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An R2R3-Type Transcription Factor OsMYBAS1 Regulates Seed Germination under Artificial Accelerated Aging in Transgenic Rice (*Oryza sativa* L.)

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Abstract: MYB-type transcription factors play an essential regulatory role in seed germination and the response to seedling establishment stress. This study isolated a rice R2R3-MYB transcription factor, OsMYBAS1, and functionally characterized its role in seed germination. There was no significant difference in the germination rate of each transgenic line in the standard germination test. However, compared to the germination rate of the wild type (WT) measured in the artificial accelerated aging test, the germination rates of the overexpression lines OE-OsMYBAS1-1 and OE-OsMYBAS1-2 were significantly increased by 25.0% and 21.7%, respectively. In contrast, the germination rates of the knockout mutants *osmybas1-1* and *osmybas1-2* were decreased by 21.7% and 33.3%, respectively. Additionally, the above data indicated that OsMYBAS1 possibly plays a positive role in rice seed germination. Moreover, the antioxidant enzyme activities of OsMYBAS1-overexpressing plants were enhanced by 38.5% to 151.0% while the superoxide dismutase (SOD) enzyme activity of *osmybas1* mutants was decreased by 27.5%, and the malondialdehyde (MDA) content was increased by 24.7% on average. Interestingly, the expression of the antioxidation-related genes OsALDH3, OsAPX3, and OsCATC was enhanced in the OsMYBAS1 overexpression lines, which is consistent with the above results. Furthermore, transcriptome sequencing determined 284 differentially expressed genes (DEGs), which were mainly involved in the carbohydrate metabolic process, glycerolipid metabolism, and glycerophospholipid metabolism. Therefore, these findings provide valuable insight into the breeding of new rice varieties with high seed germination.

Keywords: rice; seed germination; *OsMYBAS1*; antioxidant enzyme; RNA-Seq

Citation: Wu, R.; Ding, Y.; Li, C.; Wu, B.; Huang, Z.; Li, Z.; Wang, X.; Zhao, G. An R2R3-Type Transcription Factor OsMYBAS1 Regulates Seed Germination under Artificial Accelerated Aging in Transgenic Rice (*Oryza sativa* L.). *Agronomy* **2022**, *12*, 1955. https://doi.org/10.3390/agronomy12081955

Academic Editor: Ali Raza

Received: 14 July 2022
Accepted: 15 August 2022
Published: 19 August 2022

1. Introduction

Seed germination ability is an important expression of seed vigor. Seed vigor is an essential trait of seed quality, which relates to crop yield and quality. Seeds with poor vigor are characterized by rapid seed aging and fission during storage, which is accompanied by seed nucleic acid denaturation, membrane system damage, endogenous hormone imbalance, and the accumulation of harmful substances, resulting in a low seed emergence rate, slow seedling growth, and poor stress resistance. Thus, it is necessary to improve seed germination to meet the urgent needs of modern agriculture for high-quality seeds [1–3].

It is well known that long-term storage of seeds destroys the metabolic balance of free radicals in cells, produces a large number of reactive oxygen species [4], and oxidizes unsaturated fatty acids to produce toxic substances such as MDA, resulting in loss of the germination ability of seeds. The fatty acid hydroxylase gene *OsFAH2* is one of the candidate genes in the qSS3.1 locus. Yuan et al. [5] confirmed that OsFAH2 can reduce the level of lipid peroxidation and the accumulation of toxic substances, including MDA; so, the seed vigor of *OsFAH2*-overexpressing rice lines was significantly higher than that of the

wild type (WT). Moreover, Huang et al. [6] and Xu et al. [7] pointed out that knockdown of the lipoxygenase gene LOX-2 or LOX-3 in rice endosperm inhibits the peroxidation of unsaturated fatty acids, prevents cell membrane damage, and ameliorates impaired cellular function, thereby improving rice seed vigor. In addition, it has been reported that the enzymatic system, composed of SOD, CAT, and POD, and the non-enzymatic system, composed of antioxidant substances such as ascorbic acid, glutathione, and proline (Pro), in cells can scavenge ROS or reduce superoxide anion free radicals, inhibit the levels of hydrogen peroxide and lipid peroxidation, and maintain the integrity of the membrane [8]. For example, MDA can be eliminated by inducing the expression of the acetaldehyde dehydrogenase gene OsALDH7 [9]. OsAPX3 and OsAPX4 play a negative regulatory role in the pathway of leaf senescence mediated by ROS signaling, in addition to an essential role controlling intracellular H_2O_2 levels [10]. Therefore, reducing lipid peroxidation is a critical way to improve seed vigor.

MYB transcription factors are one of the most prominent families of plant transcription factors with the most diverse functions, which participate in many life activity pathways. Accumulated evidence has shown that MYB transcription factors play an essential regulatory role in abiotic stresses such as high/low temperatures and drought [11–14]. MYB-TFs can activate anthocyanin biosynthesis to improve plant stress resistance [15]. The transcriptional activation of cutin deposition and antioxidant defense by AtMYB49 contributed to salt tolerance in *Arabidopsis thaliana* [16]. Elevated expression of MYB25 reduces sensitivities toward abscisic acid, osmotic, and salt stress in Arabidopsis [17]. Overexpression of OsMYBS1 can enhance the tolerance of rice seeds to abiotic stresses and improve seed vigor, thereby promoting seed development and the accumulation of storage substances [18]. Overexpression of OsMYBS3 can significantly enhance the cold stress tolerance of rice, which is involved in regulating the CBF/DERB cold stress signal transduction pathway [11]. Additionally, the rice MYB transcription factor can effectively alleviate seed damage caused by adversity by increasing the activities of SOD, CAT, and POD and reducing the MDA content [13,19,20]. Yang et al. [13] reported that overexpression of *OsMYBAS1* in rice significantly increased the activities of antioxidant system enzymes (including CAT, SOD, and POD); reduced the accumulation of hydrogen peroxide and MDA; and enhanced the salt tolerance, dehydration tolerance, and low-temperature tolerance of rice seeds. Thus, OsMYBAS1 plays a vital role in the stress tolerance of rice. The current authors previously isolated an R2R3-MYB transcription factor, designated *OsMYBAS1*, in rice and reported that OsMYBAS1 could increase the germination rate of rice seeds under deep sowing conditions [21]; however, this was proven only at the physiological level. The molecular mechanism of its influence on seed germination has not been studied. This study aimed to determine the antioxidant capacity of *OsMYBAS1* transgenic seeds under artificial accelerated ageing treatment, analyze the RNA-Seq data of germinating seedlings after aging treatment, and mine the downstream differential genes and related pathways regulated by OsMYBAS1. It was expected that the results would preliminarily reveal the molecular mechanism of OsMYBAS1 in improving the germination of rice seeds, and provide key genes or germplasm resources for the breeding of new varieties of high-germination rice.

2. Materials and Methods

2.1. Plant Materials, Growth Conditions, and Treatment Methods

Nipponbare (*Oryza sativa* L. ssp. *Japonica*) was used as WT in different treatments. The artificial accelerated aging test consisted of three treatments: WT, *OsMYBAS1* overexpression lines (OE-OsMYBAS1-1, OE-OsMYBAS1-2), and *osmybas1* mutants (*osmybas1-1*, *osmybas1-2*). The full-length cDNA of *OsMYBAS1* was amplified from rice. The product was ligated into the pGEM-T Easy vector and sequenced. Then, the pEXT06-*OsMYBAS1* construct was obtained. *OSMYBAS1* was driven by the cauliflower mosaic virus 35S (CaMV 35S) promoter. The embryogenic calli of Asahi were transformed by the Agrobacterium-mediated method, and the overexpression transgenic lines were obtained. The *osmybas1* mutant lines were obtained by the CRISPR/CAS9 editing system. The pCAMBIA1300-

OsMYBAS1-sgRNA-Cas9 construct was transformed into the calli of the rice cultivar Nipponbare through Agrobacterium-mediated transformation. This work was completed by Baige Gene Technology Co., Ltd. (Changzhou, China); the specific gene editing methods are detailed on the website www.biogle.cn (accessed on 30 June 2022). They were identified and propagated to obtain homozygous lines. In total, 3 replicates were taken from the seedlings of 3-day-old WT, OE-OsMYBAS1-1, and OEOsMYBAS1-2 overexpression lines under artificial accelerated aging treatment for transcriptomic sequencing analysis. Each experiment consisted of three biological replications.

2.2. Artificial Accelerated Aging Test

WT, *OsMYBAS1* overexpression lines, and *osmybas1* mutants were treated at 45 °C and 100% relative humidity for 10 days and then incubated in a growth chamber at 30 °C with 16-h light/8-h dark for 14 days, referred to as the standard germination test.

2.3. Standard Germination Test

Standard germination experiments were carried out in a growth chamber at 30 °C with a 16-h light/8-h dark, and the sample was 20 seeds with 3 biological replications [21]. After 14 days, the total germinated seeds were counted, and the germination rate was calculated according to the following formula:

$$\text{Germination rate} = \frac{\text{number of germinated seeds}}{\text{total number of seeds}} \quad (1)$$

2.4. Determination of Enzyme Activity and the Malondialdehyde and Proline Content

In total, 0.5 g (fresh weight) of rice leaf tissue was ground in 5 mL of 0.05 mol/L sodium phosphate buffer (pH = 7.8) and then centrifuged at 10,000× *g* for 15 min to retain the supernatant. The content of MDA was determined according to the method of Wang et al. [22]. SOD activity determination was described by Pinhoro et al. [23]. The CAT and POD activities were measured according to the method described by Li et al. [24]. The Pro content was determined according to the ninhydrin method described by Zhang et al. [25]. The determination of each biochemical index included three biological replicates.

2.5. RNA Extraction, cDNA Library Preparation, and Transcriptome Sequencing

Total RNA was extracted from the samples using Trizol reagent, and then reverse transcribed into cDNA using a PrimeScript™ RT reagent kit with gDNA Eraser (TaKaRa, Dalian, China). First, the gDNA removal reaction was performed (2 μL 5× gDNA Eraser Buffer, 1 μL gDNA Eraser, 5 μL Total RNA, 2 μL RNase Free dH_2O) at 42 °C for 2 min. Then, the reverse transcription reaction was performed by the TB Green qPCR, reaction solution of step 1, 1 μL of PrimeScript RT Enzyme Mix I, 4 μL of RT Primer Mix, 4 μL of 5× PrimeScript Buffer 2, and 1 μL of RNase Free dH_2O, with a total of 20 μL, at 37 °C for 15 min and 85 °C for 5 s. The quality and quantity of total RNA were analyzed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The integrity was further evaluated using an Agilent 2100 Bioanalyzer (Agilent Technologies Co., Ltd., Santa Clara, CA, USA). High-quality RNA isolated from two independent samples (biological repeats) was pooled for the library preparation. The library and RNA-Seq were constructed by Baimike Biotechnology Co., Ltd. (Beijing, China) and the cDNA library was sequenced by an Illumina HiSeq2500™.

2.6. Differential Expression Analysis

The raw data were standardized by R-package "limma" [26]. To identify the differentially expressed genes (DEGs) in the high-germination rice varieties, the standardized rice expression matrix was differentially analyzed by R-package "edgeR" [27], and the screening threshold was set as $|\log FC| > 2$, FDR < 0.05.

2.7. GO and KEGG Enrichment Analysis

In order to identify the biological functions and signal pathways involving SRDs, the R-package "clusterProfiler" [28] was used to perform the enrichment analysis of germination-related genes. Pathways with $p < 0.05$ were considered as significantly enriched pathways. The top 10 upregulated and downregulated GO terms and KEGG pathways were visualized via R-package.

2.8. Gene Set Enrichment Analysis (GSEA)

In order to identify the difference in the biological functions between high/low-germination rice seed groups, GSEA enrichment analysis was performed on the gene sets of high/low-germination rice seeds using GSEA software ($p < 0.05$). The enrichment score and retrograde significance were analyzed by the displacement test, and the number of displacement tests was set at 1000.

2.9. qRT-PCR Detection

qRT-PCR was performed in an optical 96-well plate with a CFX ConnectTM Real-Time system (BIORAD, Singapore). Each reaction contained 10 μL of 2× TaqPro Universal SYBR qPCR MAster Mix (Vazyme, Nanjing, China), 1 μL of forward primer, 1 μL of reverse primer, 5 μL of cDNA, and 3 μL of ddH_2O. The thermal cycle used was 95 °C for 30 s and 39 cycles of 95 °C for 5 s and 60 °C for 30 s, and 95 °C for 15 s. Primers were designed by PrimerPremier5.0 software (Table S1), and each primer was diluted to 10 μmol/L and then added to the system. The *OsActin* gene was used as an internal reference, and the gene expression was calculated by the $2^{-\Delta\Delta CT}$ method.

2.10. Statistical Analysis

The obtained phenotypic or physiological data were statistically analyzed using one-way analysis of variance (ANOVA), which was carried out in SPSS 24.0 (IBM, Chicago, IL, USA), and multiple comparisons were explored using the Duncan test at a 0.05 probability level. Before analysis, the percentage data were transformed according to ŷ = arcsin [sqr (x/l00)].

3. Results

3.1. Effects of OsMYBAS1 on Rice Seed Germination

Significant differences in seed germination were recorded among the WT and *OsMYBAS1* overexpression lines and mutants. The germination rates of OE-OsMYBAS1-1, OE-OsMYBAS1-2, WT, *osmybas1-1*, and *osmybas1-2* were basically comparable under standard germination conditions (CK), ranging between 85% and 95%, while significant differences in the germination rates were obtained under artificial accelerated aging treatment (AAT). Compared to CK, the germination rates of OE-OsMYBAS1-1 and OE-OsMYBAS1-2 both decreased by 21.7%, WT decreased by 41.7%, and *osmybas1-1* and *osmybas1-2* decreased by 58.3% and 75.0%, respectively (Figure 1A,B). Moreover, the root length and seedling length measured under AAT were lower than that measured under CK. Compared to WT, the root length and seedling length of OE-OsMYBAS1-1 and OE-OsMYBAS1-2 significantly increased by 43.9% and 34.1% and 23.7% and 25.1%, respectively; and those of *osmybas1-1* and *osmybas1-2* were significantly lower by 51.2% and 65.9% and 64.4% and 85.6%, respectively, when measured under AAT (Figure 1C,D).

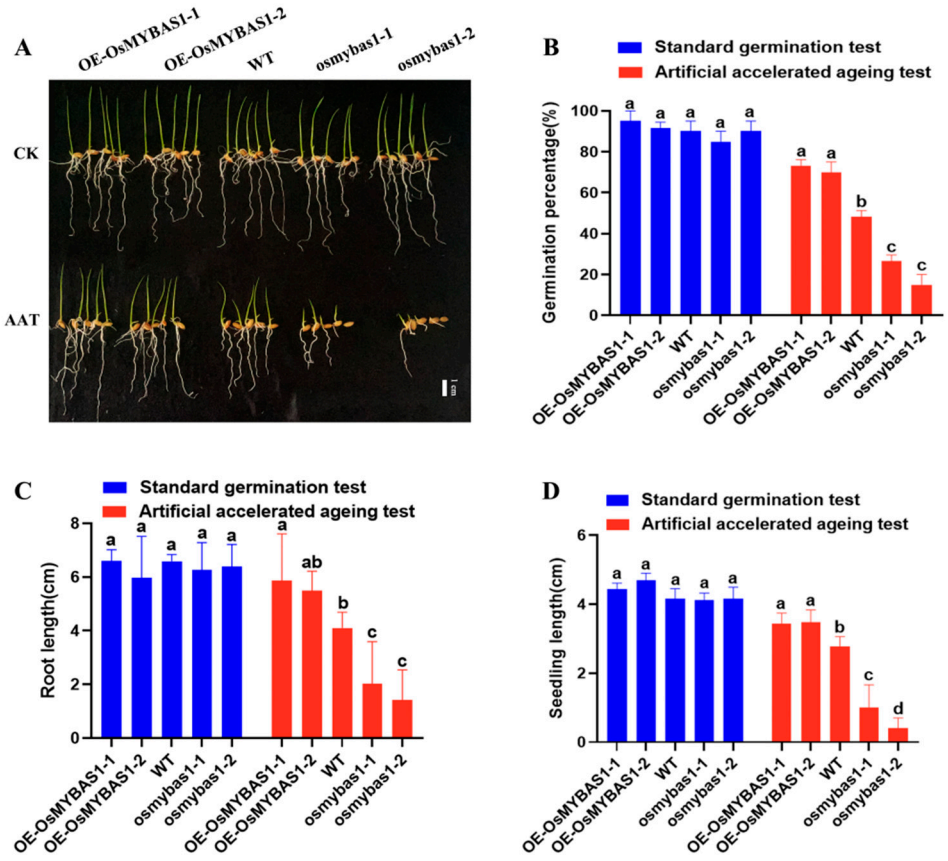

Figure 1. Effects of OsMYBAS1 on the germination characteristics and phenotypic indexes of rice seeds. (**A**) Seedling growth map of OE-OsMYBAS1-1, OE-OsMYBAS1-2, WT, *osmybas1-1*, and *osmybas1-2* for 14 days before and after aging; (**B**) Germination rates of OE-OsMYBAS1-1, OE-OsMYBAS1-2, WT, *osmybas1-1*, and *osmybas1-2*; (**C**) Root lengths of OE-OsMYBAS1-1, OE-OsMYBAS1-2, WT, *osmybas1-1*, and *osmybas1-2* after 14 days; (**D**) Seedling lengths of OE-OsMYBAS1-1, OE-OsMYBAS1-2, WT, *osmybas1-1*, and *osmybas1-2* after 14 days. The data represent the mean ± SE, and different letters represent significant differences between treatments (Duncan's test, $p < 0.05$).

3.2. Effects of OsMYBAS1 on the Antioxidant Capacity of Rice Seed

In order to determine the physiological changes in seedlings of different rice lines, we measured the activity of concentrated antioxidant enzymes, Pro content, and MDA content in the OE-OsMYBAS1 overexpression line, *osmybas1* mutant line, and WT under CK and AAT treatment, respectively. After AAT treatment, the antioxidant capacity of OE-OsMYBAS1-1, OE-OsMYBAS1-2, WT, *osmybas1-1,* and *osmybas1-2* was lower than that of CK (Figure 2A-E). Compared to WT, the POD enzyme activity of OE-OsMYBAS1-1 and OE-OsMYBAS1-2 increased by 151.0% and 64.6%, CAT enzyme activity increased by 38.5% and 51.3%, Pro content increased by 8.6% and 16.7%, and MDA content decreased by 29.3% and 44.3%, respectively; while the SOD activity of *osmybas1-1* and *osmybas1-2* decreased by 25% and 30%, the Pro content decreased by 28.1% and 27.4%, and the MDA content increased by 18.1% and 31.3%, respectively. The changes in the antioxidant capacity and germination characteristics of the different rice lines were consistent, which indicated that OsMYBAS1 might improve rice seed germination by enhancing the antioxidant capacity of rice.

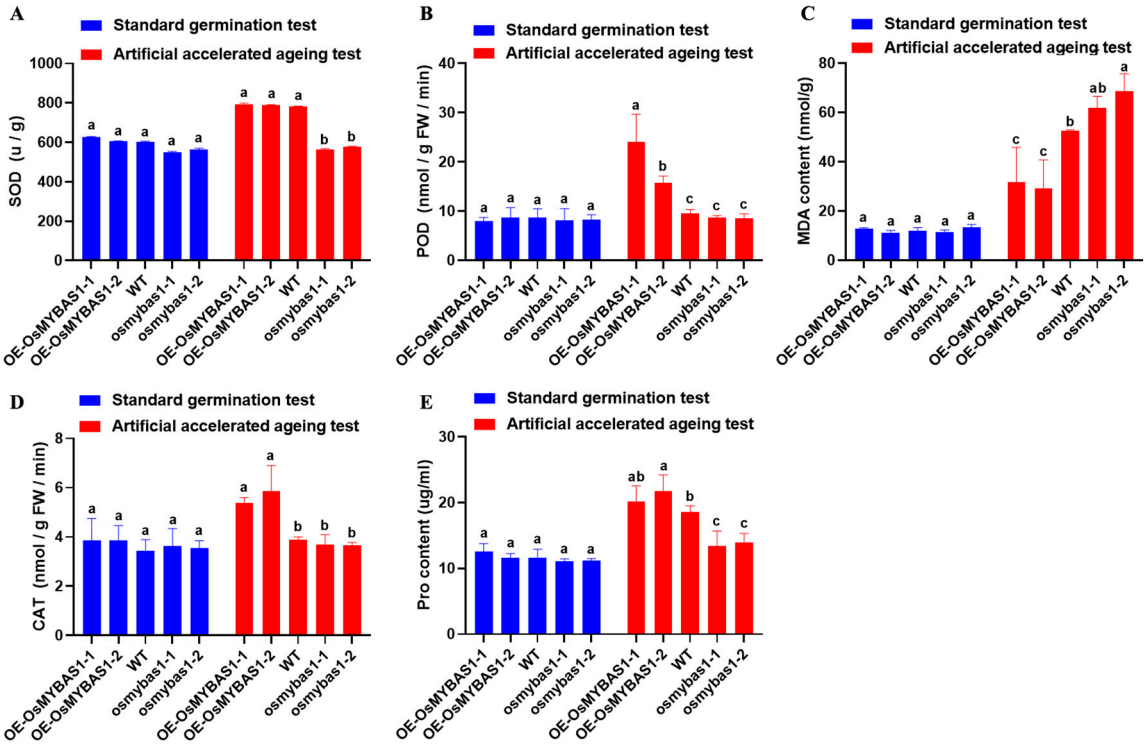

Figure 2. Effects of OsMYBAS1 on physiological and biochemical indicators. (**A**) SOD; (**B**) POD; (**C**) MDA; (**D**) CAT; and (**E**) Pro contents of OE-OsMYBAS1-1, OE-OsMYBAS1-2, WT, *osmybas1-1*, and *osmybas1-2* before and after aging. The data represent the mean ± SE, and different letters represent significant differences between treatments (Duncan's test, $p < 0.05$).

3.3. Artificial Accelerated Aging Induced Transcriptome Changes in Rice

In order to determine the reasons why *OsMYBAS1* overexpression led to increased seed germination of rice, RNA-Seq analysis was carried out with WT as the control. The difference analysis results showed that a total of 3220 DEGs were identified in OE-1_VS_WT, in which 1187 DEGs were significantly upregulated and 2033 DEGs were obviously downregulated (Figure 3A). Moreover, a total of 3149 DEGs were identified in OE-2_VS_WT, in which 966 DEGs were significantly upregulated and 2183 DEGs were significantly downregulated (Figure 3B). A total of 284 DEGs were identified in the intersection of the 2 groups, in which 78 DEGs were upregulated considerably and 206 DEGs were significantly downregulated, and these DEGs were further analyzed in the following assays (Figure 3C,D).

3.4. GO and KEGG Enrichment Analysis of DEGs

The GO biological function enrichment analysis of these DEGs (Table S2) showed that the upregulated DEGs were mainly enriched in GO terms such as carbohydrate metabolic process (GO: 0005975), lipid metabolic process (GO: 0006629), metal ion transport (GO: 0030001), and steroid-hormone-mediated signaling pathway (GO: 0043401) (Figure 4A). Regarding the cellular component module, upregulated DEGs were mainly enriched in GO terms such as Golgi apparatus (GO: 0005794), microtubule (GO: 0005874), chromosome (GO: 0005694), and epsilon DNA polymerase complex (GO: 0008622) (Figure 4B). Regarding the molecular function module, upregulated DEGs were mainly enriched in GO terms such as ATP binding (GO: 0005524) and microtubule binding (GO: 0008017) (Figure 4C). Regarding the biological process module, downregulated DEGs were mainly enriched in GO terms such as photosynthesis (GO: 0015979), hydrogen peroxide catabolic process (GO: 0042744), and brassinosteroid homeostasis (GO: 0010268) (Figure 4D). Regarding the cellular component module, downregulated DEGs were mainly enriched in GO terms such as photosystem II oxygen evolving complex (GO: 0009654), integral component of membrane (GO: 0016021),

chloroplast thylakoid membrane (GO: 0009535), and photosystem I reaction center (GO: 0009538) (Figure 4E). Regarding the molecular function module, downregulated DEGs were mainly enriched in GO terms such as oxidoreductase activity (GO: 0016491), heme binding (GO: 0020037), and monooxygenase activity (GO: 0004497) (Figure 4F). The KEGG pathway analysis of these DEGs showed that the upregulated DEGs were mainly enriched in pathways such as glycerolipid metabolism (ko00561), base excision repair (ko03410), and glycerophospholipid metabolism (ko00564) (Figure 4G) and the downregulated DEGs were primarily enriched in pathways such as phenylpropanoid biosynthesis (ko00940), carotenoid biosynthesis (ko00906), and glyoxylate and dicarboxylate metabolism (ko00630) (Figure 4H).

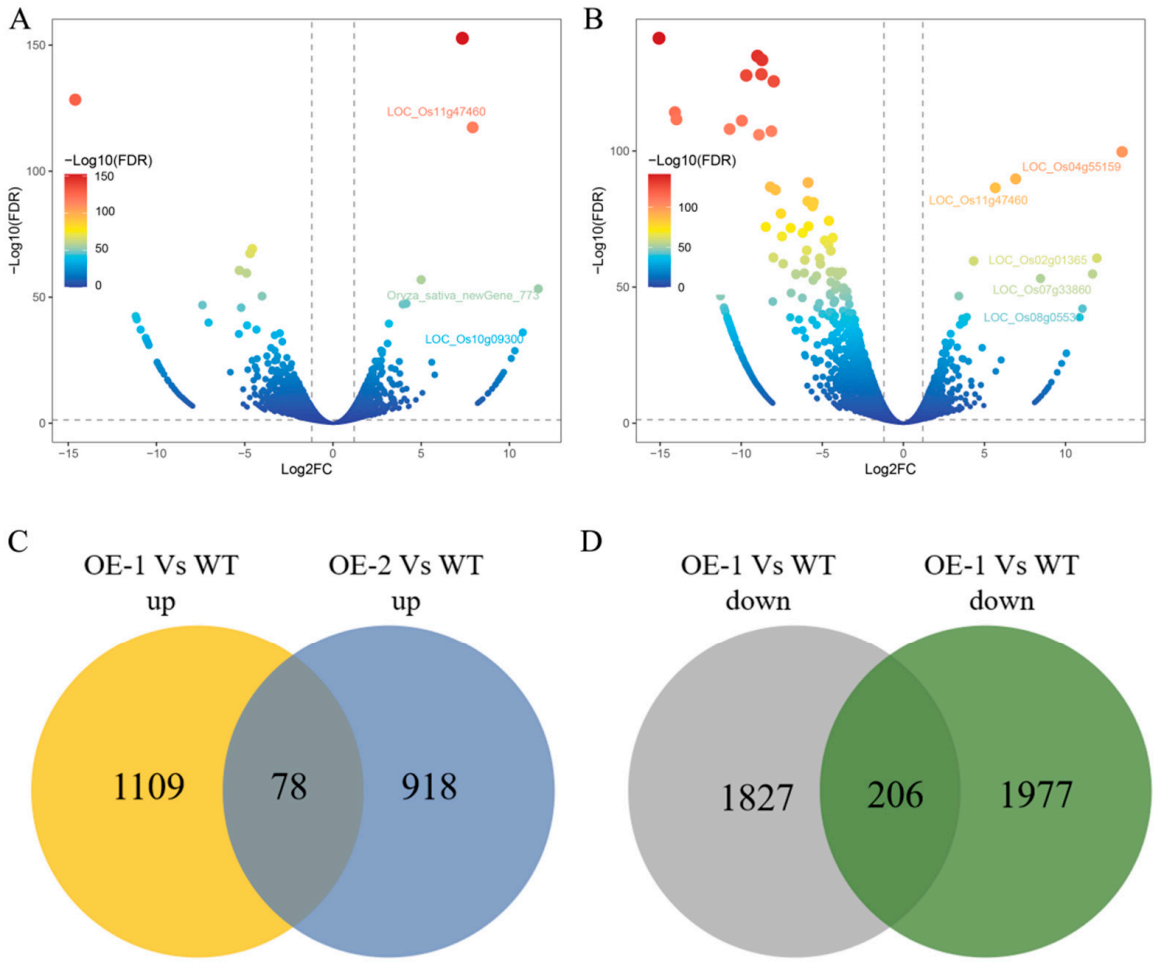

Figure 3. DEGs identification. (**A**) Volcano map of DEGs in OE-1_VS_WT; (**B**) Volcano map of DEGs in OE-2_VS_WT; (**C**) Venn map of upregulated DEGs in OE-1_VS_OE-2_VS_WT; (**D**) Venn map of downregulated DEGs in OE-1_VS_OE-2_VS_WT.

3.5. GSEA Analysis

Based on transcriptome sequencing data, GSEA analysis of the high/low-germination groups showed that significant differences were recorded in the regulation of seed germination, endomembrane organization, photosystem II oxygen evolving complex, DNA replication, and other pathways (Figure 5A–D), which may be the reason for the differences in seed germination between the high/low-germination groups.

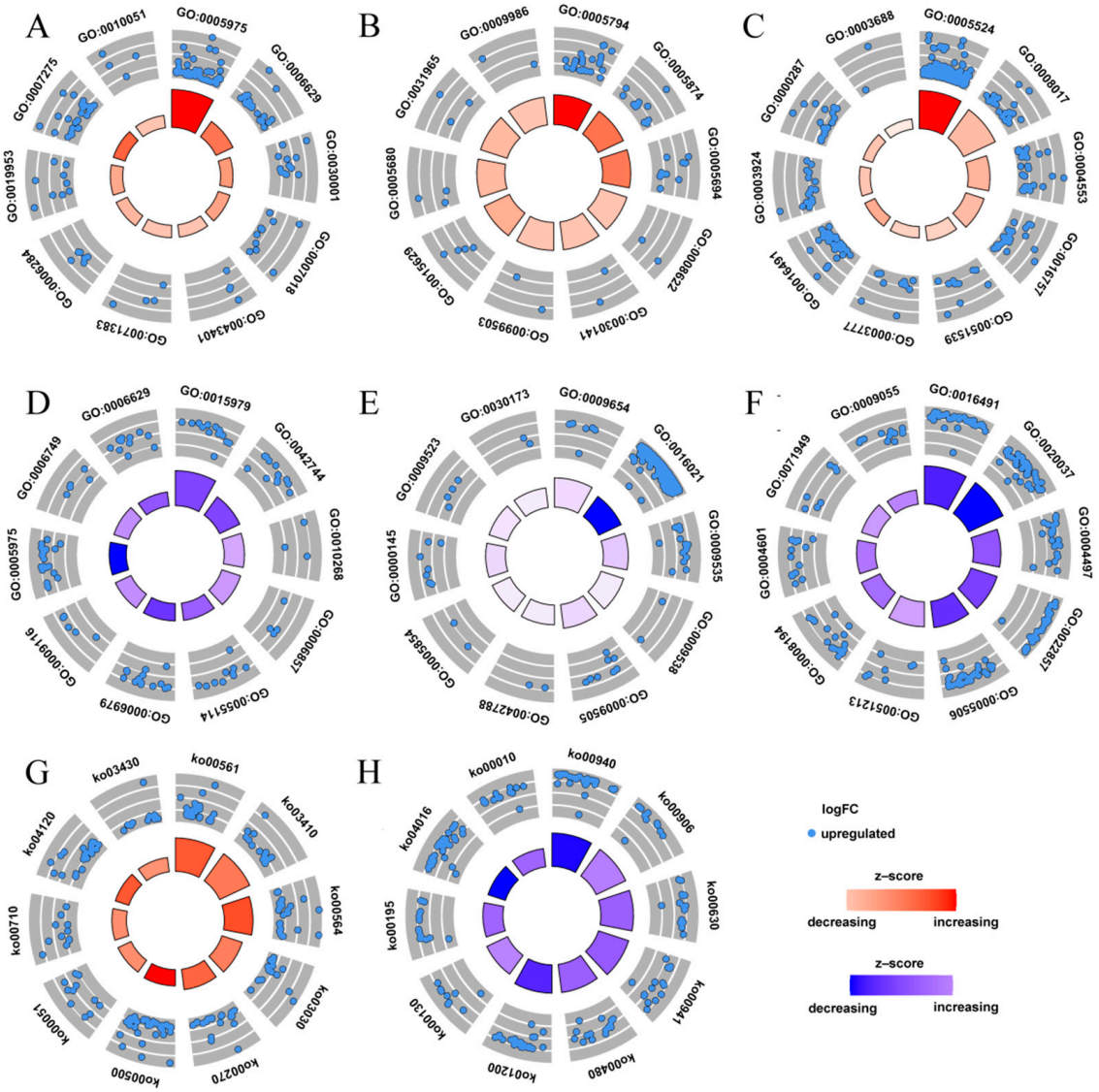

Figure 4. GO and KEGG enrichment analysis of DEGs. (**A**) Biological process annotation in GO functional enrichment analysis of upregulated DEGs; (**B**) Cellular component annotation in GO functional enrichment analysis of upregulated DEGs; (**C**) Molecular function annotation in GO functional enrichment analysis of upregulated DEGs; (**D**) Biological process annotation in GO functional enrichment analysis of downregulated DEGs; (**E**) Cellular component annotation in GO functional enrichment analysis of downregulated DEGs; (**F**) Molecular function annotation in GO functional enrichment analysis of downregulated DEGs; (**G**) KEGG enrichment analysis of upregulated DEGs; (**H**) KEGG enrichment analysis of downregulated DEGs.

3.6. Identification and Validation of Antioxidation-Related Genes and Downstream Transcription Factors

The antioxidation-related genes *LOC_Os02g43280* (*OsALDH3*), *LOC_Os04g14680* (*OsAPX3*), and *LOC_Os03g03910* (*OsCATC*) were selected for qRT-PCR verification, and their expression was increased by 6.0, 3.0, and 1.2 times, respectively (Figure 6A). These results are consistent with the RNA-Seq data.

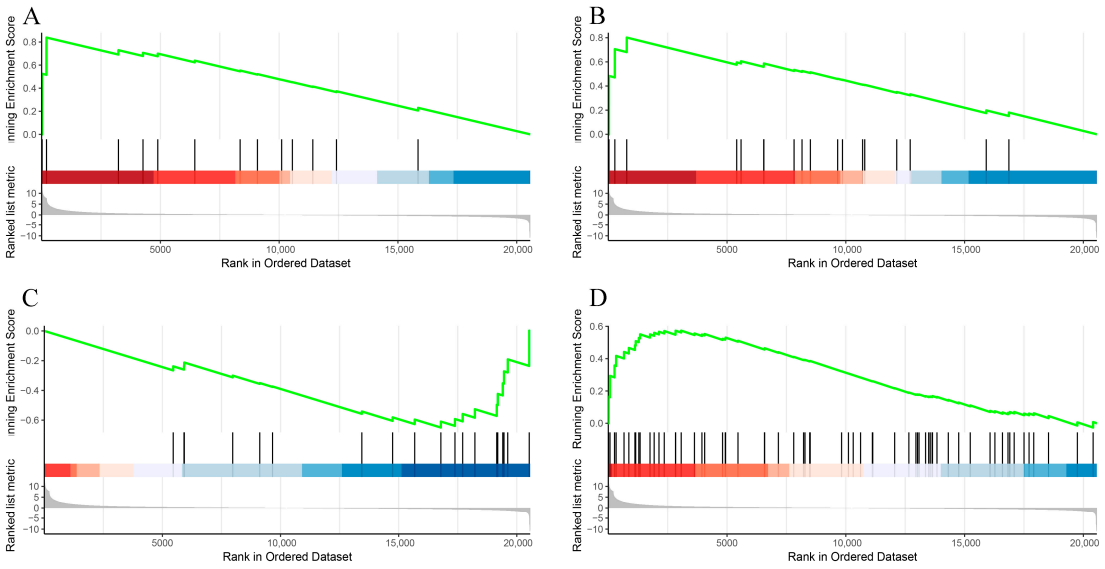

Figure 5. GSEA enrichment analysis of gene sets for high/low-germination rice seeds. (**A**) regulation of seed germination; (**B**) endomembrane organization; (**C**) photosystem II oxygen evolving complex; (**D**) DNA replication. The red line is upregulated DEGs, the gray line is the genes that have no difference, and the blue line is downregulated DEGs. The darker the color, the more significant the difference.

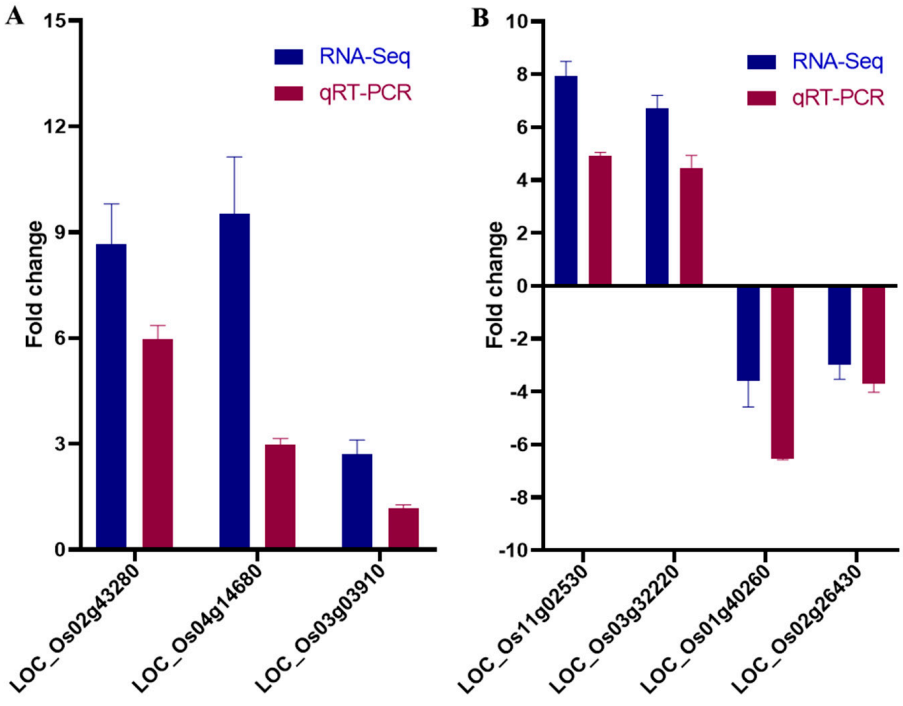

Figure 6. qRT-PCR validation results of the RNA-Seq data. (**A)** Three antioxidation-related genes; (**B**) Downstream transcription factor verified by qRT-PCR and compared with the expression obtained from RNA-Seq.

In order to identify more target genes regulated by OsMYBAS1, the rice transcription factor gene set was downloaded from Plant TFDB (accessed on 13 February 2022, http://planttfdb.gao-lab.org/) and intersected with DEGs of the high/lowg-ermination groups to obtain the 11 downstream transcription factors of OsMYBAS1 (Table S3). Then, four transcription factors related to stress resistance were selected for qRT-PCR validation.

The expression of *LOC_Os11g02530* and *LOC_Os03g32220* increased by 4.9 and 4.7 times, respectively, in the overexpression lines; and *LOC_Os01g40260* and *LOC_Os02g26430* decreased by 6.5 and 3.7 times, respectively. It is basically consistent with the RNA-Seq data (Figure 6B), indicating that the current sequencing results were accurate and reliable.

4. Discussion

Seed vigor is not only an important quantitative trait of seeds but also a specific expression of seed quality. Seed germination ability is closely related to it. High-germination seeds have a high sowing quality and strong stress tolerance, with a distinct growth advantage and production potential. Low-germinationvigor seeds are slow to germinate and have a low growth rate under suitable conditions, and their emergence is not neat or does not occur under poor environmental conditions, which makes it difficult to meet the requirements of direct rice seeding in agricultural production. Therefore, the discovery of germination-related genes in rice using bioinformatics technology is essential for rice breeding and development.

In this study, *OsMYBAS1* overexpression lines and WT were used for transcriptome sequencing analysis to analyze the gene regulatory network caused by *OsMYBAS1* expression changes and clarify its molecular mechanism. By differential expression analysis of genes in overexpression lines and a wild type, 284 DEGs were obtained from the intersection. The GO enrichment results showed that these DEGs were mainly involved in biological functions such as the carbohydrate metabolic process, lipid metabolic process, and oxidoreductase activity. The carbohydrate metabolic process is one of the vital life activities of organisms and the key for plants to withstand abiotic stress [29]. When plants are subjected to abiotic stress, it leads to significant changes in the metabolic state, which is usually characterized by impaired growth and induced or inhibited expression of various genes [30]. Lipase can dissolve the lipid bonds in hydrolyzed lipids, which plays a key role in lipid turnover in higher plants and participates in lipid oxidation events and activities such as reactions [31,32]. These results are consistent with the current physiological and biochemical indexes. After aging, the activities of antioxidant enzymes (SOD, POD, and CAT), MDA, and Pro of OE-OsMYBAS1-1, OE-OsMYBAS1-2, WT, *osmybas1-1*, and *osmybas1-2* were significantly higher than those of the CK. In comparison, the activities of antioxidant enzymes (SOD, POD, and CAT), MDA, and Pro of *osmybas1-1* and *osmybas1-2* were significantly lower than those of WT, OE-OsMYBAS1-1, and OE-OsMYBAS1-2. These results suggest that when plants are under biological and abiotic stress, the activities of ROS and antioxidant enzymes in the body increase to enhance plant resistance, which was evidenced by the study about *AtMYBAS1* [33]. *OsALDH7*, from the *OsALDHs* gene family, can scavenge MDA [9]. As a homologous gene, the expression of *LOC_Os02g43280* (*OsALDH3*) increased under the regulation of *OsMYBAS1*, which may exercise the same function. *LOC_Os04g14680* (*OsAPX3*) increases POD activity and slows down rice leaf senescence through ROS signaling. Therefore, we speculate that *LOC_Os04g14680* (*OsAPX3*) enhances seed germination by increasing POD activity [10]. *LOC_Os03g03910* (*OsCATC*) is a catalase gene, and its expression was consistent with the CAT content measured in this experiment, indicating that it may increase CAT activity and enhance the germination of rice seeds. It can be seen that *OsMYBAS1* overexpression may enhance seed germination and have better environmental adaptability by regulating these DEGs to regulate carbohydrate and lipid metabolisms.

Exploration of the pathways involved in genes is of great significance for clarifying their regulatory mechanism. We conducted KEGG pathway enrichment analysis on the identified DEGS. The results showed that these DEGS are mainly involved in signal pathways such as the lipid metabolic process, glycophospholipid metabolism, starch and sucrose metabolism, phylpropanoid biosynthesis, carotenoid biosynthesis, and glycoxylate and dicarboxylate metabolism. MYB transcription factors trigger differential adjustments of the lipid metabolism process, with enhanced triacylglycerol accumulation, indicating that there is an internal relationship between them and the regulation mechanism of lipid

metabolism [34]. WRKY2/WRKY34 regulate GLUCOSE-6-PHOSPHATE/PHOSPHATE TRANSLOCATOR 1 and activate starch and/or fatty acid biosynthesis [35]. Glyceride is the main form of lipid in plants, and its carboxyl group is connected to the carboxyl ester of glycerol [36]. A variety of lipids in plants form the hydrophobic barrier of the cell membrane, which is very important for maintaining the integrity of cells and organelles. In addition, lipids are also stored in seeds in the form of chemical energy and act as signal molecules to regulate cell metabolism [4,37]. Studies have found that the phenylpropionic acid pathway in higher plants can produce lignin and flavonoid metabolites, which are regulated by R2R3-MYB transcription factors [38]. Under abiotic stress conditions (drought, heavy metals, salinity, high and low temperatures), the biosynthetic pathway of phenylpropionic acid is activated to produce various phenolic compounds, which improves the stress resistance of plants by removing harmful reactive oxygen species from seeds [39]. Dai et al. [40] found that in the phosphate environment, *OsMYB2P-1* may act as a Pi-dependent regulator to control the expression level of the Pi transporter in rice and strengthen the adaptability of rice to Pi stress. Abe et al. [41] found that under drought stress, both *AtMYC2* and *AtMYB2* proteins function as transcriptional activators in ABA-induced gene expression in plants, participating in the abscisic acid signaling pathway in Arabidopsis. It is well known that abscisic acid induces the formation of the biofilm system's protective membrane by regulating plant stomatal opening, decreasing leaf cell membrane permeability, and increasing the leaf-cell-soluble protein content to reduce the degree of membrane lipid peroxidation and protect plant cell membrane integrity to enhance the antioxidant capacity of plants under stress and in turn improve plant stress resistance [42,43]. Interestingly, *OsMYBAS1*, in this study, is a homologous gene of *OsMYB2P-1* and *AtMYC2*. Therefore, OsMYBAS1 may actively participate in the process of lipid metabolism in plants, resist oxidation conditions by maintaining the integrity of the rice cell membrane structure, and improve the germination of rice seeds. Moreover, these results are also consistent with the phenotype identification results and plant physiological analysis results. OSMYBAS1 plays an important positive regulatory role in rice seed germination. Combined with the GSEA enrichment analysis of the high/low-germination group, there were differences in the regulation of seed germination, endomembrane organization, photosystem II oxygen evolving complex, and other signal pathways between gene sets. So, there were differences in seed germination regulation and cell membrane component regulation between the high/low-germination gene sets. It is hypothesized that the DEGs identified by bioinformatics may be the key genes of rice that regulate its germination rate under stress conditions.

We identified 11 downstream transcription factors of OsMYBAS1, belonging to C2H2, WRKY, MYB, bZIP, CO-like, GRAS, ARR-B, and bHLH family transcription factors. Studies have proved that in Arabidopsis, the JMJ17–WRKY40 and HY5–ABI5 modules integrate light signals and ABA signals at various levels through fine transcriptional regulation to ensure that plants can better adapt to environmental changes during the seed germination and seedling stage [44]. Based on this, we speculate that *LOC_Os11g02530* (*OsWRKY40*) may perform the same function in rice seeds. Viana et al. [45] compared the transcriptomic changes in rice during cold stress and found that *LOC_Os01g40260* (*OsWRKY77*) was a potential negative regulator. Transgenic rice lines overexpressing *LOC_Os02g26430* (*OsWRKY42*) showed the phenotype of early leaf senescence, accumulation of ROS and hydrogen peroxide, and a decrease in the chlorophyll content [46]. In this study, the expression level of *LOC_Os01g40260* (*OsWRKY77*) and *LOC_Os02g26430* (*OsWRKY42*) decreased under the action of OsMYBAS1, and the trend after qRT-PCR validation was consistent with the RNA-Seq data. The function of the Os03g32220 transcription factor has not been reported. Combined with this study, it is speculated that the *LOC_Os03g32220* transcription factor regulates rice seed germination under the regulation of OsMYBAS1. In conclusion, the downstream transcription factors identified herein are actively involved in abiotic stress under the regulation of OsMYBAS1, jointly regulating the germination of rice seeds.

In conclusion, this study identified DEGs in rice using the transcriptome data and retrograde analysis of *OsMYBAS1* overexpression and wild-type lines. GO and KEGG enrichment analysis revealed that these DEGs are involved in intracellular energy metabolism and lipid metabolism and play a role in plant stress. In addition, GSEA enrichment analysis showed differences in pathways such as regulation of seed germination, endomembrane organization, photosystem II oxygen evolving complex, DNA replication, and so on. Our study preliminarily clarifies the molecular mechanism of *OsMYBAS1* regulating seed germination.

5. Conclusions

This study found that OsMYBAS1 plays a vital role in positively regulating rice seed germination under aging conditions by measuring germination traits and antioxidant enzyme activities, and some key pathways and candidate genes closely related to rice seed germination were screened. The current results suggest that differences in the germination of rice seeds may result from differences in the signaling pathways involved in the regulation of seed germination, endomembrane organization, and photosystem II oxygen evolving complex. This study provides a theoretical basis for further elucidation of the complex regulatory mechanisms of rice seed germination and the breeding of rice varieties with high seed germination. In future study, we will continue to mine downstream genes, determine their response elements, create further downstream target gene genetic transformation materials, and analyze the germination of their seeds under aging conditions.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12081955/s1, Table S1: The primer for qRT-PCR; Table S2: Depiction of GO functional and KEGG enrichment analysis; Table S3: Downstream transcription factors of OsMYBAS1.

Author Contributions: G.Z. and X.W. conceived and designed research. R.W. analyzed the data and wrote the manuscript. Y.D., Z.H., B.W. and Z.L. conducted experiments, C.L. responsible for rice seeds propagate. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Key Research and Development Program of Zhejiang Province (No. 2019C02013), the Science and Technology Planning Program of Quzhou (No. 2021K13), the Basic Public Welfare Research Program of Zhejiang Province (LGN22C130003), the General Scientific Research Program of Education Department of Zhejiang Province (No. 2044100005), the Talent Initiation Program of Zhejiang Agriculture and Forestry University (No. 2021FR036).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in the graphs and tables provided in the manuscript.

Conflicts of Interest: The authors have no conflict of interest to declare that are relevant to the content of this article.

References

1. El-Maarouf-Bouteau, H.; Mazuy, C.; Corbineau, F.; Bailly, C. DNA alteration and programmed cell death during ageing of sunflower seed. *J. Exp. Bot.* **2011**, *62*, 5003–5011. [CrossRef] [PubMed]
2. Yin, G.K.; Xin, X.; Song, C.; Chen, X.L.; Zhang, J.M.; Wu, S.H.; Li, R.F.; Liu, X.; Lu, X.X. Activity levels and expression of antioxidant enzymes in the ascorbate–Glutathione cycle in artificially aged rice seed. *Plant Physiol. Biochem.* **2014**, *80*, 1–9. [CrossRef] [PubMed]
3. Jeevan, K.; Rajendra, P.S.; Rintu, B.; Chakradhar, T. Seed birth to death: Dual functions of reactive oxygen species in seed physiology. *Ann. Bot.* **2015**, *116*, 663–668. [CrossRef] [PubMed]
4. Ewelina, R.; Malecka, A.; Ciereszko, I.; Staszak, A.M. Mitochondria Are Important Determinants of the Aging of Seeds. *Int. J. Mol. Sci.* **2019**, *20*, 1568. [CrossRef]

5. Yuan, Z.Y.; Fan, K.; Xia, L.F.; Ding, X.L.; Tian, L.; Zhang, W.Q.; He, H.Z.; Yu, S.B. Genetic Dissection of Seed Storability and Validation of Candidate Gene Associated with Antioxidant Capability in Rice (*Oryza sativa* L.). *Int. J. Mol. Sci.* **2019**, *20*, 4442. [CrossRef]
6. Huang, J.X.; Cai, M.H.; Long, Q.Z.; Liu, L.L.; Lin, Q.Y.; Jiang, L.; Chen, S.H.; Wan, J.M. OsLOX2, a rice type I lipoxygenase, confers opposite effects on seed germination and longevity. *Transgenic Res.* **2014**, *23*, 643–655. [CrossRef]
7. Xu, H.B.; Wei, Y.D.; Zhu, Y.S.; Lian, L.; Xie, H.G.; Cai, Q.H.; Chen, Q.S.; Lin, Z.P.; Wang, Z.H.; Xie, H.A.; et al. Antisense suppression of *LOX3* gene expression in rice endosperm enhances seed longevity. *Plant Biotechnol. J.* **2015**, *13*, 526–539. [CrossRef]
8. Finch-Savage, W.E.; Bassel, G.W. Seed vigour and crop establishment: Extending performance beyond adaptation. *J. Exp. Bot.* **2016**, *67*, 567–591. [CrossRef]
9. Shin, J.H.; Kim, S.R.; An, G. Rice aldehyde dehydrogenase 7 is needed for seed maturation and viability. *Plant Physiol.* **2009**, *149*, 905–915. [CrossRef]
10. Ribeiroa, C.W.; Korbesb, A.P.; Garighanb, J.A.; Jardim-Messederb, D.; Carvalhoc, F.E.L.; Sousac, R.H.V.; Caverzanb, A.; Teixeirab, F.K.; Silveirac, J.A.G.; Margis-Pinheiroa, M. Rice peroxisomal ascorbate peroxidase knockdown affects ROS signaling and triggers early leaf senescence. *Plant Sci.* **2017**, *263*, 55–65. [CrossRef]
11. Su, C.F.; Wang, Y.C.; Hsieh, T.H.; Lu, C.A.; Tseng, T.H.; Yu, S.M. A novel MYBS3-dependent pathway confers cold tolerance in rice. *Plant Physiol.* **2010**, *153*, 145–158. [CrossRef] [PubMed]
12. Fávero Peixoto-Junior, R.; Mara de Andrade, L.; dos Santos Brito, M.; Macedo Nobile, P.; Palma Boer Martins, A.; Domingues Carlin, S.; Vasconcelos Ribeiro, R.; Helena de Souza Goldman, M.; Felipe Nebó Carlos de Oliveira, J.; Vargas de Oliveira Figueira, A.; et al. Overexpression of ScMYBAS1 alternative splicing transcripts differentially impacts biomass accumulation and drought tolerance in rice transgenic plants. *PLoS ONE* **2018**, *13*, e0207534. [CrossRef] [PubMed]
13. Yang, A.; Dai, X.Y.; Zhang, W.H. A R2R3-type MYB gene, *OsMYB2*, is involved in salt, cold, and dehydration tolerance in rice. *J. Exp. Bot.* **2012**, *63*, 2541–2556. [CrossRef] [PubMed]
14. El-Kereamy, A.; Bi, Y.M.; Ranathunge, K.; Beatty, P.H.; Good, A.G.; Rothstein, S.J. The rice R2R3-MYB transcription factor OsMYB55 is involved in the tolerance to high temperature and modulates amino acid metabolism. *PLoS ONE* **2012**, *7*, e52030. [CrossRef]
15. Yan, H.; Pei, X.; Zhang, H.; Li, X.; Zhang, X.; Zhao, M.; Chiang, V.L.; Sederoff, R.R.; Zhao, X. MYB-mediated regulation of anthocyanin biosynthesis. *Int. J. Mol. Sci.* **2021**, *22*, 3103. [CrossRef]
16. Zhang, P.; Wang, R.; Yang, X.; Ju, Q.; Li, W.; Lü, S.; Tran, L.P.; Xu, J. The R2R3-MYB transcription factor AtMYB49 modulates salt tolerance in Arabidopsis by modulating the cuticle formation and antioxidant defence. *Plant Cell Environ.* **2020**, *43*, 1925–1943. [CrossRef]
17. Beathard, C.; Mooney, S.; Saharin, R.A.; Goyer, A.; Hellmann, H. Characterization of Arabidopsis thaliana R2R3 S23 MYB Transcription Factors as Novel Targets of the Ubiquitin Proteasome-Pathway and Regulators of Salt Stress and Abscisic Acid Response. *Front. Plant Sci.* **2021**, *12*, 629208. [CrossRef]
18. Chen, Y.S.; Ho, T.D.; Liu, L.H.; Lee, D.H.; Lee, C.H.; Chen, Y.R.; Lin, S.Y.; Lu, C.A.; Yu, S.M. Sugar starvation-regulated MYBS2 and 14-3-3 protein interactions enhance plant growth, stress tolerance, and grain weight in rice. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 21925–21935. [CrossRef]
19. Mehrotra, S.; Verma, S.; Kumar, S.; Kumari, S.; Mishra, B.N. Transcriptional regulation and signalling of cold stress response in plants: An overview of current understanding. *Environ. Exp. Bot.* **2020**, *180*, 104243. [CrossRef]
20. Zhu, N.; Cheng, S.F.; Liu, X.Y.; Du, H.; Dai, M.Q.; Zhou, D.X.; Yang, W.J.; Zhao, Y. The R2R3-type MYB gene *OsMYB91* has a function in coordinating plant growth and salt stress tolerance in rice. *Plant Sci.* **2015**, *236*, 146–156. [CrossRef]
21. Wang, X.M.; Wu, R.; Shen, T.S.; Li, Z.N.; Li, C.Y.; Wu, B.K.; Jiang, H.Y.; Zhao, G.W. An R2R3-MYB Transcription Factor OsMYBAS1 Promotes Seed Germination under Different Sowing Depths in Transgenic Rice. *Plants* **2022**, *11*, 139. [CrossRef] [PubMed]
22. Wang, Y.; Hu, J.; Qin, G.C.; Cui, H.W.; Wang, Q.T. Salicylic acid analogues with biological activity may induce chilling tolerance of maize (*Zea mays*) seeds. *Botany* **2012**, *90*, 845–855. [CrossRef]
23. Pinhero, R.G.; Rao, M.V.; Paliyath, G.; Murr, D.P.; Fletcher, R.A. Changes in Activities of Antioxidant Enzymes and Their Relationship to Genetic and Paclobutrazol-Induced Chilling Tolerance of Maize Seedlings. *Plant Physiol.* **1997**, *114*, 695–704. [CrossRef] [PubMed]
24. Li, Z.; Xu, J.G.; Gao, Y.; Wang, C.; Guo, G.Y.; Luo, Y.; Huang, Y.T.; Hu, W.M.; Sheteiwy, M.S.; Guan, Y.J.; et al. The Synergistic Priming Effect of Exogenous Salicylic Acid and H_2O_2 on Chilling Tolerance Enhancement during Maize (*Zea mays* L.) Seed Germination. *Front. Plant Sci.* **2017**, *8*, 1153. [CrossRef]
25. Zhang, X.H.; Shen, L.; Li, F.J.; Zhang, Y.X.; Meng, D.; Sheng, J. Up-regulating arginase contributes to amelioration of chilling stress and the antioxidant system in cherry tomato fruits. *J. Sci. Food Agric.* **2010**, *90*, 2195–2202. [CrossRef]
26. Ritchie, M.E.; Phipson, B.; Wu, D.; Hu, Y.F.; Law, C.W.; Shi, W.; Smyth, G.K. limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **2015**, *43*, e47. [CrossRef]
27. Robinson, M.D.; McCarthy, D.J.; Smyth, G.K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **2010**, *26*, 139–140. [CrossRef]
28. Yu, G.C.; Wang, L.G.; Han, Y.Y.; He, Q.Y. clusterProfiler: An R package for comparing biological themes among gene clusters. *OMICS J. Integr. Biol.* **2012**, *16*, 284–287. [CrossRef]

29. Pommerrenig, B.; Ludewig, F.; Cvetkovic, J.; Trentmann, O.; Klemens, P.A.W.; Neuhaus, H.E. In Concert: Orchestrated Changes in Carbohydrate Homeostasis Are Critical for Plant Abiotic Stress Tolerance. *Plant Cell physiol.* **2018**, *59*, 1290–1299. [CrossRef]
30. Gupta, A.K.; Kaur, N. Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants. *J. Biosci.* **2005**, *30*, 761–776. [CrossRef]
31. Kelly, A.A.; Feussner, I. Oil is on the agenda: Lipid turnover in higher plants. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2016**, *1861*, 1253–1268. [CrossRef] [PubMed]
32. Alché, J.D.D. A concise appraisal of lipid oxidation and lipoxidation in higher plants. *Redox Biol.* **2019**, *23*, 101136. [CrossRef] [PubMed]
33. Kiran, U.; Abdin, M.Z. Computational predictions of common transcription factor binding sites on the genes of proline metabolism in plants. *Bioinformation* **2012**, *8*, 886–890. [CrossRef] [PubMed]
34. Xing, G.L.; Li, J.Y.; Li, W.L.; Lam, S.M.; Yuan, H.L.; Shui, G.H.; Yang, J.S. AP2/ERF and R2R3-MYB family transcription factors: Potential associations between temperature stress and lipid metabolism in *Auxenochlorella protothecoides*. *Biotechnol. Biofuels* **2021**, *14*, 22. [CrossRef]
35. Zheng, Y.P.; Deng, X.X.; Qu, A.L.; Zhang, M.M.; Tao, Y.; Yang, L.Y.; Liu, Y.D.; Xu, J.; Zhang, S.Q. Regulation of pollen lipid body biogenesis by MAP kinases and downstream WRKY transcription factors in Arabidopsis. *PLoS Genet.* **2018**, *14*, e1007880. [CrossRef]
36. Chapman, K.D.; Ohlrogge, J.B. Compartmentation of triacylglycerol accumulation in plants. *J. Biol. Chem.* **2012**, *287*, 2288–2294. [CrossRef]
37. Ohlrogge, J.; Browse, J. Lipid biosynthesis. *Plant Cell* **1995**, *7*, 957–970.
38. Ma, D.; Constabel, C.P. MYB Repressors as Regulators of Phenylpropanoid Metabolism in Plants. *Trends Plant Sci.* **2019**, *24*, 275–289. [CrossRef]
39. Sharma, A.; Shahzad, B.; Rehman, A.; Bhardwaj, R.; Landi, M.; Zheng, B. Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants under Abiotic Stress. *Molecules* **2019**, *24*, 2452. [CrossRef]
40. Dai, X.Y.; Wang, Y.Y.; Yang, A.; Zhang, W.H. *OsMYB2P-1*, an R2R3 MYB transcription factor, is involved in the regulation of phosphate-starvation responses and root architecture in rice. *Plant Physiol.* **2012**, *159*, 169–183. [CrossRef]
41. Abe, H.; Urao, T.; Ito, T.; Seki, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell* **2003**, *15*, 63–78. [CrossRef] [PubMed]
42. Chen, K.; Li, G.J.; Bressan, R.A.; Song, C.P.; Zhu, J.K.; Zhao, Y. Abscisic acid dynamics, signaling, and functions in plants. *J. Integr. Plant Biol.* **2020**, *62*, 25–54. [CrossRef]
43. Hsu, P.K.; Dubeaux, G.; Takahashi, Y.; Schroeder, J.I. Signaling mechanisms in abscisic acid-mediated stomatal closure. *Plant J.* **2021**, *105*, 307–321. [CrossRef]
44. Wang, T.J.; Huang, S.Z.; Zhang, A.; Guo, P.; Liu, Y.T.; Xu, C.M.; Cong, W.X.; Liu, B.; Zheng, Y.X. JMJ17-WRKY40 and HY5-ABI5 modules regulate the expression of ABA-responsive genes in Arabidopsis. *New Phytol.* **2021**, *230*, 567–584. [CrossRef] [PubMed]
45. Viana, V.E.; Maia, L.; Busanello, C.; Pegoraro, C.; Oliveira, A. When rice gets the chills: Comparative transcriptome profiling at germination shows WRKY transcription factor responses. *Plant Biol.* **2021**, *23*, 100–112. [CrossRef] [PubMed]
46. Han, M.H.; Kim, C.Y.; Lee, J.; Lee, S.K.; Jeon, J.S. *OsWRKY42* represses *OsMT1d* and induces reactive oxygen species and leaf senescence in rice. *Mol. Cells* **2014**, *37*, 532–539. [CrossRef] [PubMed]