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Overexpression of *NB-LRR* Gene *AtRPM1*(*D505V*) Improved Drought and Salt Resistance and Decreased Cold Tolerance in Transgenic Rice

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Abstract: Abiotic stimuli severely restrict the growth and development of plants, resulting in massive losses in the quality and yield of crops. Exploring genes that can improve crop tolerance to abiotic stress is important. In a previous study, we found that overexpression of the *Arabidopsis nucleotide-binding domain leucine-rich repeat* (*NB-LRR*) gene *AtRPM1*(*D505V*) increased disease resistance in rice. In this research, we found that *AtRPM1*(*D505V*) transgenic plants were more sensitive to abscisic acid (ABA) than wild type (WT) plants. Abiotic-stress resistance in *AtRPM1*(*D505V*) transgenic plants was investigated. We found that *AtRPM1*(*D505V*) transgenic plants exhibited improved resistance to drought and salt stress; the phonotype and survival rates of transgenic rice were better than WT plants. The expression of stress responsive genes including *OsDREB2A*, *OsDREB2B*, *OsRD22*, and *OsRD29A* were significantly upregulated in *AtRPM1*(*D505V*) overexpressed plants than in WT plants. Moreover, the activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were significantly increased in *AtRPM1*(*D505V*) overexpressed plants than in WT plants under drought and salt stress. Under cold stress, the expression of stress responsive genes and the activities of antioxidant enzymes in *AtRPM1*(*D505V*) transgenic plants were significantly lower than in WT plants. Our research demonstrated that *AtRPM1*(*D505V*) confers drought and salt resistance to transgenic rice. Therefore, *AtRPM1*(*D505V*) could act as a potential candidate gene to cultivate drought- and salt-tolerant plants.

Keywords: *AtRPM1*(*D505V*); abscisic acid; abiotic stress; transgenic rice

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1. Introduction

Rice (*Oryza sativa* L.) is an important worldwide cultivated crop, and it has made a remarkable contribution to the food safety of the world. However, its production in many regions is threatened by various abiotic stimuli, such as heat, drought, salinity, and cold. Drought stress is the leading adverse factor that inhibits crop quality and production. Statistics showed that drought caused over USD 30 billion in crop production losses in the last decade [1]. Because the growth and development of rice requires a large amount of water, 48–94% of economic losses from rice production were due to drought stress. At the grain-filling stage, about 60% of economic losses in rice were related to water deficient [2]. Globally, over 40 million hectares of farmland and 25–30% of crop yield is threatened by salt stress [3]. Rice is a salt-sensitive crop. Salt stress negatively affects rice growth at the early vegetative and reproductive stages, increasing the mortality rate of leaves and decreasing

the panicle sterility, respectively [4]. Cold stress is often encountered during the whole life cycle of rice, including the germinating, booting, and flowering stages [5]. The root growth and development of rice were decreased, ranging from 2% to 87%, under cold stress [6]. Therefore, improving the tolerance of rice to abiotic stress is important for ensuring stable yields and food security.

During their evolution, plants have obtained a highly plastic and complicated system to adapt to abiotic stress. A large number of genes are related to plant responses and tolerance to abiotic stress. Transcription factors (TFs) can control the expression level of downstream genes which can then participate in the response to diverse abiotic stress in plants [7]. The NAC TF family has numerous members and features the presence of an NAC domain. NAC TFs play important roles in the responses of plants to biotic and abiotic stressors [8]. For example, expression of *OsNAC045* was induced by drought, salt, cold, and ABA treatments. Drought- and salt-stress resistance were increased in *OsNAC045* transgenic rice [9]. The WRKY TFs can regulate plant growth and the development of plants, and they also participate in plant abiotic-stress resistance [10]. Overexpression of *TaWRKY2* increased drought-stress resistance in wheat, and the expression of dehydration-responsive element-binding genes (*DREBs*) were significantly upregulated in transgenic plants [11]. Ectopic expression of *GmWRKY16* in *Arabidopsis* improved resistance to drought and salt stress, and the expression of dehydration-responsive gene *RD22* and desiccation-responsive gene *RD29A* were upregulated in *GmWRKY16* transgenic plants [12]. *OsWRKY87* has been located in the nucleus of plant cells, and transgenic rice overexpression of *OsWRKY87* significantly enhanced drought and salt tolerance [13].

With the rapid development of genetic transformation methods, it is feasible to enhance abiotic-stress resistance in rice by using genetic engineering. For example, overexpression of *Cajanus cajan CDR* (*CcCDR*) in rice significantly improved tolerance to drought, salt, and cold stress, and the transgenic plants were more hypersensitive to ABA and had lower seed germination rates than WT plants. Moreover, the expression of ABA signaling–related genes was significantly upregulated in *CcCDR* transgenic plants than in WT plants under stress conditions [14]. Cytochrome P450 genes are related to the production of various secondary metabolites and participate in stress responses in plants. Overexpression of *Arabidopsis* cytochrome P450 gene *AtCYP78A7* in rice enhanced drought tolerance and increased seed size [15]. Overexpression of *GmNAC20* in rice enhanced salt- and cold-stress resistance, probably due to upregulating the expression of abiotic-stress-related genes. In addition, the expression of genes involved in auxin signaling was also upregulated in *GmNAC20* overexpressing plants, which induced the formation of lateral roots in transgenic plants [16].

The cross-talk signaling pathways in plants between biotic and abiotic stress are well-documented, and the stress hormones ABA, salicylic acid (SA), MeJA, and ethylene synergistically or antagonistically regulate the signaling pathways associated with biotic and abiotic stress [17,18]. In rice, many genes participate in biotic and abiotic stress responses. The transcription of *OsWRKY11* was induced by pathogens, heat, and drought stress, and ectopic expression of *OsWRKY11* enhanced heat and drought tolerance [19]. In addition, ectopic expression of *OsWRKY11* increased the expression of defense-related genes, which in turn conferred resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *Oryzae* (*Xoo*), one of the major bacterial diseases in rice [20]. *OsWRKY45-1* isolated from *japonica* rice positively regulates resistance to *Magnaporthe oryzae* and negatively regulates resistance to *Xoo*, cold, and drought stress. *OsWRKY45-2* isolated from *indica* rice positively regulates resistance to *Xoo* and negatively regulates resistance to salt, cold, and drought stress [21,22].

At present, research on plant abiotic-stress resistance mainly focuses on TFs, and studies on the involvement of disease resistance genes in abiotic-stress resistance are rarely reported. *Arabidopsis NB-LRR* gene-based *resistance to Pseudomonas syringae* pv. *maculicola* 1 (*RPM1*) is involved in the disease response. AtRPM1 recognizes the *P. syringae* effectors AvrRpm1 and AvrB through the guarded protein RIN4 [23]. The activation of AtRPM1 triggers downstream signal transductions, including the accumulation of reactive oxygen species (ROS), the influx of Ca^{2+}, and stomata closure [24]. *AtRPM1*(*D505V*) is autoactive

in *rin4* knock out mutant, and its autoactivation can induce a hypersensitive response when transiently expressed in *Nicotiana benthamiana* [25]. We previously reported that overexpression of *AtRPM1*(*D505V*) in rice confers broad-spectrum disease resistance [26], while the functions of *AtRPM1*(*D505V*) in response to abiotic stress remains unclear. In this study, the abiotic-stress tolerance of *AtRPM1*(*D505V*) transgenic rice plants was investigated with a physiological index and gene expression analysis.

2. Materials and Methods

2.1. Plant Growth Conditions

The rice variety Nipponbare (*Oryza sativa* ssp. *Japonica*) was used throughout the study; rice seeds were surface-sterilized and germinated in a nursery tray with vermiculite. Seedlings with similar shoot lengths were cultured into plastic pots (10 cm in diameter and 8 cm in depth) filled with the same weight of vermiculite, and then grown in a greenhouse (28/25 °C, 14/10 h, 10,000 Lux of light intensity, and 60% relative humidity).

2.2. Generation of Transgenic Rice

AtRPM1(*D505V*) was cloned into the expression vector pCAMBIA1380 with a *Zea may* ubiquitin promoter, and the resulting overexpression vector was transformed into the *Agrobacterium tumefaciens* (*EHA105*). The recombinant strain was transformed into Nipponbare according to the *Agrobacterium*-mediated transformation [27]. Two overexpression lines (#D and #E) were used for subsequent studies.

2.3. Abiotic Stress Treatments

For ABA stress treatment, three-day-old seedlings gronw in 1/2 MS medium were transferred to 1/2 MS medium supplemented with 3 μM ABA. Shoot and root lengths were measured and analyzed after ten days of treatment.

For drought-stress treatment, four-week-old plants were deprived of water until one group of plants became wilted, the surviving seedlings were recorded and photographed after rewatered for three days.

For salt-stress treatment, three-day-old seedlings in 1/2 MS medium were transferred to 1/2 MS medium supplemented with 200 mM NaCl. Shoot and root length were measured and analyzed after ten days of treatment. Furthermore, four-week-old plants were irrigated with 200 mM NaCl for ten days; phenotypes were recorded and photographed after recovery with water for three days.

For cold-stress treatment, four-week-old plants were transferred to a growth chamber with a cycle of 14 h light and 10 h dark at 4 °C for two weeks; the survival rates were recorded after recovery at 28 °C for three days.

Twenty plants of each line were used with three independent biological replicates.

2.4. Analysis of Water Loss Rate and Relative Water Content

Water loss rates of detached leaves (the second leaf) from WT plants and transgenic plants were analyzed with the previously reported protocol [28]. Plant RWC was measured after drought treatment that lasted three days and was calculated with the following formula: RWC = (fresh weight − dry weight)/(saturated weight − dry weight) × 100%. Briefly, twenty leaves from WT plants or transgenic plants were weighed to obtain the fresh weight and then soaked in deionized water for 4 h to obtain the saturated weight. Finally, the saturated leaves were dried for 24 h at 75 °C to obtain the dry weight.

2.5. Measurement of Electrolyte Leakage

Relative electrolyte leakage was measured with the method described previously [24]. Twenty leaves from WT plants or transgenic plants were detached and placed in deionized water and then shaken for 3 h; the conductivity was recorded as C1 with an ion-leakage meter (METTLER TOLEDO, Zurich, Switzerland). Next, the leaves were boiled for 15 min

and shaken for 1 h to cool thoroughly; the conductivity was recorded as C2. The relative electrolyte leakage was calculated as follows: (C1/C2) × 100%.

2.6. Measurement of CAT, SOD, and POD Activities

The activities of catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) were analyzed with the previously described method [29]. A total of 0.05 M potassium phosphate buffer (pH 7.0) was used to extract total protein; the suspension was centrifuged at 12,000× *g* at 4 °C for 10 min. The obtained supernatant was used to measurement the CAT, SOD, and POD activity using detection kits (Jiancheng Bioengineering Institute, Nanjing, China).

2.7. RNA Isolation and qRT-PCR

Total RNA was isolated from rice leaves with a TRIzol reagent (Invitrogen, Carlsbad, CA, USA). First-strand cDNA was generated with a cDNA Synthesis Kit (Vazyme, Nanjing, China) with 1μg of total RNA. Quantitative real time PCR (qRT-PCR) was conducted with Bio-Rad CFX96 (Bio-Rad, Hercules, CA, USA) with SYBR Green dye (Vazyme, Nanjing, China). PCR reactions were carried out with the following settings: 1 min incubation step at 95 °C, then 40 cycles of 10 s at 95 °C and 30 s at 65 °C. The rice *Actin* gene was chosen as the internal reference; the $2^{-\Delta\Delta CT}$ method was used for relative gene expression analysis [30]. Sequences of the primers used are listed in Table S1.

2.8. Statistical Analysis

All experiments were conducted with three independent biological replicates. Data were analyzed with GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). To perform the statistical analysis, statistical significance was analyzed using analysis of variance (ANOVA) and Duncan's multiple tests. Varied letters indicate significant differences ($p < 0.05$, one-way ANOVA).

3. Results

3.1. ABA Sensitivity Analysis of AtRPM1(D505V) Transgenic Plants

We overexpressed *AtRPM1*(*D505V*) in rice under the control of a *ZmUbi* promoter; transcription levels of *AtRPM1*(*D505V*) in different transgenic lines were analyzed with qRT-PCR (Figure S1). Two representative overexpression lines (#D and #E) were used for experiments.

ABA is an important plant hormone that plays a role in plant biotic and abiotic stress responses. Therefore, we analyzed the ABA sensitivity of *AtRPM1*(*D505V*) transgenic plants and WT plants. The growth between *AtRPM1*(*D505V*) transgenic plants and WT plants was similar when grown in medium without ABA (Figure 1A). After treatment with 3 μM ABA, the growth of *AtRPM1*(*D505V*) transgenic plants and WT plants was retarded (Figure 1B). Although *AtRPM1*(*D505V*) transgenic plants and WT plants had similar root lengths, the shoot lengths of transgenic plants were significantly shorter than the WT plants (Figure 1C,D). These results showed that the *AtRPM1*(*D505V*) transgenic plants exhibited stronger ABA sensitivity than WT plants at the seedling stage.

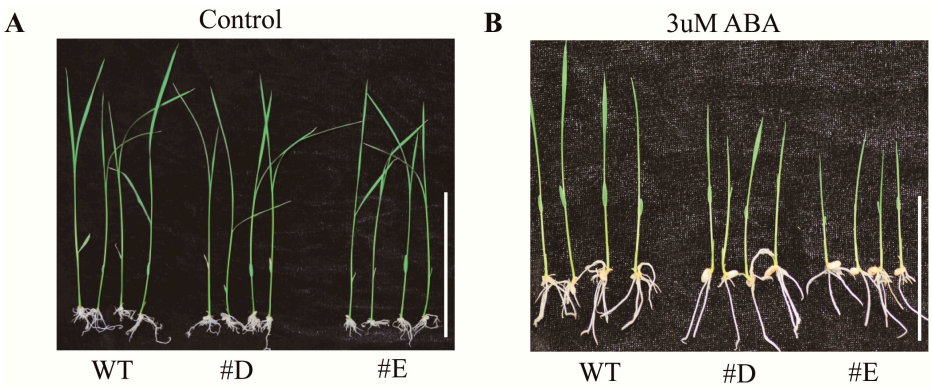


Figure 1. *Cont.*

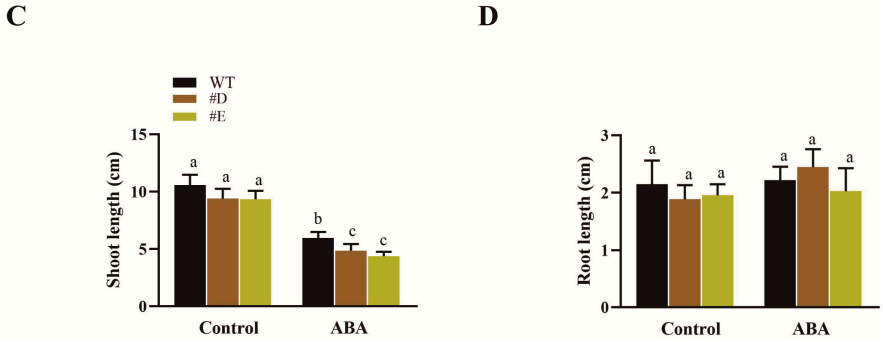


Figure 1. ABA sensitivity phenotypes of *AtRPM1*(*D505V*) transgenic plants. (**A**,**B**) The growth performance of transgenic plants and WT plants under normal and 3 μM ABA treatments, Scale bar = 5 cm, (**C**) shoot length, and (**D**) root length. Data are shown as means ± SD (n = 20). Different lowercase letters represent the significant difference ($p < 0.05$).

3.2. Drought Tolerance Analysis of AtRPM1(D505V) Transgenic Plants

To investigate the potential roles of *AtRPM1*(*D505V*) in abiotic-stress resistance, we first tested the drought tolerance of *AtRPM1*(*D505V*) transgenic plants at the four-leaf stage. Under normal conditions, *AtRPM1*(*D505V*) transgenic plants and WT plants showed no significant difference in growth (Figure 2A). After continuous drought treatment, WT plants showed the typical stress symptoms of leaf wilting and drying (Figure 2B). The survival rate of WT plants was 20%, while the survival rate of *AtRPM1*(*D505V*) transgenic lines were 41% and 60%, respectively (Figure 2C). In addition, the water loss rate of *AtRPM1*(*D505V*) transgenic plants was lower than WT plants, particularly when examined after 60 min (Figure 2D). The leaf relative water content (RWC) reflects the water holding capability of plants; the RWC of both *AtRPM1*(*D505V*) transgenic plants and WT plants were reduced under drought treatment, and the RWC in transgenic plants was higher than in WT plants (Figure 2E). *AtRPM1*(*D505V*) transgenic plants were more sensitive to ABA (Figure 1); drought stress could increase the accumulation of ABA and then the accumulated ABA could promote stomatal closure and reduce water loss. These results indicated that *AtRPM1*(*D505V*) transgenic plants had stronger viability and can better adapt to growth under drought stress, and this phenotype might be due to the alteration of the ABA signal pathway.

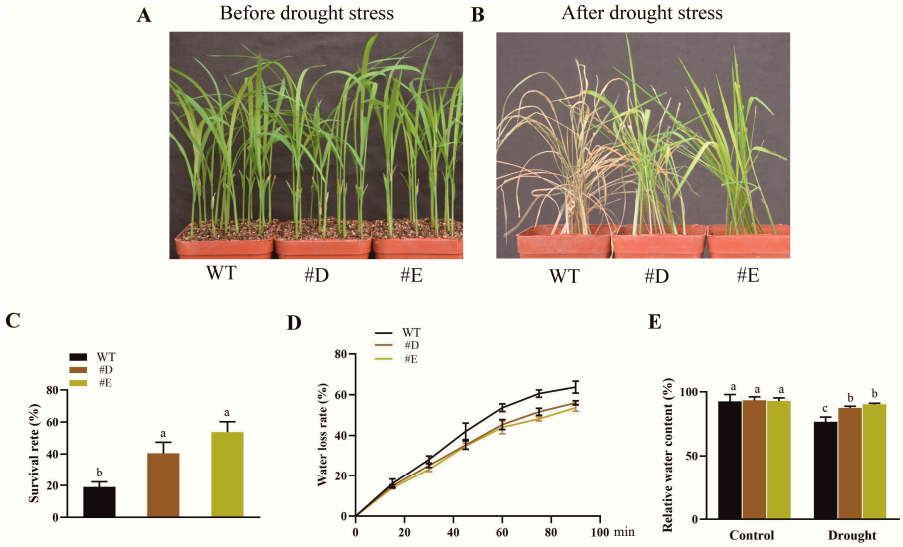


Figure 2. Performance of *AtRPM1*(*D505V*) transgenic plants under drought stress. (**A**,**B**) The growth performance of transgenic plants and WT plants under normal and drought treatments, (**C**) survival rates of transgenic plants and WT plants, (**D**) water loss rate, and (**E**) relative water content (RWC). Data are shown as means ± SD (n = 20). Different lowercase letters represent a significant difference ($p < 0.05$).

3.3. Salt Tolerance Analysis of AtRPM1(D505V) Transgenic Plants

Salt stress is one of the major adverse factors restricting plant growth and development. The tolerance of *AtRPM1*(*D505V*) transgenic plants to salt stress was subsequently investigated. Under normal conditions, the phenotypes between *AtRPM1*(*D505V*) transgenic plants and WT plants were similar. However, after ten days of 200 mM NaCl treatment, transgenic plants had longer shoot lengths than WT plants (Figure 3A–D). Additionally, *AtRPM1*(*D505V*) transgenic plants had higher survival rates than WT plants after being watered with 200 mM NaCl for ten days (Figure 3E). Approximately 40–45% of *AtRPM1*(*D505V*) transgenic plants survived, while only 15–20% of WT plants survived, and the leaf tissues of *AtRPM1*(*D505V*) transgenic plants were greener than WT plants (Figure 3F,G). These results confirmed that overexpression of *AtRPM1*(*D505V*) in rice improved salt-stress resistance.

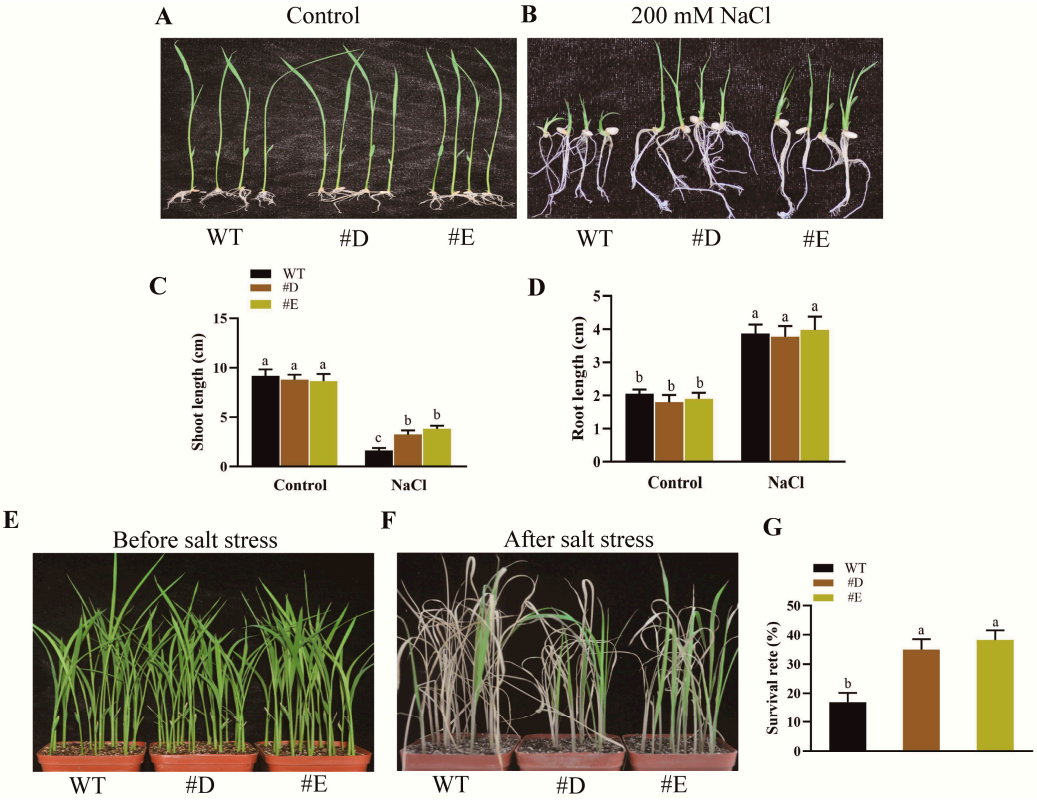


Figure 3. Performance of *AtRPM1*(*D505V*) transgenic plants against salt stress. (**A**,**B**) The growth performance of transgenic plants and WT plants under normal and 200 mM NaCl treatment, (**C**) shoot length, and (**D**) root length. (**E**,**F**) Phenotypes of transgenic plants and WT plants under normal irrigation and salt stress. (**G**) Survival rate of transgenic plants and WT plants. Data are shown as means ± SD (n = 20). Different lowercase letters represent a significant difference ($p < 0.05$).

3.4. Cold Tolerance Analysis of AtRPM1(D505V) Transgenic Plants

To investigate the cold tolerance of *AtRPM1*(*D505V*) transgenic plants, four-week-old transgenic plants and wild-type plants were exposed to low temperature (4 °C) stress. Before cold treatment, *AtRPM1*(*D505V*) transgenic plants had similar phenotypes compared to the WT plants (Figure 4A). However, the *AtRPM1*(*D505V*) transgenic plants showed severe wilting and died more quickly when compared with WT plants at 4 °C for two weeks (Figure 4B). After three days of recovery, WT plants had a higher survival rate than *AtRPM1*(*D505V*) transgenic plants (Figure 4C). The *AtRPM1*(*D505V*) transgenic plants and WT plants had similar relative electrolyte leakage levels under control conditions, but the relative electrolyte leakage of *AtRPM1*(*D505V*) transgenic plants was significant higher than WT plants after cold treatment (Figure 4D). These results indicated that *AtRPM1*(*D505V*) transgenic plants were more sensitive to cold stress.

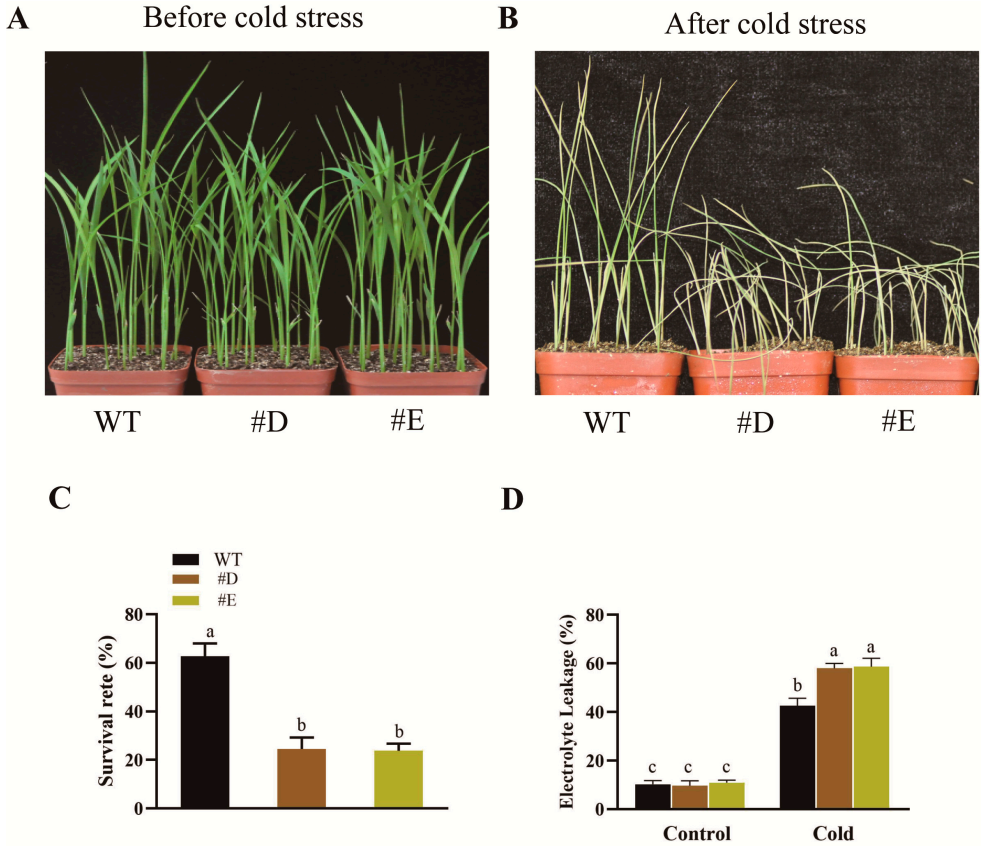
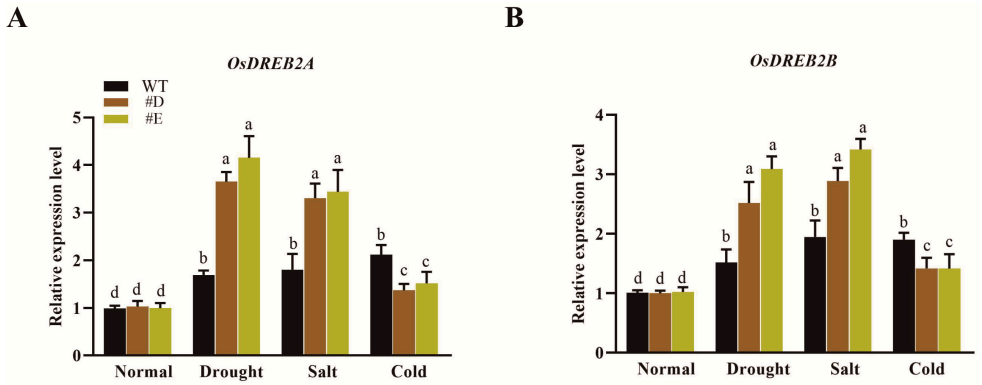


Figure 4. Performance of *AtRPM1*(*D505V*) transgenic plants under cold stress. (**A**,**B**) The growth performance of transgenic plants and WT plants under normal and cold treatment, (**C**) survival rate of transgenic plants and WT plants, and (**D**) relative electrolyte leakage. Data are shown as means ± SD (n = 20). Different lowercase letters represent a significant difference ($p < 0.05$).

3.5. Expression of Stress-Related Genes in AtRPM1(D505V) Transgenic Plants

To reveal the molecular mechanisms of *AtRPM1*(*D505V*) transgenic plants in response to abiotic stress, the expressions of *OsDREB2A*, *OsDREB2B*, *OsRD22*, and *OsRD29A* were analyzed with qRT-PCR. Under normal conditions, there was no difference in the expression of the tested genes between transgenic plants and WT plants. Under drought and salt treatments, the expressions of these genes were significantly upregulated in *AtRPM1*(*D505V*) transgenic plants compared to WT plants. However, when exposed to cold stress, the expression of *OsDREB2A*, *OsDREB2B*, *OsRD22*, and *OsRD29A* was significantly downregulated in *AtRPM1*(*D505V*) transgenic plants compared to WT plants (Figure 5A–D). These results implied that the enhanced drought- and salt-stress resistance of *AtRPM1*(*D505V*) transgenic plants may be attributable to the higher expression of stress-related genes.



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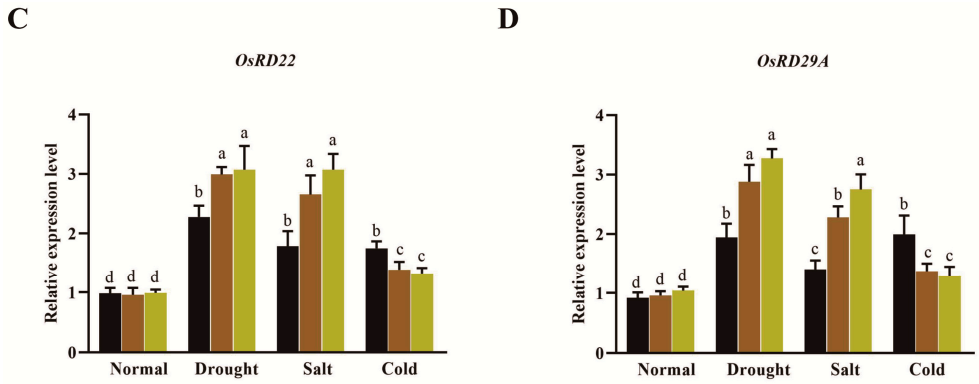

Figure 5. qRT-PCR analysis of stress-responsive genes under drought, salt, and cold stress in *AtRPM1*(*D505V*) transgenic plants. (**A**) Relative expression level of *OsDREB2A*, (**B**) relative expression level of *OsDREB2B*, (**C**) relative expression level of *OsRD22*, and (**D**) relative expression level of *OsRD29A*. Data are shown as means ± SD (n = 3). Different lowercase letters represent the significant difference ($p < 0.05$).

3.6. Activities of Antioxidant Enzymes in AtRPM1(D505V) Transgenic Plants

CAT, SOD, and POD are antioxidant enzymes, and play crucial roles in the homeostasis of ROS. Therefore, we measured the activities of CAT, SOD, and POD in the leaves of *AtRPM1(D505V)* transgenic plants and WT plants after drought, salt, and cold stress. We found that the activities of antioxidant enzymes were similar between the *AtRPM1*(*D505V*) transgenic plants and WT plants under normal conditions. Drought and salt stress enhanced the activities of the antioxidant enzymes and the activities of CAT, SOD, and POD in *AtRPM1*(*D505V*) transgenic plants; levels of activity for these enzymes were increased more significantly than in WT plants. The increased activities of antioxidant enzymes are beneficial for reducing oxidative damage in transgenic plants (Figure 6A–C). However, after cold-stress treatment, the activities of CAT, SOD, and POD in *AtRPM1*(*D505V*) transgenic plants were significantly lower than WT plants (Figure 6A–C), which was consistent with the greater sensitivity to cold stress of transgenic plants (Figure 4B).

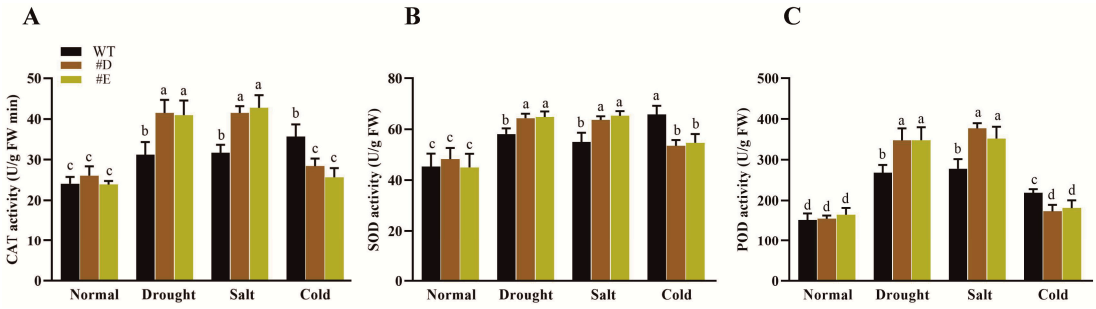

Figure 6. Evaluation of antioxidant enzymes activities in *AtRPM1*(*D505V*) transgenic plants. (**A**) CAT, (**B**) SOD, and (**C**) POD. Data are shown as means ± SD (n = 3). Different lowercase letters represent a significant difference ($p < 0.05$).

4. Discussion

Plants are often threatened by various biotic and abiotic stressors during their life cycle. Therefore, plants have evolved complex systems to adapt to external environmental changes. In the last two decades, more and more studies have indicated that plants can coordinate their responses to biotic and abiotic stress. In *Gossypium hirsutum*, the transcript of *GhMYB36* was induced with polyethylene glycol (PEG), ABA, and *Verticillium dahlia* infection. Overexpression of *GhMYB36* in *Arabidopsis* and cotton improved drought tolerance and *Verticillium* wilt resistance by enhancing the expression of *PR1* [31]. *GmERF3* was isolated from soybean, and overexpression of *GmERF3* in tobacco induced the expression of some *PR* genes, increased resistance to tobacco mosaic virus (TMV), *Alternaria alternata*, and

Ralstonia solanacearum, and further increased tolerance to drought and salt stress [32]. The expression of *OsWRKY30* was rapidly induced with SA and MeJA treatment; constitutive expression of *OsWRKY30* increased resistance to the fungal pathogen *Magnaporthe grisea* and to drought stress [33,34]. AtRPS2 is a NB-LRR immune receptor, and overexpression of *AtRPS2* in rice induced the expression of stress-related genes and enhanced antioxidant capacity, thereby improving the drought- and salt-stress resistance of transgenic plants [35].

In a previous study, we found that overexpression of the *Arabidopsis NB-LRR* gene *AtRPM1*(*D505V*) in rice significantly improved biotic-stress resistance [26]. In this study, the abiotic-stress resistance of rice lines with an overexpression of *AtRPM1*(*D505V*) was further investigated. Drought and salt stress are the main adverse factors threatening the survival of plants. Previous studies have shown that drought and salt stress can cause water stress, ionic stress, or osmotic stress [36,37]. Drought and salt tolerance assays indicated that *AtRPM1*(*D505V*) transgenic plants had higher survival rates and lower water loss rates in leaves compared with WT plants (Figures 3 and 4). The expressions of the stress-related genes *OsDREB2A*, *OsDREB2B*, *OsRD22*, and *OsRD29A* were upregulated in *AtRPM1*(*D505V*) transgenic plants. DREBs belong to the AP2/ERF transcription factor family and play important roles in abiotic-stress tolerance [38]. Overexpression of *OsDREB2A* in soybean improved salt-stress tolerance [39]. Similarly, overexpression of *OsDREB2B* in rice significantly improved drought-stress tolerance [40]. The expression of *OsRD22* was associated with stress responses, and overexpression of rat neuronal NO synthase (nNOS) in rice increased the expression of *OsRD22*, and the drought- and salt-stress tolerance was improved in nNOS transgenic plants [41]. *RD29A* is considered as an indicator of stress tolerance, and the induction of *RD29A* is associated with improved salt tolerance in transgenic plants [42]. Therefore, *AtRPM1*(*D505V*) contributes to drought- and salt-stress tolerance in rice, possibly through the induction of a series of stress-related genes.

ROS are known to be involved in signal transduction and physiological processes in plants [43,44]. Abiotic stressors, such as drought, salt, and cold, often cause ROS accumulation, but a high content of ROS often leads to cell membrane damage and can even cause death [45,46]. It is believed that the antioxidant enzymes, including CAT, SOD, and POD, play a crucial role in regulating the homeostasis of ROS [47]. Overexpression of *VvASMT1* in *Nicotiana benthamiana* increased the activity of antioxidant enzymes (CAT, SOD, and POD) and decreased the content of ROS, which in turn improved the salt- and osmotic-stress tolerance of the transgenic plants [48]. Overexpression of a pea *SOD* gene in tobacco improved photosynthetic capacity, and the higher photosynthetic rates of transgenic plants reduced oxidative damage, thus increasing their tolerance to cold stress [49]. Overexpression of *ThNAC7* in *Arabidopsis* improved the activity of SOD and POD, increased ROS scavenging capabilities, and enhanced tolerance to salt and osmotic stress [50]. In this study, the activities of CAT, SOD, and POD in *AtRPM1*(*D505V*) transgenic plants were analyzed. We found that the activities of antioxidant enzymes in transgenic plants and wild-type plants did not show obvious differences under normal conditions. Under drought and salt stress, the activities of antioxidant enzymes in transgenic plants and wild-type plants were increased, but the activities of the enzymes in transgenic plants were significantly higher than in WT plants (Figure 6A–C). These results suggested that the increased activities of SOD, POD, and CAT are beneficial for enhancing drought- and salt-stress tolerance in *AtRPM1*(*D505V*) transgenic plants.

Plant growth and development are also greatly impacted by cold stress, and low temperatures can affect plant respiration, cell membrane fluidity, and cytoskeleton integrity and can ultimately lead to plant death [51]. Signaling pathways involved in drought, salt, and cold stress can be antagonistic or synergistic [52,53]. *PsHAT5* is a transcription factor isolated from *Pyrus sinkiangensis*; overexpression of *PsHAT5* improved drought and salt tolerance but also increased cold sensitivity in tomatoes [54]. *SikCOR413PM1* is a member of the *COR* family in *Saussurea involucrate*; the expression of *SikCOR413PM1* is significantly increased with drought and cold stress. Furthermore, overexpression of *SikCOR413PM1* improved drought and cold tolerance in cotton [52]. In this study, we found

that *AtRPM1*(*D505V*) transgenic plants were more sensitive to cold stress (Figure 4B), and the relative electrolyte leakage was dramatically increased in *AtRPM1*(*D505V*) transgenic plants than in WT plants (Figure 4D). In addition, when the plants suffered from cold stress, the expression of stress-related genes and the activities of CAT, SOD, and POD were significantly lower in *AtRPM1*(*D505V*) transgenic plants than in WT plants (Figures 5 and 6). These results indicated that *AtRPM1*(*D505V*) positively contributes to drought- and salt-stress tolerance, while it negatively regulates cold-stress resistance in rice.

In summary, the results of this work indicated that overexpression of *AtRPM1*(*D505V*) improved drought- and salt-stress tolerance in transgenic rice, which may be due to a decreased water loss rate, higher expression of stress-related genes, and stronger activities of antioxidant enzymes. These results are helpful for understanding the biological functions of *NB-LRR* genes and also provide a candidate gene for improving drought- and salt-stress tolerance in rice and other crops.

5. Conclusions

Drought, salt, and cold stress are the main reasons for reduced crop yields. Plants have also developed complex regulatory mechanisms to overcome environmental changes. In this study, the abiotic-stress tolerance of *AtRPM1*(*D505V*) transgenic rice plants was investigated. Phenotypic observations and stress-related physiological and biochemical results suggested that *AtRPM1*(*D505V*) transgenic plants exhibited improved resistance to drought and salt stress but decreased tolerance to cold stress. Overall, our research demonstrated that *AtRPM1*(*D505V*) has potential application value for increasing the drought- and salt-stress tolerance of crops through genetic engineering.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14051050/s1, Figure S1: Relative expression of *AtRPM1(D505V)* in transgenic plants; Table S1: qRT-PCR primers used in this study.

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