70*, and chitinase family protein (Bra027940) (Fig. S6, S7b).

Relationship between SA response and the induction level of systemic acquired resistance

We found that the induction level of *BrPR1*, *BrPR2* and *BrPR4* by SA treatment was higher in ‘Nanane’ than in ‘Misugi’ at 72 HAT (Fig. 1), suggesting that the induction levels corresponded to SAR induction level. To test this hypothesis, we selected six more commercial cultivars of *B. rapa*, which are resistant to *Foc* predicted by DNA marker basis; ‘Akimeki’, ‘CR-shirowarabe’, ‘Matsushima-jun-2-go’, ‘Minebuki505’, ‘Natsu-no-koshien’, and ‘Zaoh’ (Table S2). These lines together with ‘Misugi’ and ‘Nanane’ were treated with 1.0 mM SA and the expression levels of *BrPR1*, *BrPR2*, and *BrPR4* were examined. At 72 HAT, ‘Nanane’ and ‘Akimeki’ showed significantly higher expression of *BrPR1* compared to expression in ‘Misugi’, while ‘CR-shirowarabe’, ‘Matsushima-jun-2-go’, ‘Minebuki505’, ‘Natsu-no-koshien’ and ‘Zaoh’ showed a similar level (Fig. 7). Compared with *BrPR2* expression level of ‘Misugi’ at 72 HAT, ‘Nanane’, ‘Akimeki’, ‘Matsushima-jun-2-go’, ‘Minebuki505’ and ‘Zaoh’ showed significantly higher expression (Fig. 7). The *BrPR4* induction level of ‘Nanane’, ‘Akimeki’, ‘CR-shirowarabe’, ‘Matsushima-jun-2-go’ and ‘Zaoh’ was significantly higher than that of ‘Misugi’ (Fig. 7). In ‘Nanane’ and ‘Akimeki’, induction levels of these three genes by SA tended to be higher than in the other lines.

Discussion

We examined how many *B. rapa* genes categorized into the GO term ‘response to SA stimulus’

showed a change of expression with SA treatment; approximately 20 % of these genes were identified as BrSAIGs or BrSASGs. This low percentage might be due to the GO classification being based on an *A. thaliana* database and the actual SA-responsive genes in *B. rapa* might be different. There is a variation in SA-responsive genes between lines of *B. rapa*, and we defined the overlapped genes between lines as BrSAIGs or BrSASGs. This restriction may result in a lower percentage of overlapped genes between BrSAIGs or BrSASGs and genes categorized into ‘response to SA stimulus’. Comparison between BrSAIGs and AtSAIGs showed that only 59 BrSAIGs (about 5.6 % of total BrSAIGs) were in common, indicating that most SAIGs are different between species. There is the possibility that this difference was caused by the period of SA treatment, 72 h treatment in *B. rapa* or 2.5 h treatment in *A. thaliana*. But it suggests that most DEGs at 72 HAT include the genes induced at earlier time points (mentioned later), the difference of SAIGs variation in species might be caused by species difference, not by the difference of SA treatment period. In this study, we identified BrSAIGs at the whole genome level, and this gene list is valuable for identification of genes directly affected by SA in *B. rapa*. Although many genes did not overlap between BrSAIGs and AtSAIGs, overrepresented categories of AtSAIGs included ‘Cell rescue, defense and virulence’ and ‘Systemic interaction with the environment’ (subcategory ‘Plant/fungal specific systemic sensing and response’) (Blanco et al. 2009) and similar categories were overrepresented in BrSAIGs, suggesting that the functions of BrSAIGs and AtSAIGs could be similar. Species-conserved SAIGs include *SIB1*, which has been reported to be induced by SA and has a function in the SA- and JA-mediated defense response to *Pseudomonas syringae* in *A. thaliana* (Xie et al. 2010). *SIB1* function in SA response might have been conserved after speciation.

In *B. rapa*, the expression levels of *BrPRI* and *BrBG3* at control and 2, 5, 10, and 24 h after SA treatment have been examined (Abe et al. 2011). The expression level of *BrPRI* was increased from 5 h after treatment and was highest at 10 h, then was decreased at 24 h. The expression level of *BrBG3* was increased from 2 h after treatment and was highest at 5 h, then was decreased (Abe et al. 2011). In this study, we found that *BrPRI* had two peaks of expression level; a first peak at 12 HAT, and a second peak at 48 or 72 HAT. *BrBG3* also had two peaks of expression; a first peak at 12 HAT and a second peak at 48 or 72 HAT. Our qPCR results from 0 to 24 HAT mainly corresponded to the data of Abe et al. 2011. The expression levels of seven genes were also validated, and there were three types of gene expression

pattern; (1) expression peak only at earlier stages (3 to 12 HAT), (2) expression peak only at later stages (48 to 72 HAT), or (3) expression peaks at both earlier and later stages. In this study, RNA-seq was performed at a later stage of SA treatment (72 HAT), and genes categorized into category (1) also showed a higher expression level at a later stage (72 HAT) than before treatment in the qPCR results, suggesting that most DEGs at 72 HAT include the genes induced rapidly after SA treatment.

In general, *PR1* and *PR2* are SA-induced marker genes, and *PR3* and *PR4* are JA-induced marker genes (Thomma et al. 1998; Abe et al. 2011). In this study, *BrPR1* and *BrPR2* were induced by SA, consistent with previous reports. There are two orthologs of *BrPR1*s, and one *BrPR1* (Bra013123) was up-regulated by both SA treatment (this study) and *Foc* inoculation (Miyaji et al. 2017). But another *BrPR1* (Bra013124) did not show significant expression change in either condition. The expression level of *BrPR1* was analyzed by qPCR using a primer set, which amplifies only Bra013123, and these data indicate that Bra013123 is the main functional *BrPR1* in *B. rapa*. By contrast, a previous report showed that SA treatment repressed *BrPR4* expression in *B. rapa* (Chung et al. 2005), while in this study, we found that the expression of *BrPR4* was induced by SA.

WRKY46, *WRKY53*, and *WRKY70* positively regulate basal resistance to biotrophic pathogens such as *P. syringae* in *A. thaliana*, while *WRKY8*, *WRKY11*, *WRKY18*, *WRKY38*, *WRKY48*, and *WRKY62* negatively regulate basal resistance (Journot-Catalino et al. 2006; Xu et al. 2006; Kim et al. 2008; Xing et al. 2008; Chen et al. 2010; Hu et al. 2012). From these nine genes, *WRKY8*, *WRKY38*, *WRKY46*, *WRKY53*, *WRKY62*, and *WRKY70* were induced by SA in *A. thaliana* (Kim et al. 2008; Chen et al. 2010; Hu et al. 2012). In this study, SA induced the positive regulators, *BrWRKY46* and *BrWRKY70*, in both ‘Misugi’ and ‘Nanane’, and the negative regulators, *BrWRKY8* in ‘Nanane’ and *BrWRKY18* and *BrWRKY38* in ‘Misugi’ and ‘Nanane’. Both the positive (*BrWRKY46* and *BrWRKY70*) and negative regulators (*BrWRKY18* and *BrWRKY38*) were induced by SA treatment in both lines, suggesting that the function of these four *WRKY* genes in SA response might be fundamental. In contrast, *BrWRKY8*, which is specifically induced in the resistant line ‘Nanane’, might account for the different resistance phenotype between lines.

Some *MYB* TFs were induced by SA treatment in ‘Misugi’ or ‘Nanane’. *MYB28* positively regulates biosynthesis of methionine-derived glucosinolate and is suppressed by SA treatment in *A. thaliana* (Gigolashvili et al. 2007; Seo and Kim 2017), while in this study, we found up-regulation of

BrMYB28 in both lines following SA treatment, which is inconsistent with previous reports. This result suggests that the function of *MYB28* in the SA response is different between *B. rapa* and *A. thaliana*. *MYB59* is induced by SA treatment, and functions as a negative regulator of calcium signaling during plant growth and general stress responses in *A. thaliana* (Yanhui et al. 2006; Fasani et al. 2019). In this study, *BrMYB59* was up-regulated by SA treatment in both lines. This data suggests that *BrMYB59* has a function in SA response similar to that in *A. thaliana*, and that *BrMYB59* is a candidate for a general stress response induced genes including the stress of SA stimulus.

Using up- or down-regulated genes following SA treatment in ‘Misugi’ and ‘Nanane’, GO analysis was performed. The trends of overrepresented GO terms were different between lines. In the Fusarium yellows resistant line ‘Nanane’, genes related to response to SA stimulus were up-regulated, and genes related to response to JA and ET stimulus were down-regulated, consistent with the antagonistic relationship of SA and JA or ET (Bari and Jones 2009; Zhang et al. 2018). In the susceptible line ‘Misugi’, this trend was not detected. These results reveal that Fusarium yellows resistant and susceptible lines have specific responses to SA and that ‘Nanane’ showed a response similar to previous reports.

Previously, transcriptome analysis with and without *Foc* inoculation in Fusarium yellows resistant and susceptible lines of *B. rapa* was performed and genes categorized into the GO terms ‘response to salicylic acid stimulus’ or ‘systemic acquired resistance’ were induced at 24 HAI specifically in a resistant line (Miyaji et al. 2017). We showed that there were differences between genes categorized into GO term of ‘response to salicylic acid stimulus’ and SA-responsive genes detected in this study. We compared SA-induced genes in ‘Misugi’ or ‘Nanane’ with up-regulated genes by *Foc* inoculation at 24 HAI in Fusarium yellows resistant and susceptible lines. About half of the up-regulated genes following *Foc* inoculation in resistant and susceptible lines were identified as SA-induced genes in ‘Misugi’ or ‘Nanane’, suggesting that SA-induced genes were induced by *Foc* inoculation in susceptible lines as well as in resistant lines. 39 SA-induced genes specific to resistant lines including some *BrPRR* genes, *BrCBP60g*, and *BrDMR6* might have important functions in *Foc* resistance mediated by the SA response. CBP60g plays a role in SA accumulation after recognition of MAMPs, and also might control the defense response (Wang et al. 2009; Wang et al. 2011), and SA induced *CBP60g* transcription (Sun et al. 2020).

These data indicate that more accumulation of SA and the induction of the SA response may be important for the defense response to *Foc. DMR6/S5H* (*SALICYLIC ACID 5-HYDROXYLASE*), which encodes the enzyme that catalyzes the formation of 2,5-DHBA from SA, is induced by SA treatment with an expression peak just after the peak of total amount of SA following *P. syringae* pv *tomato* DC3000 inoculation and might have a role in the detoxification of excessive SA induced by a pathogen response (Zhang et al. 2017). These data suggest that SA accumulation by *Foc* inoculation peaked at around 24 HAI in the Fusarium yellows resistant line and SA detoxification was started at 24 HAI. Among the SA-induced genes, those categorized into ‘response to JA, ET, or gibberellin stimulus’ showed up-regulation after *Foc* inoculation in both resistant and susceptible lines. However, half of the genes are different between resistant and susceptible lines, suggesting that genes specific to the resistant line might be important for *Foc* resistance.

In *B. rapa* and *B. oleracea*, differences of SA response in gene expression levels between subspecies or between lines of the same subspecies have been reported. In *B. rapa*, six Chinese cabbage lines showed different levels of *BrPRI* induction by SA treatment (Abe et al. 2011). In *B. oleracea*, glucosinolate accumulation and expression levels of glucosinolate biosynthesis-related genes showed a subspecies-specific pattern of SA response in cabbage, broccoli, and kale (Yi et al. 2016). In this study, we found differences of SA response in *BrPRI*, *BrPR2*, and *BrPR4* expression between subspecies or between the lines of the same subspecies. Similar induction levels of *BrPR* genes in some Fusarium yellows resistant lines compared to susceptible line were found. SA regulates disease resistance to biotrophic pathogens and SA treatment induces SAR (Narusaka et al. 1999; Bari and Jones 2009; Mandal et al. 2009; Klessig et al. 2018), suggesting that the difference of SA response between lines might result in a difference of induction level in disease resistance or SAR. ‘Nanane’ and ‘Akimeki’ showed higher induction of *BrPRI*, *BrPR2*, and *BrPR4* by SA treatment compared with other lines, and this variation might be involved in the variation of PTI response. The biological meaning of SA response difference between subspecies or between lines in the same subspecies needs to be investigated in the future.

In our previous study, the transcriptome response to *Foc* inoculation at the early/seedling stages was examined because the early stages are more sensitive to *Foc* than mature stages and infection in the early stages is damaging to production. Thus, in this study the SA response at an early stage was

examined to get insights into the relationship between SA response and Fusarium yellows resistance. The cotyledons were used to examine the SA response as an initial in *B. rapa*. However, to further understand the role of SA in disease resistance, it will be necessary to examine the SA reaction and defense response at different developmental stages and in different tissues such as roots and leaves.

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Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data

The RNA-sequencing data have been deposited with DDBJ (<https://www.ddbj.nig.ac.jp/index.html>) under DRA010766.

Authors' contributions

RF, ESD, and TTY designed research. NM performed research. NM and MS analyzed the RNA-seq data. NM, RF, and ESD wrote the paper.

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Figure legends

Fig. 1 Gene expression levels in 'Misugi' and 'Nanane' before and after salicylic acid (SA) treatment. (a) Gene expression levels of *PATHOGENESIS-RELATED 1* (*BrPRI*), *BETA-1,3-GLUCANASE 3* (*BrBG3*) and *GLUTATHIONE S-TRANSFERASE 25* (*BrGST25*). (b) Gene expression levels of *ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 15* (*BrERF15*), *MYB DOMAIN PROTEIN 59* (*BrMYB59*), *BrPR2*, *BrPR4*, *UDP-GLUCOSE TRANSFERASE 1* (*BrUGT1*) and *WRKY DNA-BINDING PROTEIN 70* (*BrWRKY70*). Values are mean $\pm$ SE (three biological and technical replicates) of relative expression levels of each gene compared with *Bractin* quantitated by real-time RT-PCR (qPCR) at 0, 3, 6, 12, 24, 48 and 72 h after SA treatment (HAT). Expression levels before and after SA treatments were compared using Dunnett's test (*; $p < 0.05$, **; $p < 0.01$).

Fig. 2 Venn diagrams of differentially expressed genes (DEGs) compared between salicylic acid (SA)-treated and non-treated samples. Each number represents the number of DEGs categorized into each category. Up-regulated DEGs in SA-treated samples between 'Misugi' and 'Nanane' by normal (a) and stringent criteria (c) and ortholog of *A. thaliana* SA induced genes (AtSAIGs) are compared. Down-regulated DEGs in SA-treated samples between 'Misugi' and 'Nanane' by normal (b) and stringent criteria (d) are compared. Misugi_up/down, up-/down-regulated DEGs in SA-treated samples compared with genes in non-treated sample in 'Misugi' at 72 h after treatment. Nanane_up/down, up-/down-regulated DEGs in SA-treated samples compared with genes in non-treated sample in 'Nanane'

at 72 h after treatment.

Fig. 3 Expression levels of *Pathogenesis-related (BrPR)* genes following salicylic acid treatment in ‘Misugi’ and ‘Nanane’. Fragments per kilobase per million (FPKM) ‘0’ showed as white color, and min to max values higher than 0 were converted into log10 value and scaled from blue to red. Up and down arrow indicate up- and down-regulated differentially expressed genes (DEGs), respectively.

Fig. 4 Up- or down-regulated genes by salicylic acid treatments categorized into Gene ontology (GO) terms related to hormone response genes, and systemic acquired resistance. Up/down; up-/down-regulated DEGs, no; not identified as DEGs. SA; genes categorized into GO term ‘response to salicylic acid stimulus’, JA; genes categorized into GO term ‘response to jasmonic acid stimulus’, ET; genes categorized into GO term ‘response to ethylene stimulus’, GA; genes categorized into GO term ‘response to gibberellin stimulus’, SAR; genes categorized into GO term ‘response to systemic acquired resistance’.

Fig. 5 GO terms overrepresented in salicylic acid (SA)-induced/suppressed genes in ‘Misugi’/‘Nanane’. Ratio compared with expected values were shown. Ratio ‘0’ showed as white color, and min to max values higher than 0 were converted into log10 value and scaled from blue to red. Misugi_up/down; up-/down-regulated DEGs by stringent criteria in SA-treated samples compared with genes in non-treated sample in ‘Misugi’ at 72 h after treatment, Nanane_up/down; up-/down-regulated DEGs by stringent criteria in SA-treated samples compared with genes in non-treated sample in ‘Nanane’ at 72 h after treatment, SAIGs; SA-induced genes, SASGs; SA-suppressed genes.

Fig. 6 Salicylic acid-induced genes conserved in *Brassica rapa* and *Arabidopsis thaliana*. Fragments per kilobase per million (FPKM) ‘0’ showed as white color, and min to max values higher than 0 were converted into log10 value and scaled from blue to red.

Fig. 7 *PATHOGENESIS-RELATED* genes (*BrPR1*, *BrPR2* and *BrPR4*) expression level after salicylic

acid (SA) treatment in eight *B. rapa* lines. Values are mean $\pm$ SE (three biological and technical replicates) of relative expression levels of each gene compared with *Bractin* quantitated by real-time RT-PCR (qPCR) at 0 and 72 h after SA treatment (HAT). All data are statistically analyzed using Student's *t* test compared with the expression level of 'Misugi' at 72 HAT (*; $p < 0.05$, **; $p < 0.01$).

Fig. S1 Gene expression levels of *PATHOGENESIS-RELATED 1 (BrPRI)* in 'Misugi' and 'Nanane' at 72 h after salicylic acid (SA) treatment. (a) Expression level of *BrPRI* at 0.010, 0.10, and 1.0 mM SA treatments in 'Nanane'. Expression levels before and after SA treatment were compared using Dunnett's test (n.s.; no significant, **; $p < 0.01$). (b) Expression level of *BrPRI* at 1.0 mM SA treatment in 'Misugi' and 'Nanane'. All data are statistically analyzed using Student's *t* test (**; $p < 0.01$). Values are mean $\pm$ SE (three biological and technical replicates) of relative expression levels of *BrPRI* compared with *Bractin* quantitated by real-time RT-PCR (qPCR).

Fig. S2 Differentially expressed genes (DEGs) compared between samples before and after salicylic acid treatment categorized into transcription factors. (a) Number of up-/down-regulated DEGs categorized into transcription factors in each criteria. Up/down; up-/down-regulated DEGs, no; not identified as DEGs. (b) Gene family of DEGs categorized into transcription factors. Top three categories (including tied numbers) are only shown.

Fig. S3 Gene expression levels in 'Misugi' and 'Nanane' before and after salicylic acid (SA) treatment. The expression of *BETA-1,3-GLUCANASE 3 (BrBG3)*, *ETHYLENE-RESPONSIVE ELEMENT BINDING FACTOR 15 (BrERF15)*, *GLUTATHIONE S-TRANSFERASE 25 (BrGST25)*, *MYB DOMAIN PROTEIN 59 (BrMYB59)*, *PATHOGENESIS-RELATED 1 (BrPRI)*, *BrPR2*, *BrPR4*, *UDP-GLUCOSE TRANSFERASE 1 (BrUGT1)* and *WRKY DNA-BINDING PROTEIN 70 (BrWRKY70)* were validated. The values of fragments per kilobase per million (FPKM) were calculated by data from RNA-seq analysis. Values are mean $\pm$ SE (three biological and technical replicates) of relative expression levels of each gene compared with *Bractin* quantitated by real-time RT-PCR (qPCR) at 0, 3, 6, 12, 24, 48 and 72 h after SA treatment (HAT). All data are statistically analyzed using Dunnett's test (*; $p < 0.05$, **; $p < 0.01$).

Misugi/Nanane_0h, samples before SA treatment in 'Misugi' or 'Nanane', respectively.
Misugi/Nanane_72h, samples at 72 h after SA treatment in 'Misugi' or 'Nanane', respectively.

Fig. S4 Venn diagrams compared overrepresented GO terms in 'Misugi' and 'Nanane'. Each number represents the number of GO terms in each category. Misugi_up/down; GO terms overrepresented in up-/down-regulated DEGs in 'Misugi' at 72 h after salicylic acid (SA) treatment, Nanane_up/down; GO terms overrepresented in up-/down-regulated DEGs in 'Nanane' at 72 h after SA treatment.

Fig. S5 Enrichment pathways following salicylic acid (SA) treatment by Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. Pathways classified with 10 or more genes into at least one category are presented in the y-axis. The x-axis represents the percentage of query annotated genes in the reference annotated genes. The numbers shown at right side of bars indicate the actual number of the annotated genes. Misugi_up/down; up-/down-regulated DEGs by stringent criteria in SA-treated samples compared with genes in non-treated sample in 'Misugi' at 72 h after treatment, Nanane_up/down; up-/down-regulated DEGs by stringent criteria in SA-treated samples compared with genes in non-treated sample in 'Nanane' at 72 h after treatment. SAIGs; SA-induced genes, SASGs; SA-suppressed genes.

Fig. S6 Overlapped genes between salicylic acid (SA)-induced genes in 'Misugi' or 'Nanane' and up-regulated genes following *Fusarium oxysporum* f. sp. *conglutinans* (*Foc*) infection in the Fusarium resistant (RJKB-T23) or the susceptible lines (RJKB-T24). Misugi_up; up-regulated genes at 72 hours after SA treatment (HAT) in 'Misugi', Nanane_up; up-regulated genes by SA treatment at 72 HAT in 'Nanane', R-24h_up; up-regulated genes by *Foc* inoculation in RJKB-T23 at 24 hours after inoculation (HAI), S-24h_up; up-regulated genes by *Foc* inoculation in RJKB-T24 at 24 HAI. Numbers indicate the overlapped genes between categories.

Fig. S7 Genes overlapped between up-regulated differentially expressed genes (DEGs) in 'Misugi' or 'Nanane' by salicylic acid (SA) treatment and that in RJKB-T23 or RJKB-T24 by *Fusarium oxysporum* f. sp. *conglutinans* inoculation. (a) SA-induced genes (BrSAIGs) overlapped with up-regulated genes in the

Fusarium yellows resistant (RJKB-T23) and the susceptible lines (RJKB-T24). (b) Overlapped genes between SA-induced genes in the Fusarium yellows resistant line 'Nanane' and up-regulated genes by *Foc* inoculation in RJKB-T23. Fragments per kilobase per million (FPKM) '0' showed as white color, and min to max values higher than 0 were converted into log10 value and scaled from blue to red.

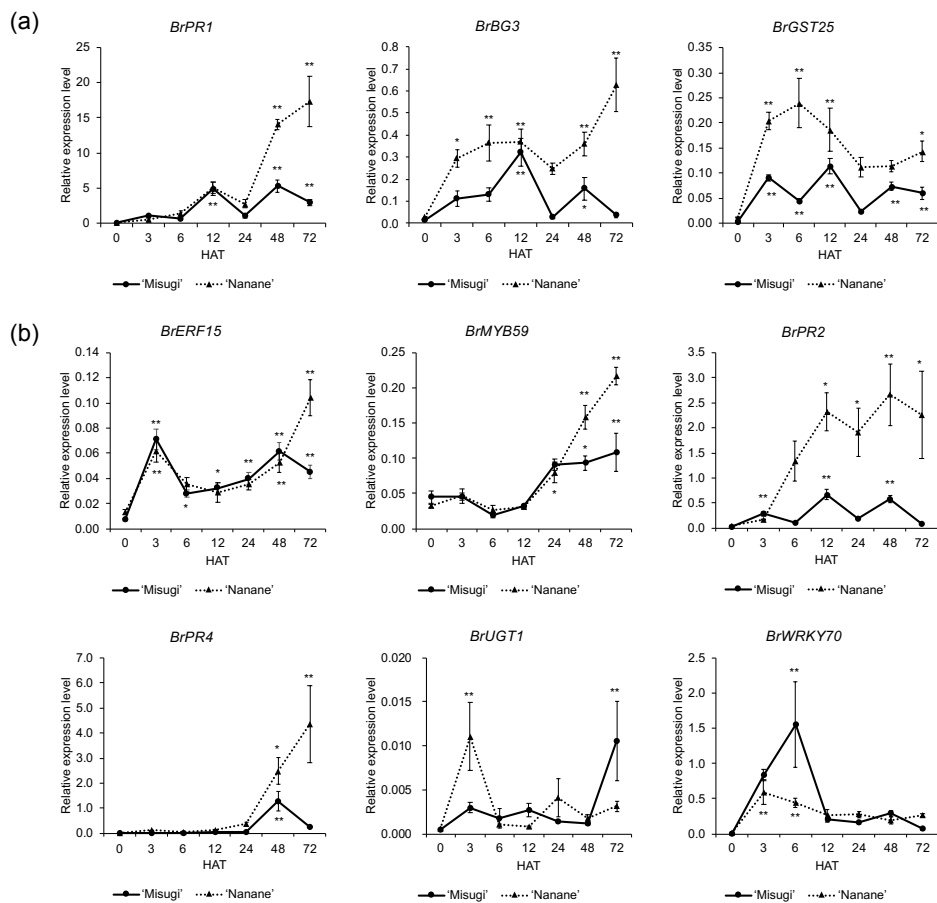


Fig. 1

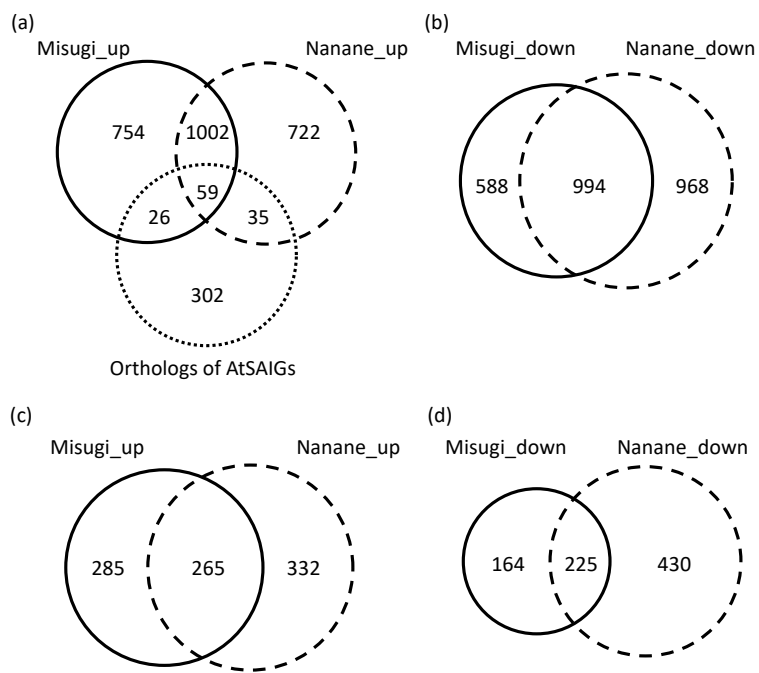


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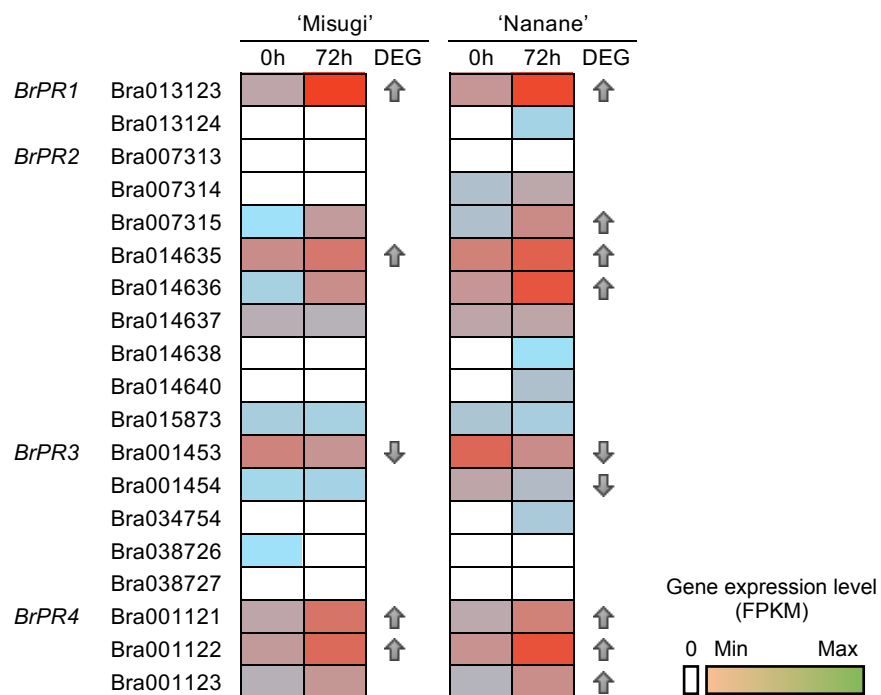


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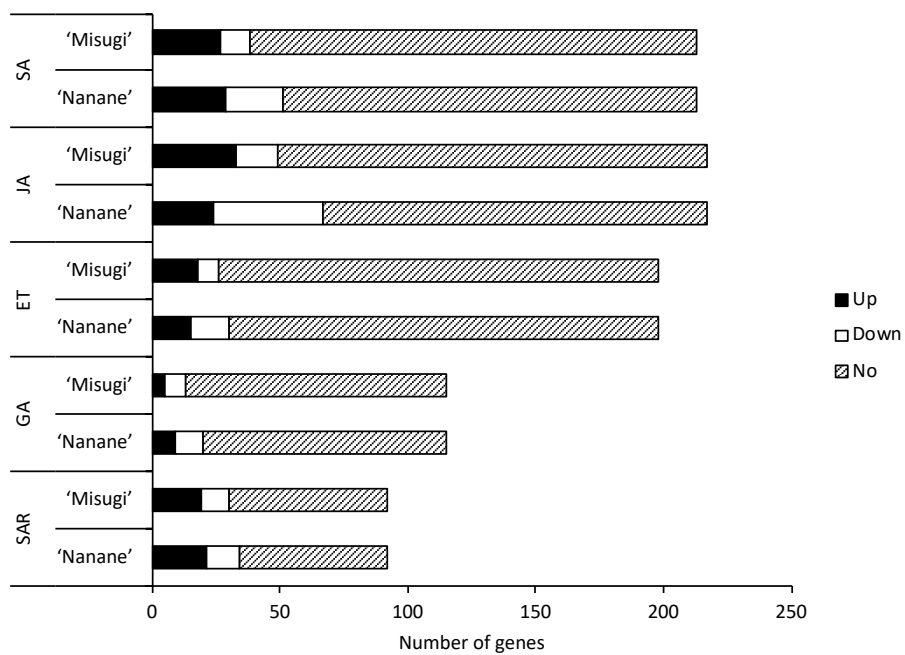


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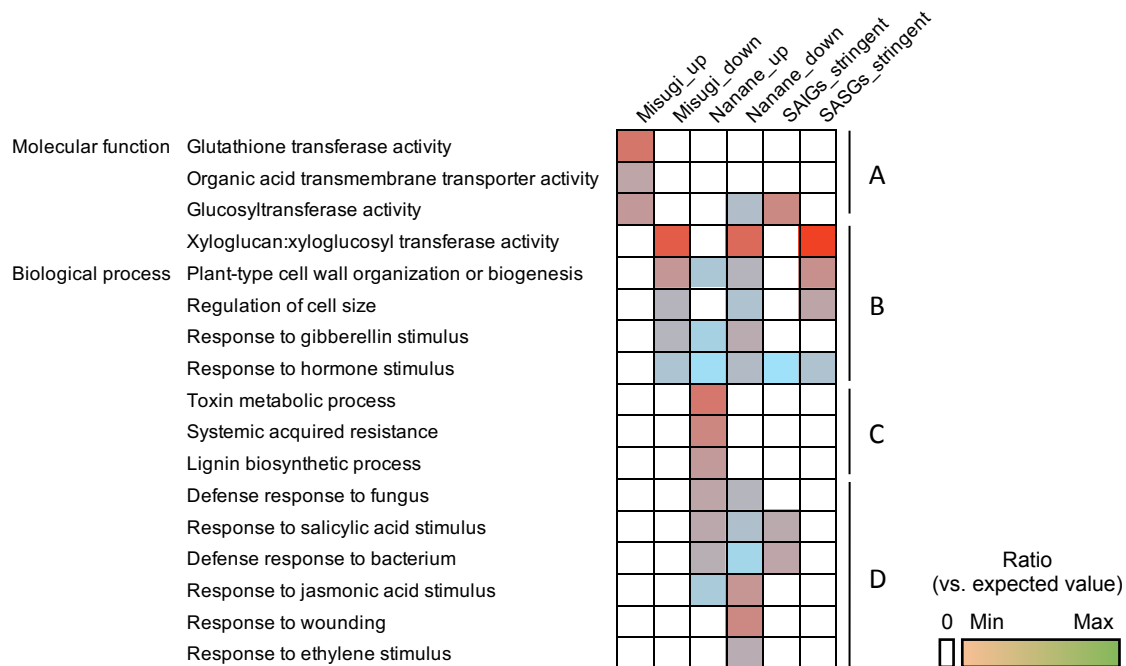


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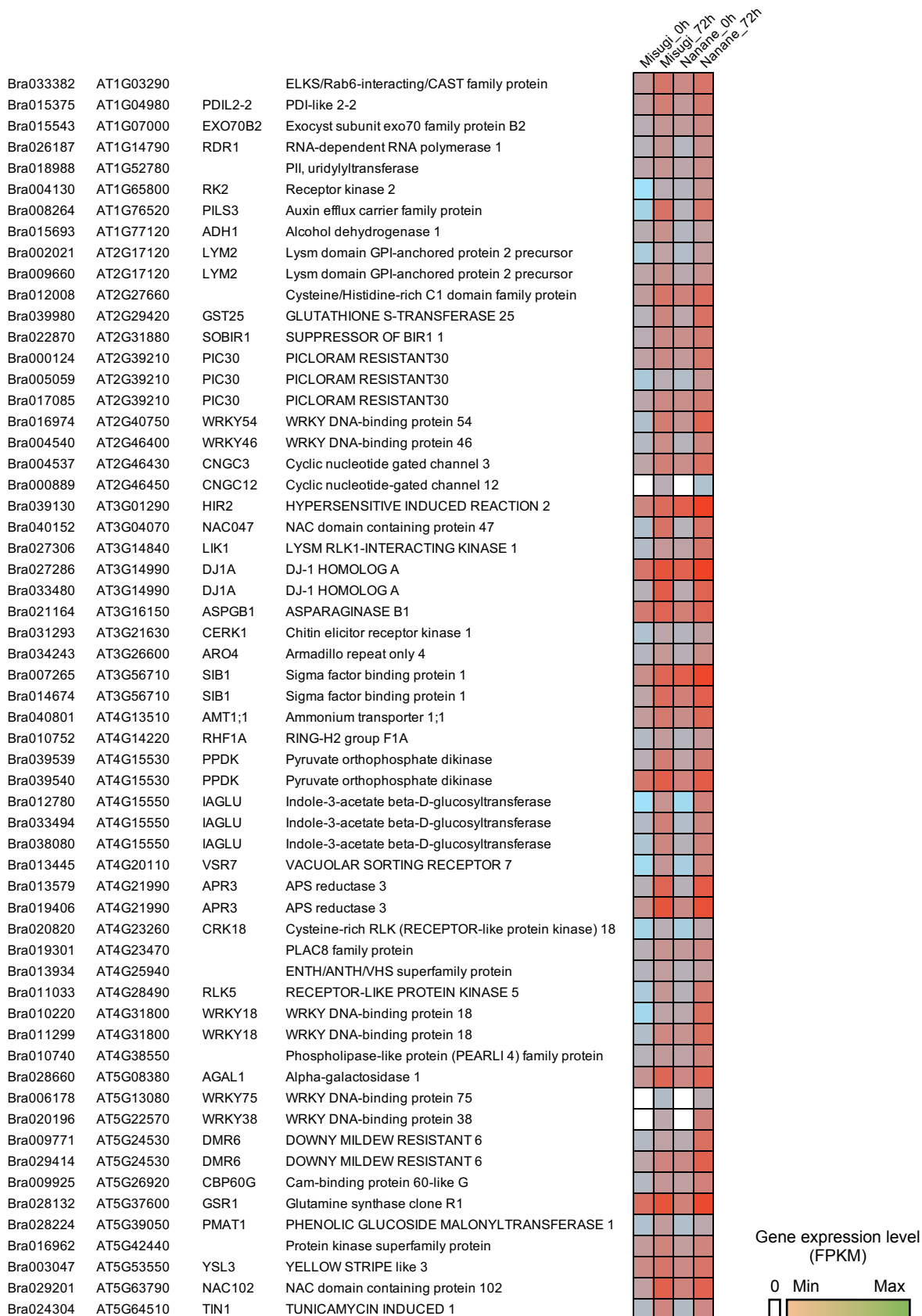


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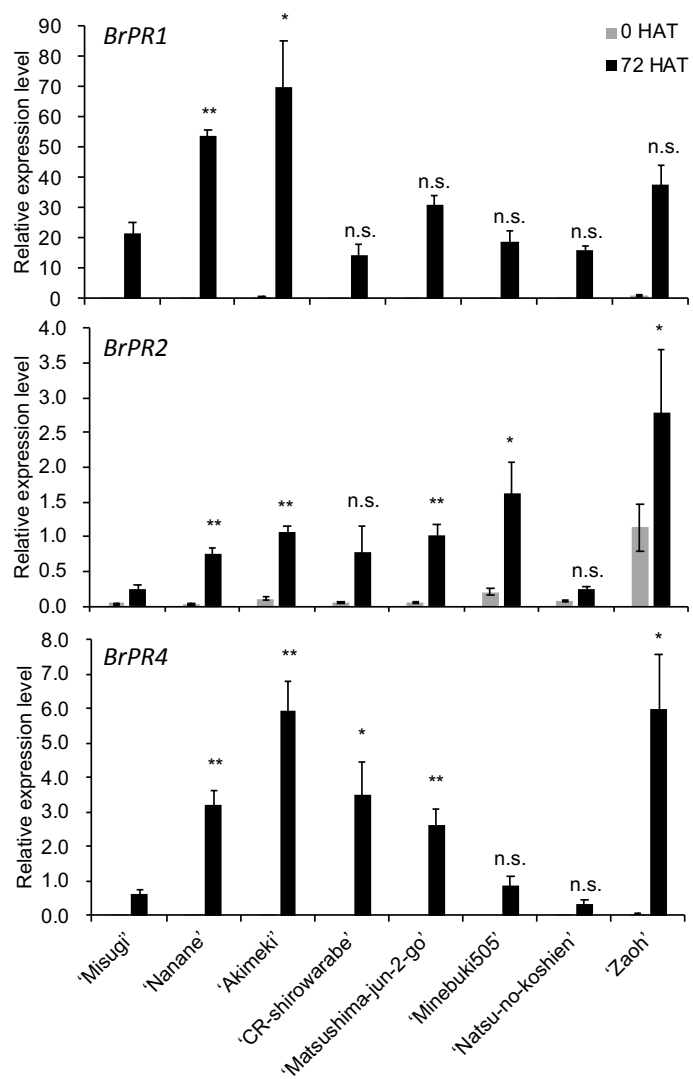


Fig. 7