



Genome Identification and Expression Analysis of GRAS Family Related to Development, Hormone and Pathogen Stress in *Brachypodium distachyon*

Zejun Tang^{1,2†}, Na Song^{1,2†}, Weiye Peng^{1,2}, Yang Yang^{1,2}, Tian Qiu^{1,2}, Chenting Huang^{1,2}, Liangying Dai^{1,2*} and Bing Wang^{1,2*}

¹ Hunan Provincial Key Laboratory for Biology and Control of Plant Diseases and Insect Pests, Hunan Agricultural University, Changsha, China, ² College of Plant Protection, Hunan Agricultural University, Changsha, China

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*Correspondence:

Bing Wang
zhufu@hunau.edu.cn
Liangying Dai
daily@hunau.net

†These authors have contributed
equally to this work

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GRAS transcription factors are widely present in the plant kingdom and play important roles in regulating multiple plant physiological processes. *Brachypodium distachyon* is a model for grasses for researching plant-pathogen interactions. However, little is known about the *BdGRAS* family genes involved in plant response to biotic stress. In this study, we identified 63 genes of the GRAS family in *B. distachyon*. The phylogenetic analysis showed that *BdGRAS* genes were divided into ten subfamilies and unevenly distributed on five chromosomes. qRT-PCR results showed that the *BdGRAS* family genes were involved in the growth and development of *B. distachyon*. Moreover, the expression of the HAM subfamily genes of *BdGRAS* changed during the interaction between *B. distachyon* and *Magnaporthe oryzae*. Interestingly, *BdGRAS31* in the HAM subfamily was regulated by miR171 after inoculation with *M. oryzae*. These results provide insight into the potential functions of the *BdGRAS* family in disease resistance.

Keywords: GRAS family, transcription factor, *Brachypodium distachyon*, *Magnaporthe oryzae*, HAM subfamily

INTRODUCTION

The GRAS protein family is an important transcription factor family named after the function of three members: Gibberellic acid insensitive (GAI), Repressor of GAI (RGA), and Scarecrow (SCR; Chen et al., 2019). Genome-wide analysis of the family showed that GRAS genes are also widely distributed in the plant kingdom (Tian et al., 2004). GRAS proteins typically consist of 400–700 amino acids. The sequences at the C-terminus are highly conserved and are divided into five conserved motifs: LHRI, VHIID, LHRII, PFYRE, and SAW (Pysh et al., 1999; Tian et al., 2004). VHIID, PFYRE, and SAW are important for maintaining the structural stability and function of GRAS proteins (Itoh et al., 2002; Smit et al., 2005; Hofmann, 2016; Li et al., 2016); the VHIID sequence can interact with other proteins (Gao et al., 2004). LHRI and VHIID have abundant leucine repeats, which affect protein dimerization. The N-terminal sequence is highly variable and can be used as bait in molecular research activities (Tian et al., 2004). Some GRAS proteins contain the DELLA domain, which plays important roles in gibberellin signaling (Silverstone et al., 1998).

The GRAS family is widely involved in regulating plant growth and development, including gibberellin signaling (Peng et al., 1997; Ikeda et al., 2001; Hirsch and Oldroyd, 2009), formation of

axillary meristems (Greb et al., 2003; Li et al., 2006), root radial patterning (Helariutta et al., 2000), photosensitive signal transduction (Bolle et al., 2000), and male gametogenesis (Morohashi et al., 2003). For example, two GRAS proteins are required for root rot formation in legumes (Kaló et al., 2005; Heckmann et al., 2006). Moreover, the GRAS family has been shown to participate in the hormone signaling pathway that regulates plant growth and development (Davière and Achard, 2016; Van De Velde et al., 2017). DELLA proteins also participate in the signaling pathways of auxin, brassinosteroids (BRs), abscisic acid (ABA), and ethylene (Chen et al., 2019). *AtSCL3* (an *Arabidopsis thaliana* GRAS gene) mediates the gibberellic acid pathway by attenuating the DELLA repressors during root development (Heo et al., 2011). In addition, GRAS genes participate in the regulation of jasmonic acid (JA) signaling by interacting with the JAZ1 protein (repressor of JA signal pathway) (De Lucas et al., 2008; Feng et al., 2008; Hou et al., 2010).

GRAS genes also participate in the process of stress resistance. For example, the expression of *GRAS6*, *GRAS37*, *GRAS50*, *GRAS68*, and *GRAS69* was significantly induced in sweet potato under salt stress treatment (Chen et al., 2019). *OsGRAS23*-overexpressing plants demonstrated less H₂O₂ accumulation under antioxidant stress and enhanced drought resistance (Xu et al., 2015). Moreover, GRAS genes are involved in the interaction between plants and fungi. The GRAS transcription factor CIGR2 activates *OsHsf23* to cause hypersensitive cell death and inhibits excessive cell death in the incompatible interaction between rice (*Oryza sativa*) and *Magnaporthe oryzae* (Tanabe et al., 2016).

Brachypodium distachyon is a new model of gramineous plants and has a short growth cycle, small plant size, small genome, and easy growth. *B. distachyon* can be infected by *M. oryzae*, which causes rice blast, a devastating disease of rice. In the process of *M. oryzae* infecting *B. distachyon*, conidia first germinated and formed appressoria and invaded epidermal cells. Invasive hyphae were found in the cells, and the hyphae continued to develop and spread to adjacent cells, causing cell death (Routledge et al., 2004). The infection process and symptoms developed on *B. distachyon* were very similar to those in rice, including the degree of occurrence, time of emergence, size of the lesions, and growth rate (Routledge et al., 2004).

In this study, we performed chromosomal mapping, constructed a phylogenetic tree, and performed gene structure analysis of the *BdGRAS* family. Furthermore, we analyzed the expression levels of *BdGRAS* family of *B. distachyon* after inoculation with *M. oryzae*. Our results will provide evidence for further study of GRAS gene function in disease resistance.

MATERIALS AND METHODS

Experimental Materials and Treatments

B. distachyon (Bd21-3 genotype) seeds were grown in the soil (nutrient soil and stone 1:1) in a greenhouse under a 16 h light/8 h dark photoperiod, at a constant temperature of 23°C and humidity of 60%. Two-month-old *B. distachyon* seedlings were used in the experiment (Wang et al., 2015). Four different

B. distachyon tissues (roots, stems, leaves, and seeds) were used for total RNA extraction.

The seedlings were treated with jasmonic acid (JA100 μmol/mL), salicylic acid (SA, 100 μmol/mL), indoleacetic acid (IAA, 100 μmol/mL), and abscisic acid (ABA, 100 μmol/mL). Fifteen seedlings were used for each treatment. Three leaves from the treated samples were harvested at 0 and 1 h post-treatment (hpt).

The rice blast fungus (*M. oryzae*) strain RO1-1 was grown on an oat medium at 28°C in the light in an incubator. After 20 days, the spores were washed with sterile water and filtered through gauze. The seedlings were challenged with *M. oryzae* spore (1×10^5 spores mL⁻¹) by spraying (Wei et al., 2013). Inoculated leaves were harvested at 0, 24, and 48 h post inoculation (hpi).

Identification of *BdGRAS* Genes and Phylogenetic Analysis

All *B. distachyon* GRAS genes were derived from the Plant Transcription Factor Database (<http://planttfdb.gao-lab.org/>). The sequences of the GRAS genes of rice, wheat and *A. thaliana* for analysis of the phylogeny of the *BdGRAS* family were also obtained from the Plant Transcription Factor Database (Supplementary Table 1).

The protein sequences of the *BdGRAS*, *OsGRAS*, and *AtGRAS* family were analyzed to generate a phylogenetic tree using MEGA7.0 (Kumar et al., 2016). ClustalW was used for sequence alignment to infer the evolutionary history of multiple sequences. The neighbor-joining method was used to set the parameters to the P-distance model, and 1,000 bootstrap replicates were used to generate phylogenetic trees (Wu et al., 2021). We performed miRNA target analysis using the webtool psRNATarget (<http://plantgrn.noble.org/psRNATarget/>).

Analysis of Conserved Domains and Motifs for the Chromosomal Location of *BdGRAS* Protein

We used NCBI to search for each gene annotation in the *BdGRAS* family to confirm the specific location of each gene on the chromosome. The genetic map of the *BdGRAS* family was visualized using MapChart software. We used Multiple Em for Motif Elicitation Version 5.1.1 (MEME) (<http://meme-suite.org/tools/meme>) to analyze *BdGRAS* family motifs. For the analysis of the conserved motifs of *BdGRAS* family genes, we first used the NCBI Web CD-search Tool (<https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi>) to predict the conserved domains of genes and then used TBtools to realize visualization. We also performed *cis*-acting regulatory element analysis, found the 2000 bp DNA promoter sequence upstream of the start site of each gene, and used PlantCare (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>).

RT-PCR and qRT-PCR Analysis

Total RNA (roots, stems, leaves and seeds, and leaves treated by hormone and rice blast) was extracted using TRIzol Reagent (Ambion, Waltham, USA). cDNA synthesis was performed using TransScript One-Step gDNA Removal and cDNA Synthesis

SuperMix (TransGen Biotech, Beijing, China) for RT-PCR (reverse transcription-polymerase chain reaction). The specific primers for *BdGRAS* family genes were designed using Primer5.0 (Singh et al., 1998) (**Supplementary Table 2**). The *BdUBC* gene (*Bradi4g00660*) was used as the internal reference. For PCR amplification, 2× EasyTaq PCR SuperMix (+dye) (TransGen Biotech, Beijing, China) was used. The reaction procedure was as follows: 94°C for 3 min, 34 cycles of 15 s at 94°C, 30 s at 56°C, and 72°C for 1 min (Chen et al., 2015).

First-strand cDNA was synthesized using the RevertAid First Stand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, USA) for qRT-PCR. The qRT-PCR reaction sample adopts 20 μl systems, of which 10 μM forward and reverse primers are 0.4 μl each, TransStart Tip Green qPCR SuperMix 10 μl, template 2 μl, 7.2 μl ddH₂O. qRT-PCR conditions were as follows: 94°C for 1 min, 30 cycles (94°C for 20 s, 56°C for 15 s, and 1 min at 72°C), and 72°C for 5 min. qRT-PCR was performed using the CFX 96 qPCR instrument (BioRad, California, USA) (Tong et al., 2020). The *BdUBC* gene was used as the internal reference. Similar results were obtained from three biological replicates.

Transcriptional Activation Assay

The GRAS genes were constructed on the pGBKT7 vector and then transformed into AH109 yeast competent cells. Transformed cells were streaked onto SD/-Trp and SD/-Trp-His-Ade medium. The growth status of AH109 transformed cells was evaluated after incubation at 30°C for 2 days. Transformed cells (OD₆₀₀ = 1.0) were tested on the selection medium (SD/-Trp and SD/-Trp-His-Ade medium). The α-galactosidase activity assay was performed using X-α-Gal as a substrate (Wang et al., 2018a).

RESULTS

Phylogenetic Evolution Analysis and Chromosome Location of GRAS Family

Through *in silico* prediction, 63 candidate GRAS genes were identified in *B. distachyon*. The proteins of the BdGRAS family ranged from 185 to 806 amino acids with molecular weights ranging from 30 to 89 kDa. The isoelectric point of BdGRAS was between 4.6 and 9.8, with an average of 6.7 (**Supplementary Table 3**). A phylogenetic tree was constructed using 60 GRAS genes from *B. distachyon*, 50 GRAS genes from *O. sativa*, and 33 GRAS genes from *A. thaliana*. Atypical GRAS family genes, including, 3 *BdGRAS* genes, 10 *OsGRAS* genes, and 1 *AtGRAS* gene, were also observed and were not included in the phylogenetic tree. The phylogenetic analysis showed they were divided into ten subfamilies, namely, DELLA, SCL3, SCL4/7, LAS, SCR, HAM, PAT1, SHR, DLT, and LISCL. Among these, the LISCL subfamily contained the greatest number of *BdGRAS* genes (11 members), the DLT subfamily contained only three genes. Moreover, 16 *BdGRAS* family genes were not classified into any subfamilies (**Figure 1A**). According to the clad support values and the classification of orthologs in wheat and *B. distachyon*. They can also be divided into 10 subfamilies using the phylogenetic analysis

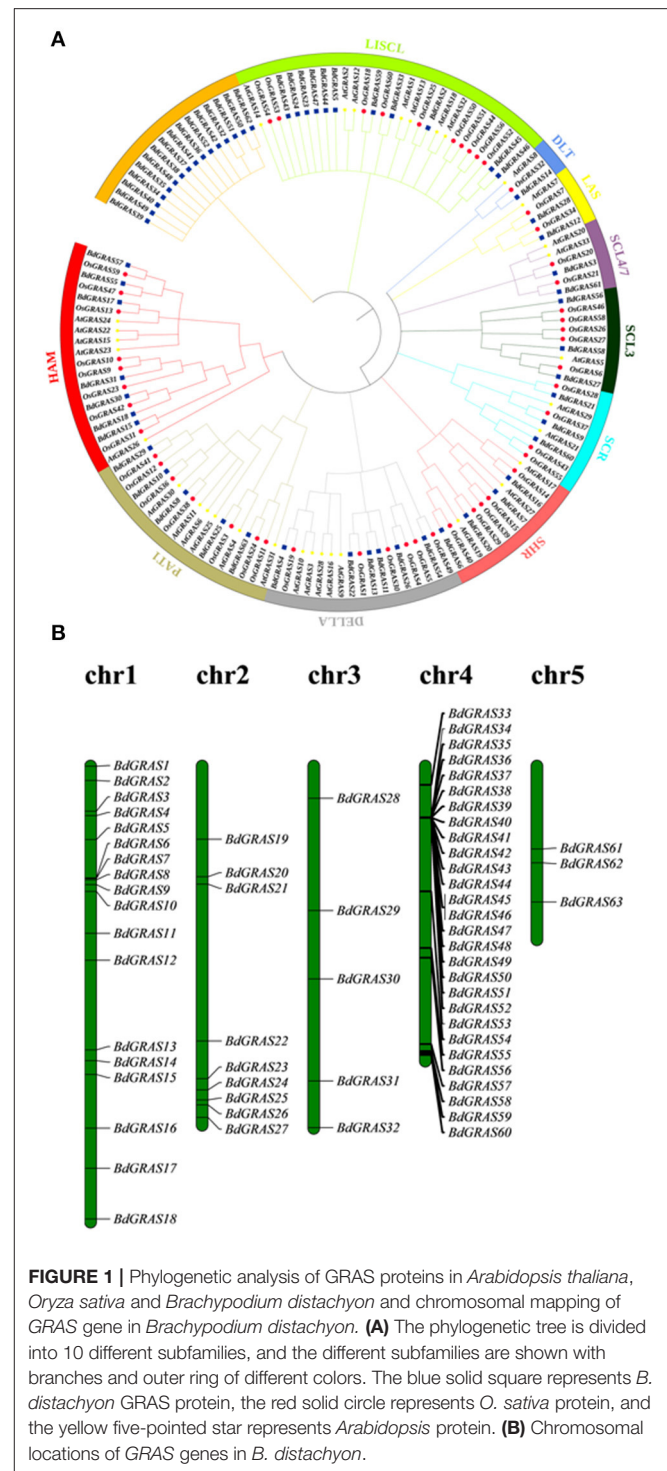


FIGURE 1 | Phylogenetic analysis of GRAS proteins in *Arabidopsis thaliana*, *Oryza sativa* and *Brachypodium distachyon* and chromosomal mapping of GRAS gene in *Brachypodium distachyon*. **(A)** The phylogenetic tree is divided into 10 different subfamilies, and the different subfamilies are shown with branches and outer ring of different colors. The blue solid square represents *B. distachyon* GRAS protein, the red solid circle represents *O. sativa* protein, and the yellow five-pointed star represents *Arabidopsis* protein. **(B)** Chromosomal locations of GRAS genes in *B. distachyon*.

(**Supplementary Figure 1**). These *BdGRAS* genes were unevenly distributed on five chromosomes. Chr4 contained the greatest number of *BdGRAS* genes ($n = 28$), followed by Chr1 ($n = 18$). Furthermore, nine genes were distributed on Chr2, and five genes were distributed on Chr3. Only three genes were distributed on Chr5 (**Figure 1B**).

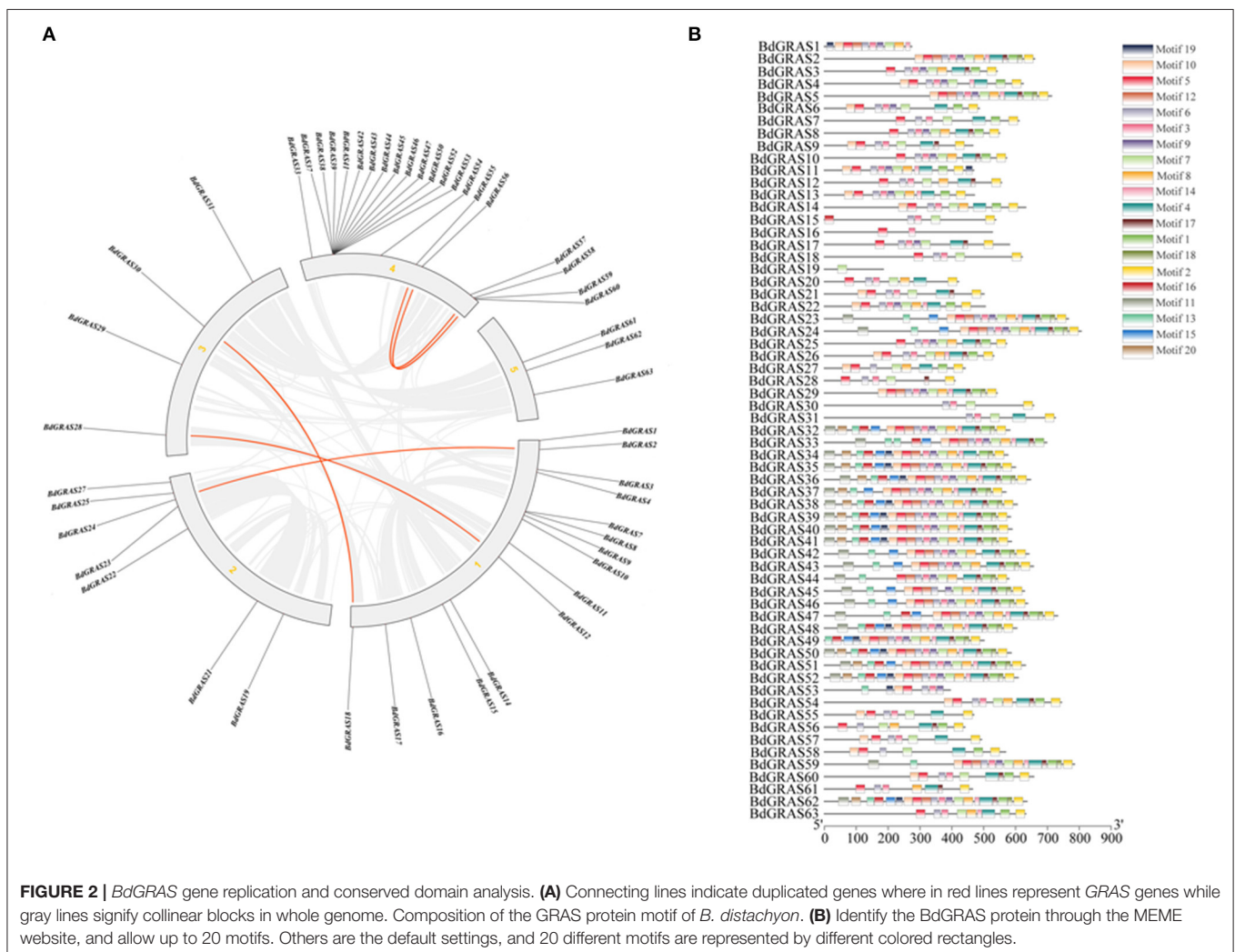
B. distachyon GRAS Family Collinearity Analysis and Structural Domain Analysis

Protein domain analysis showed that most members contained typical GRAS regions (**Supplementary Figure 2**). In each subfamily, the amino acid sequences of BdGRAS proteins showed high homology. All BdGRAS proteins have five conserved motifs in the C-terminus, namely, LHRI, VHIID, LHRII, PFYRE, and SAW; meanwhile, the N-terminus of BdGRAS proteins was highly variable (**Figure 2B**). To compare the genetic relationship of BdGRAS in *B. distachyon*, we used TBtools to identify paralogous genes in the *B. distachyon* genome. Five pairs of homologous genes were identified in BdGRAS, indicating that *B. distachyon* has experienced genome replication events during evolution (**Figure 2A**). Through the analysis of the transcriptional regulation mechanism of GRAS gene, it is found that BdGRAS gene mainly involves 15 biological pathways, most of which are involved in stress response, such as low temperature and light signal. It has been detected that BdGRAS gene is related to hormones, such as salicylic acid and jasmonic acid (**Supplementary Figure 3**).

Expression Profiles of the BdGRAS Family Genes in Different Tissues and Hormone Treatments

We tested the expression levels of BdGRAS family genes in different tissues of *B. distachyon*, including the roots, stems, leaves, and seeds, by RT-PCR. It was found that most of the genes were identified in a wide range of tissues, such as BdGRAS33, BdGRAS57, and BdGRAS58 (**Figure 3**). Moreover, some genes were highly expressed in some tissues. For instance, the expression of BdGRAS34 to BdGRAS40 was high in the roots and seeds. In contrast, the expression of BdGRAS31 was low in the seeds.

We also investigated the expression levels of BdGRAS genes under four different hormone treatments by qRT-PCR (SA, JA, IAA, and ABA). The expression levels of BdGRAS48, BdGRAS49, BdGRAS51, BdGRAS52, and BdGRAS55 significantly increased after SA hormone treatment at 1 hpt; BdGRAS2, BdGRAS8, BdGRAS10, BdGRAS23, BdGRAS47, and BdGRAS59 were significantly induced after JA hormone treatment. Moreover, BdGRAS47 expression levels significantly increased after ABA



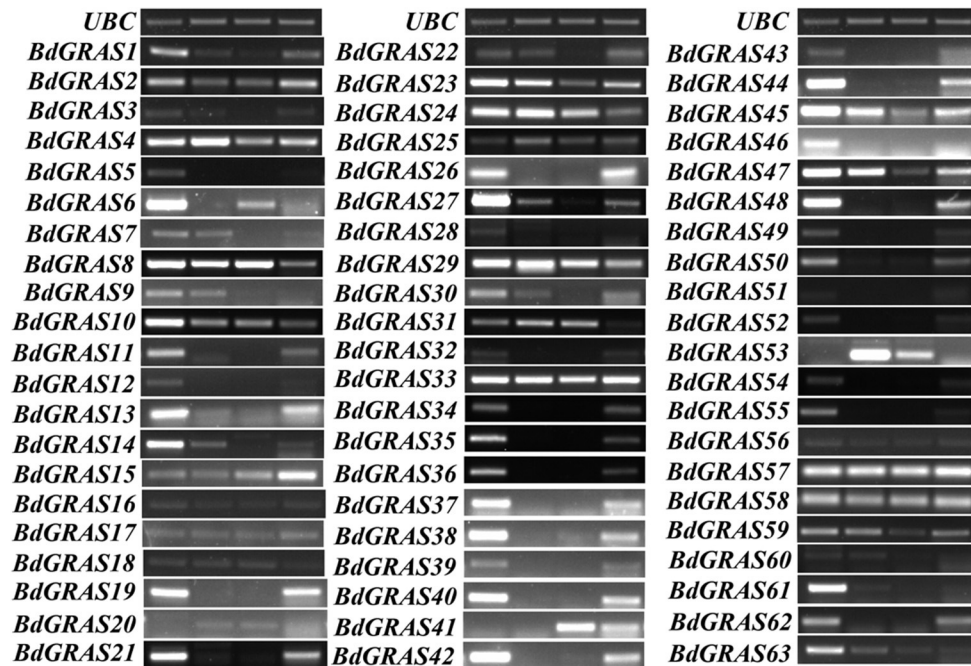


FIGURE 3 | The GRAS genes expression pattern in different tissues of *Brachypodium distachyon*. The expression level of the *BdGRAS* gene in the root, stem, leaf, and seeds of *B. distachyon*, using UBC as a control.

hormone treatment. After IAA treatment, the expression levels of most GRAS genes significantly reduced. The expression of *BdGRAS34*, *BdGRAS36*, *BdGRAS37*, *BdGRAS38*, *BdGRAS39*, *BdGRAS40*, *BdGRAS41*, *BdGRAS42*, and *BdGRAS45* was inhibited under all four different hormone treatments (Figure 4). These results indicated that the GRAS family genes participate in different hormone signaling pathways.

Expression Levels of *BdGRAS* Family Genes in *B. distachyon* and *M. oryzae* Interaction

To investigate the roles of *BdGRAS* genes in response to biotic stress, we analyzed the expression levels of *BdGRAS* genes in *B. distachyon* inoculated with *M. oryzae* RO1-1 at 0, 24, and 48 hpi. The results showed that the expression of *BdGRAS4*, *BdGRAS8*, *BdGRAS10*, *BdGRAS30*, and *BdGRAS31* gradually decreased after inoculation with *M. oryzae*. Moreover, expression levels of *BdGRAS2*, *BdGRAS9*, *BdGRAS18*, *BdGRAS23*, *BdGRAS33*, *BdGRAS34*, *BdGRAS47*, *BdGRAS48*, *BdGRAS49*, *BdGRAS51*, *BdGRAS52*, *BdGRAS57*, and *BdGRAS58* significantly increased at 24 hpi (Figure 5). These results indicate that the *BdGRAS* family participates in the interaction between *B. distachyon* and *M. oryzae*, and plays a role in the resistance of *B. distachyon* to rice blast.

BdGRAS31 Is the Target of miR171 in *B. distachyon*

Some members of the GRAS gene family are potential regulatory targets of miRNAs. We predicted that 23 *BdGRAS* genes were

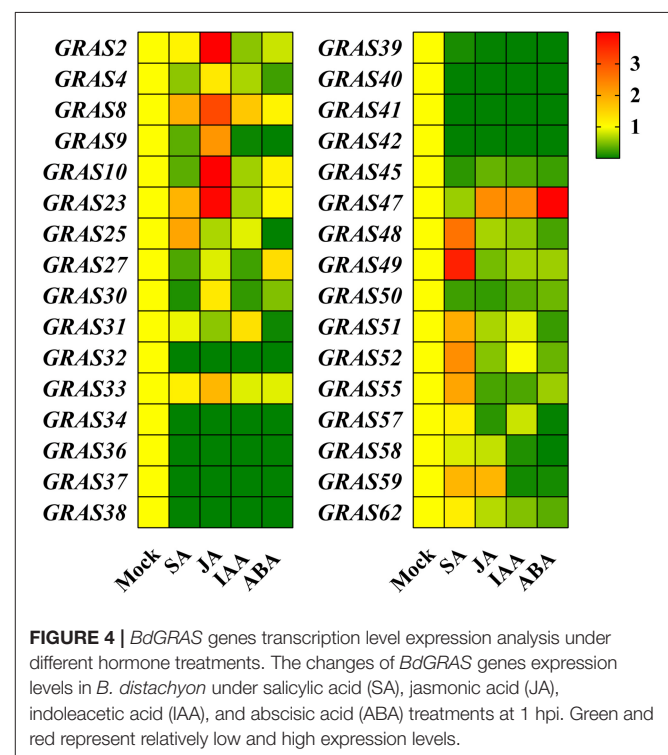
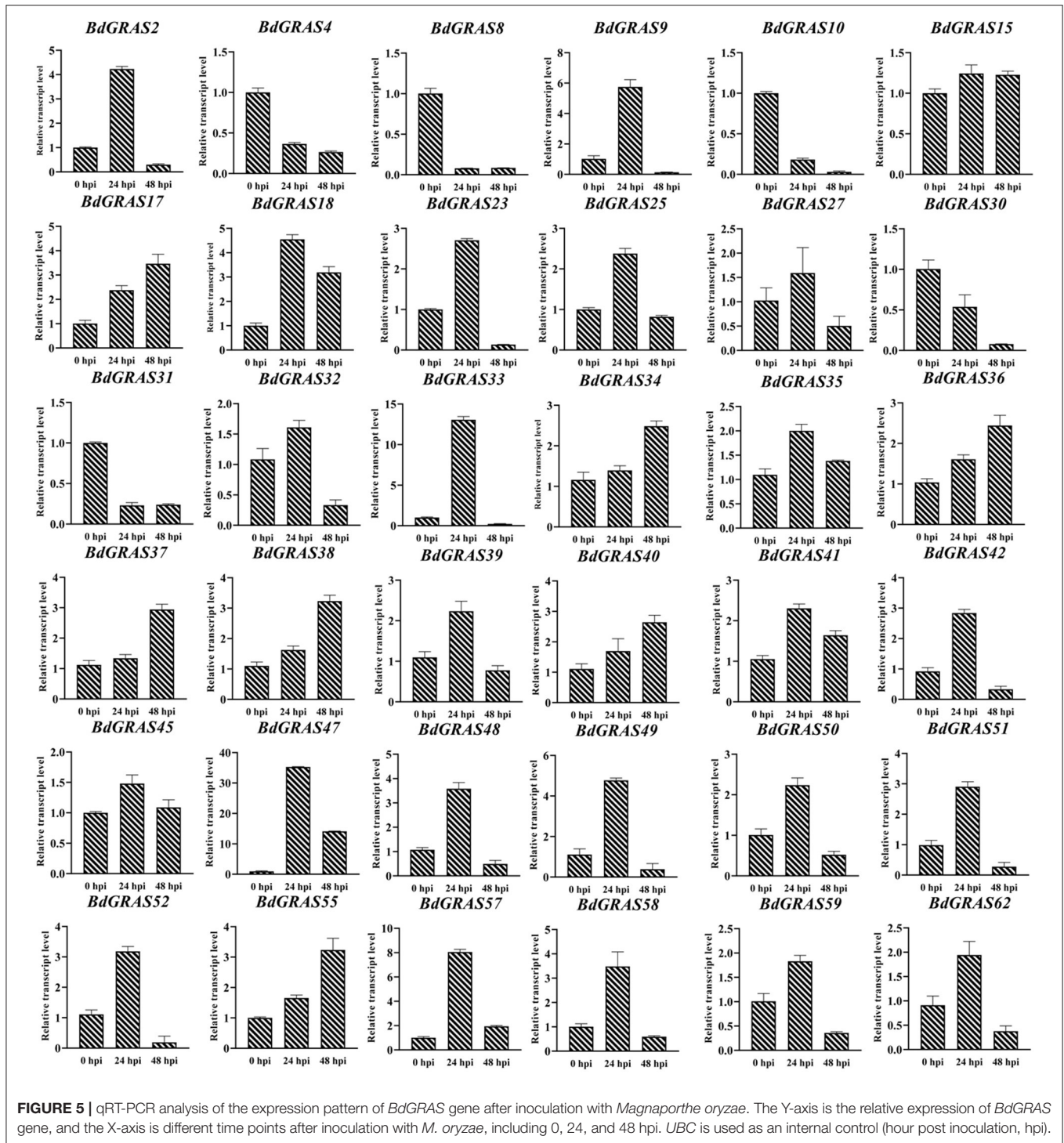


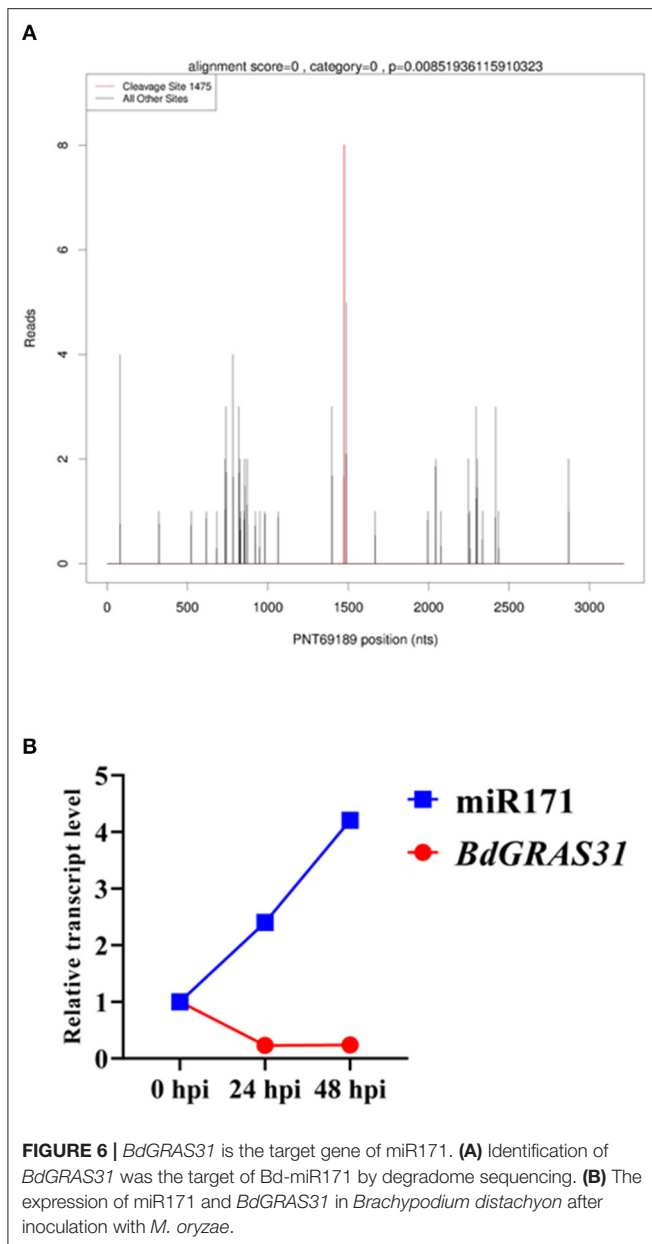
FIGURE 4 | *BdGRAS* genes transcription level expression analysis under different hormone treatments. The changes of *BdGRAS* genes expression levels in *B. distachyon* under salicylic acid (SA), jasmonic acid (JA), indoleacetic acid (IAA), and abscisic acid (ABA) treatments at 1 hpi. Green and red represent relatively low and high expression levels.

targeted by six miRNA families (Supplementary Table 4). Furthermore, we identified that *BdGRAS15*, *BdGRAS18*, and



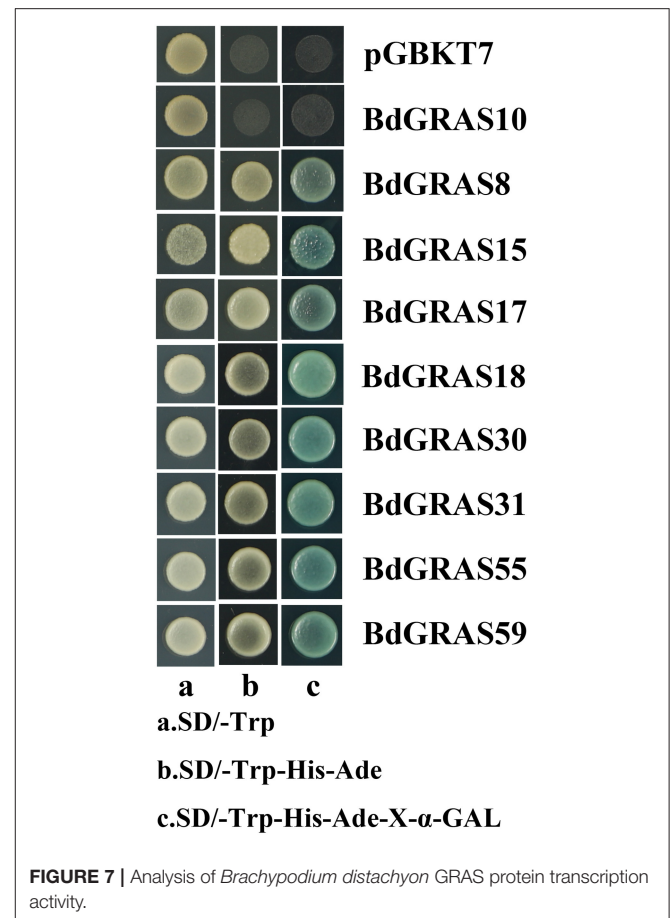
BdGRAS31 were candidate targets of *Bd-miR171* by degradome deep sequencing (Figure 6A). We used qRT-PCR to detect the expression levels of the three candidate target genes and *Bd-miR171* after inoculation with *M. oryzae* (Figure 6B). The expression of *Bd-miR171* gradually increased within 48 hpi with RO1-1, while that of *BdGRAS31* gradually decreased within

48 hpi. The expression level of *BdGRAS15* did not change significantly, and the expression of *BdGRAS18* increased at 24 hpi and began to decrease at 48 hpi (Supplementary Figure 4). Therefore, we inferred that *BdGRAS31* is regulated by *Bd-miR171* in the interactions between *B. distachyon* and *M. oryzae*.



Verification of Transcriptional Activity of *BdGRAS* Genes

To detect whether the *BdGRAS* family has transcriptional activity, we selected nine genes—*BdGRAS8*, *BdGRAS10*, *BdGRAS515*, *BdGRAS17*, *BdGRAS18*, *BdGRAS30*, *BdGRAS31*, *BdGRAS55*, and *BdGRAS59* for testing transcriptional activation. It was found that eight transformants harboring *BdGRAS* genes could grow on SD/-Trp and SD/-Trp-His-Ade and turned blue on SD/-Trp-His-Ade-X- α -GAL, indicating that *BdGRAS8*, *BdGRAS15*, *BdGRAS17*, *BdGRAS18*, *BdGRAS30*, *BdGRAS31*, *BdGRAS55*, and *BdGRAS59* show transcriptional activity (**Figure 7**). However, *BdGRAS10* did not show transcriptional activity.



DISCUSSION

The GRAS family is an important plant-specific transcription factor family divided into multiple subfamilies (Bolle, 2004). Recent studies have divided the GRAS family into 10–17 subfamilies in multiple plants (Bolle, 2016; Wang et al., 2018b,c). It is reported there were 48 *BdGRAS* genes, which were divided into ten known subfamilies (Niu et al., 2019). In this study, 63 *BdGRAS* family genes were identified by domain analysis. The phylogenetic tree showed that *BdGRAS* family genes were divided into ten subfamilies according to their genetic relationship. Moreover, most *B. distachyon* GRAS genes are divided into the same clade as that of *Arabidopsis* or rice (Niu et al., 2019). For example, the LISCL subfamily contains 11 *BdGRAS* genes. The conserved function of putative orthologs in each subfamily was indicated by the functional characterization of GRAS proteins. Therefore, we inferred that *BdGRAS* genes are also involved in multiple plant physiological processes by different regulation.

Each subfamily of GRAS genes has specific functions in plant development (Chen et al., 2019). For example, in *Lilium longiflorum*, the LISCL gene is involved in regulating microsporogenesis (Morohashi et al., 2003). The LISCL subfamily members were identified in a wide range of tissues in *Brassica napus* (Guo et al., 2019). The HAM subfamily

is considered to play an important role in the growth and development of root tips, stem tips, and shoot apex meristem (Stuurman et al., 2002). In this study, we identified some LISCL genes, such as *BdGRAS2*, *BdGRAS23*, *BdGRAS45*, and *BdGRAS59*, in multiple tissues. Moreover, we found that members of the HAM subfamily showed differences in tissue expression. *BdGRAS55* is only highly expressed in roots. Therefore, the results suggested that *BdGRAS* genes play an important role in controlling the growth and development of *B. distachyon*.

Multiple plant physiological processes are regulated by a diverse group of hormones (Huang et al., 2015). IAA is involved in almost all aspects of plant growth and development. JA and SA play important roles in biotic stresses. The *HcSCL13* gene of *Halostachys Caspica* was overexpressed in *Arabidopsis*, under the treatment of hormone ABA, the *HcSCL13* gene was induced rapidly, reached the highest peak at 1 h, and then decreased to the control level (Zhang et al., 2020). In the potato, the expression levels of *StGRAS34*, *StGRAS36*, and *StGRAS46* are increased under the treatment of IAA, ABA, and GA₃ hormones (Wang et al., 2019). In this study, we observed that *BdGRAS47* expression increased significantly under ABA and IAA treatment; this gene may play important roles in plant development. Moreover, the expression of *BdGRAS2*, *BdGRAS8*, *BdGRAS10*, *BdGRAS23*, and *BdGRAS47* increased significantly under JA treatment. The expression of *BdGRAS48*, *BdGRAS49*, *BdGRAS51*, *BdGRAS52*, and *BdGRAS55* significantly increased after SA treatment. These results suggest that *BdGRAS* genes may be involved in the plant response to biotic stress.

GRAS genes play important roles in the plant response to pathogens. For example, *VviRGA5* is upregulated in grape berries at the initial stage of fungal infection (Agudelo-Romero et al., 2014). *SIGRAS1* is involved in the plant response to biotic stress (Mayrose et al., 2006). CIGR2 negatively regulated resistance to *M. oryzae* in rice (Tanabe et al., 2016). In this study, the expression levels of 28 *BdGRAS* genes changed after inoculation with rice blast fungus. We found that the expression levels of most members of the HAM subfamily, including *BdGRAS17*, *BdGRAS18*, *BdGRAS55*, and *BdGRAS57*, increased significantly after inoculation with *M. oryzae*, while the expression levels of *BdGRAS30* and *BdGRAS31* significantly decreased. In *B. napus*, HAM subfamilies comprise two clades and perform different functions (Guo et al., 2019). We inferred that *BdGRAS* genes of HAM subfamilies may play multiply function in *B. distachyon* and *M. oryzae* interaction. Moreover, HAM genes are candidate targets of miR171 (Fan et al., 2015). We found 4 HAM subfamilies genes as candidate targets of Bd-miR171, and confirmed that *BdGRAS31* could be regulated by degradome deep sequencing and qRT-PCR. We also identified the transcriptional activity of the members of GRAS genes. Most *BdGRAS* genes showed transcriptional activity, except *BdGRAS10*. These results suggested that *BdGRAS* regulated target genes through multiple approaches.

In this study, we found that the HAM subfamily of *BdGRAS* genes may play essential roles in disease resistance. These results

provide a basis for further research on the *BdGRAS* family and also provide a reference for the study of disease resistance and stress resistance of grasses.

DATA AVAILABILITY STATEMENT

The data presented in the study are deposited in the Plant Transcription Factor Database (<http://planttfdb.gao-lab.org/index.php>) repository, accession number Bradi1g00219.1.p-Bradi5g19190.1.p; LOC_Os01g45860-LOC_Os12g38490; AT1G07520-AT5G67411; TRAES3BF021600230CFD_t1-Traes_7DL_44FCA17C4.1, see **Supplementary Table 1** for details.

AUTHOR CONTRIBUTIONS

ZT and NS are the experimental designers and executors of the experimental research, completing data analysis, and writing the first draft of the paper. WP, YY, TQ, and CH participated in the experimental design and analysis of the experimental results. LD and BW are the creators and leaders of the project, directing experimental design, data analysis, thesis writing, and revision.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2021.675177/full#supplementary-material>

Supplementary Figure 1 | Phylogenetic analysis of GRAS proteins in *Triticum aestivum* and *Brachypodium distachyon*.

Supplementary Figure 2 | The composition of the GRAS protein domain of *Brachypodium distachyon*. Green represents the GRAS domain, yellow represents the GRAS superfamily domain, pink represents the DELLA domain, and blue represents the PA28_alpha superfamily domain.

Supplementary Figure 3 | *Brachypodium distachyon* GRAS cis-acting regulatory element analysis.

Supplementary Figure 4 | The expression level of candidate targets of Bd-miR171.

Supplementary Table 1 | GRAS family genes in wheat, rice and *Arabidopsis thaliana*.

Supplementary Table 2 | *Brachypodium distachyon* GRAS family genes specific primers for qRT-PCR and RT-PCR analysis.

Supplementary Table 3 | Parameters of *Brachypodium distachyon* GRAS family genes.

Supplementary Table 4 | Prediction of target RNA of GRAS family in *Brachypodium distachyon*.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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