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Overexpression of the zinc finger protein gene *OsZFP350* improves root development by increasing resistance to abiotic stress in rice

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The root system of rice is influenced by various environmental factors. However, how the root system responds to abiotic stress has not yet been fully understood. A zinc finger protein gene, *OsZFP350*, is exclusively expressed in the rice root, but its biological function needs to be investigated. Expression of *OsZFP350* was up-regulated by salt, drought and high temperature, indicating that it might be a regulator in response to abiotic stress in rice root. The primary root length, the number of adventitious and lateral roots was significantly increased in *OsZFP350* transgenic plants when compared to the wild-type. In addition, our results also show that the up-regulated *OsZFP350* could significantly increase the germination rate of seeds under abiotic stress, and attenuate the heat, salinity and drought stress during the development of rice roots. Based on these findings, it could be concluded that *OsZFP350* plays a positive role in the adaptability of rice roots to abiotic stress.

Key words: abiotic stress, *OsZFP350*, rice, root system, zinc finger protein

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Abbreviations: ABA, abscisic acid; JA, jasmonic acid; SA, salicylic acid; α-NAA, α-naphthylacetate; ZFPs, zinc finger proteins; ORF, open reading frame; DAG, day after germination; GA, gibberellic acid; qPCR, quantitative PCR reactions

INTRODUCTION

Root is an essential organ for the growth and development of rice, which plays a vital role in absorbing, transporting and storing water and nutrients. Therefore, it directly determines many agronomic traits, such as the yield, quality, stress resistance and wide adaptability of rice. Morphogenesis of the root system in rice is influenced by both, the endogenous genes and exogenous environmental stimulus. Mutation of those intrinsic genes would have mainly resulted in such a wide phenotype as shorter main root, decreased number of adventitious and lateral roots, no lateral root, shorter or even no root hairs (Hossain *et al*., 2017; Zhang *et al*., 2017; Zhang *et al*., 2018; Parry *et al*., 2018). Environmental factors affecting the root morphology mainly include the temperature (thermotropism), humidity (hydrotropism), gravity (gravitropism), light (phototropism), touch (thigmotropism), metal ions and other chemicals (chemotropism) (Zhao *et al*., 2015). In addition, endogenous hormones (auxins, etc.) also play key roles in the morphogenesis of rice roots, which function together to determine the size of the apical meristem in the root tips (Sun *et al*., 2018).

To date, over thirty genes relating to root traits in rice have been well characterized, such as auxin influx carrier gene *AUX1* (Dindas *et al*., 2018), root-crown formation gene *ARL1* (Coudert *et al*., 2010; Kitomi *et al*., 2011) and *WOX11* (Zhao *et al*., 2015; Zhou *et al*., 2017; Cheng *et al*., 2018), all of which participate in the morphogenesis of the main root, adventitious and lateral root and root hairs, respectively, through regulating either the phytohormone signaling or the uptake of inorganic salt. Actually, phytohormone signaling regulated by these genes is the major factor for the root morphogenesis during postembryonic development, in which cytokinins, together with other hormones, i.e. auxin and ethylene, suppress the root growth through inhibiting cell proliferation and elongation (Street *et al*., 2016a). In detail, the auxin importer AUX1 is a positive regulator of response to cytokinins (Parry *et al*., 2018), while the complex formed by two receptor-like kinases, Receptor-like kinase FERONIA (FER) and RPM1-induced protein kinase (RIPK), transmits Rapid Alkalinization Factor 1 (RALF1) peptide to inhibit root growth in Arabidopsis, which is a typical evidence of phytohormone interaction in the root (Street *et al*., 2016b). The AP2/ERF transcription factor CROWN ROOTLESS5 (CRL5) controls the crown root initiation through the induction of *OsRR1*, which is a type-A response regulator in cytokinin signaling (Kitomi *et al*., 2011). In addition, the *OsCKX4* gene controls rice crown formation by integrating cytokinin and auxin signaling (Gao *et al*., 2014), whereas *WOX11* is involved in the canopy development of roots (Cheng *et al*., 2018). Overexpression of *WOX11* accelerated cell differentiation in the root canopy, leading to early growth and production of the outer nodes of rice by affecting the auxin and cytokinin signaling transduction (Zhou *et al*., 2017). The interaction between WOX11 and an AP2/ERF protein ERF3 promotes the development of root crown through cytokinin signaling (Zhao *et al*., 2015).

External nutrients are also important factors in the development of rice roots, uptake of which is carried out by several transporters. The IRT1 (Boonyaves *et al*., 2016) and LsiL (Khan & Gupta, 2018) control the uptake and translocation of Fe^{2+} and Si^{2+} in rice roots. Overexpression of *IRT1* enhances the ability of rice roots to absorb Fe^{2+} from the soil. When the *LsiL* gene was deleted, the storage and transport of Si^{2+} was re-

duced in the rice roots. The SKC1 encodes a member of the HKT transporter family expressed in parenchyma cells around the vascular bundle of the xylem. This type of Na^+ selective transporter regulates K^+/Na^+ balance under salt stress *in vivo* (Kobayashi *et al*., 2017). In addition, the glucosamine-6-phosphate acetyltransferase is encoded by *OsGNA1* (Jiang *et al*., 2005), without which the rice seedlings did not grow at 25°C.

During the plant growth process, the response of roots to abiotic stress is reflected by the corresponding expression of stress-responsive genes. For instance, abscisic acid (ABA) or jasmonic acid (JA) is involved in regulating genes associated with drought and injury, i.e. *salT* and JA responsive genes which belong to the *LEA* family. It had been shown that JA significantly induced the increase of pathogen-associated protein (PR-1 and PR-10) and JIRs in roots (Gonzalez *et al*., 2017). The ABA can induce the transcript accumulation of *OsLEA3* but negatively affect the expression of *salT*, and JA negatively affects ABA-induced transcription of *OsLEA3*; while both, the ABA and JA can induce the transcription of *salT* (Duan and Cai, 2012). What's more, overexpression of the *OsERF71*, a transcription factor responsive to drought stress, altered the root structure and enhanced drought resistance of rice (Lee *et al*., 2016). A very recent study had shown that four 14-3-3 isoforms, prominently express in rice roots, have exhibited diverse expression patterns in the stress response to salt and drought (Zhang *et al*., 2017). *AtUSPL1*, mostly prevalent in the Arabidopsis root, is up-regulated as part of the ABA-mediated moisture stress response (Harshavardhan *et al*., 2014). Loss of function of AtUSPL1 increases the moisture stress tolerance, suppressing in turn the drought stress response in plants. It was also found that both, PEG and heavy metals can rapidly and distinctly induce expression of *OsGSTU3* and *OsGSTU4* (glutathione S transferase genes) in rice seedling roots, besides such hormones and growth regulators as salicylic acid (SA), JA and α-naphthylacetate (α-NAA). Moreover, antioxidants are rapidly induced in rice roots, indicating that redox signaling is also involved in the regulation of these stress responses (Moons, 2003).

The zinc finger proteins (ZFPs) have derived their name from the 'finger-like' zinc finger domain formed by Zn^{2+} and the surrounding conserved cysteine (Cys) or histidine (His) residues. The ZFPs were originally found in the transcription factor TFIIIA of *Xenopus oocytes*, the most abundant class of transcription factors in eukaryotes. They are widely involved in gene transcription, translation, cytoskeleton construction, mRNA trafficking, protein folding and chromatin remodeling (Imbeault *et al*., 2017; Patel *et al*., 2018). At present, the ZFPs found in higher plants mainly possess two major functions. One is involved in abiotic stresses, such as high salt and drought, and the other is a SUPERMAN-like protein, which is basicly involved in the development of flowers (Fu *et al*., 2017). Interestingly, overexpression of such genes can sometimes cause dwarf and other abnormal phenotypes in plants (Kazama *et al*., 2009). The protein encoded by the *OsZFP350* gene in rice has a molecular weight (MW) of 35 kDa and is therefore named OsZFP350, which is exclusively expressed in roots. Specifically, several members of the C2H2-type ZFPs in rice have also been shown to be involved in the responses to drought, salinity and oxidative stress (Patel *et al*., 2018). However, until now, there was no report on the involvement of ZFPs in the process of root morphogenesis yet. In this study, *OsZFP350* gene driven by CaM 35S promoter (*35S::OsZFP350*) was overexpressed in rice, and the effects on the primary root length and the number of adventitious and lateral roots in transgenic plants had been investigated. Our results demonstrate that overexpression of *OsZFP350* significantly increased the adaptability of rice roots to abiotic stress.

MATERIALS AND METHODS

Rice and cultivation. Rice (*Oryza sativa* L. cv Japonica, Nipponbare) was used for the construction of overexpressing plants in this experiment. The rice plants were grown in a greenhouse at 30°C and 70% humidity under a photoperiod of 16 h light/8 h dark.

Vector construction and genetic transformation. The binary expression vector pCAMBIA1301 containing the *GUS* reporter gene and hygromycin resistant genes was used to construct the overexpressing vector in this study. Total RNA was extracted from rice roots, and mRNA was purified from total RNA and reversely transcribed into the first strand cDNA as PCR templates. Specific primers (forward: 5'-CGGAATTCATGGATCCAGCAAGGTACTGG-3' and reverse: 5'-CCAAGCTTCTACTGTTCTTTTGGGGCTTCC-3', restriction sites are underlined) were used to amplify the full-length open reading frame (ORF) of the *OsZFP350* gene (Os05g0286100), which was then verified by sequencing. The fragment was double digested by *Eco*RI and *Hin*dIII and inserted into plasmid pCAMBIA1301 to construct the overexpression vector *35S::OsZFP350*. Rice callus was infested with *Agrobacterium EHA105* harboring the overexpressing vector, and then screened in 1/2 MS medium containing 75 mg L^{-1} hygromycin until differentiation. The transgenic seedlings were transferred into soil for continued growth. Positive plants were detected by PCR with amplification of the *GUS* gene and 35S promoter.

Stress treatment. For stress treatment, the wild-type rice seedlings of 15 d after germination (15 DAG) were selected. For testing resistance to various stress factors, NaCl (1.5 M), PEG6000 (20%) for mimicing drought, ABA (100 mg L^{-1}), IAA (100 mg L^{-1}), GA (100 μM), cold (4°C) and heat (42°C) stresses were performed for 36 h in 1/2 MS liquid medium. Temperature stress was carried out in a growth chamber, and seedlings under the other abiotic stresses were grown in a greenhouse. To verify the resistance ability of transgenic plants to abiotic stresses, the seeds of T2 generation and wild-type were germinated for 2 d in the dark, then 15 DAG seedlings under normal growth condition were placed either in the 1/2 MS solid medium with a final concentration of 0.15 M NaCl or in 1/2 MS liquid medium containing 20% PEG6000. After 36 h of cultivation at 30°C with a photoperiod of 16 h light/8 h darkness, pictures were taken of the roots of transgenic and wild-type seedlings and the parameters of the root system were analyzed with the ImageJ software (version 8.0). For extraction of total RNA, the roots of the seedlings were rinsed with deionized water and quickly cut into pieces either for subsequent experiments, or freezed by liquid N_2 and stored at –80°C until use.

Gene expression analysis. Total RNA of transgenic and wild-type roots and leaves was extracted and reversely transcribed into cDNA which was used as templates for quantitative PCR (qPCR). Primer sequences were: forward 5'-AACGCCCCTCTTGTCTCATC-3' and reverse 5'-AGTCCCTTCTTGATCGGCAC-3'. The qPCR reaction assay was performed in 20 μL with the final concentration of 200 μM for each primer and 1×SYBR

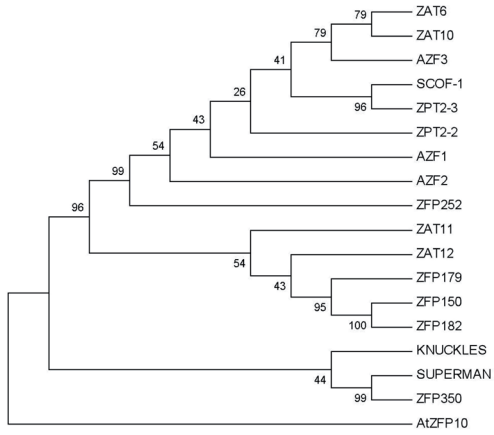

Figure 1. The phylogenetic relationship between OsZFP350 and its homologues.
Numbers on the nodes represent bootstrap values from 1000 replicates.

PremixEX Taq II (Takara, Japan). Reactions were carried out in a CFX96 thermocycler (Bio-Rad, USA). The PCR program was set as 95°C for 1 min, 95°C for 5 s, 60°C for 30 s, 40 cycles. A melting curve was applied to identify the specificity of the PCR products. The expression level was normalized by *Actin* as an internal control and then calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences of *OsLEA3, OsDREB1A* and *OsDREB1B* are the same as in Tang *et al*., (2019), and the primer for *OsHSP70* are: forward 5'-CTCCCTCCCAACTCGCTTGA-3' and reverse 5'-AACCCGTTCACAATAGATCCTC-3'.

Bioinformatics and statistical analysis. All of the experiments were repeated three times. SPSS software (version 13) and the Tukey test were used for statistical analysis and significant difference analysis, respectively. The BLASTP software was applied to search the non-redundant protein sequence databases to verify OsZFP350 homolog. Alignment of the OsZFP350 amino acid sequence for biological evolution analysis was performed using the MEGA 7.0 software.

RESULTS

Biological evolution analysis

Homologous proteins of OsZFP350 were found in rice and other species with the use of BLAST, including OsZFP252 (AAO46041.1), OsZFP182 (AAP42461.1), OsZFP179 (AAL76091.1) and OsZFP150 (AAP42460.1) in rice, ZAT6 (AT5G04340), ZAT10 (AT1G27730), ZAT11 (AT2G37430), ZAT12 (AT5G59820), AZF1 (AT5G67450), AZF2 (AT3G19580), AZF3 (AT5G43170), AtZFP10 (AT2G37740), KNUCKLES (AT5G14010), and SUPERMAN (AT3G23130) in Arabidopsis, ZPT2-2 (BAA05077.1), and ZPT2-3 (BAA05079.1) in Petunia and Soybean SCOF-1 (AAB39638.1). The analysis (Fig. 1) showed that the homologous proteins of OsZFP350 were widely distributed in higher plants, and this large family is obviously conserved in plants. Cluster analysis showed that OsZFP350 was closely related to KNUCKLES and SUPERMAN in *A. thaliana*. Plants overexpressing them behave normally, but the development of genitals is impaired in mutants. In detail, the KNUCKLES mediates *WUS* gene to inhibit the meristem determination in flowering (Sun *et al*., 2009). The *SUPERMAN* is a flower-specific gene that controls the stamen and carpel boundary (Prunet *et al*., 2017). Interestingly, AtZFP10, which is closely related to OsZFP350, is also involved in the floral development, and its transgenic plants have a dwarf phenotype (Dinkins *et al*., 2002). Other zinc finger transcription factors in these species are relatively distant from OsZFP350 but are basically related to abiotic stresses, such as heavy metals, high salinity and light. Thus, this study mainly focuses on the role of OsZFP350 in root morphology and resistance to abiotic stress in rice seedlings.

The *OsZFP350* gene had a spatio-temporal expression pattern and was up-regulated by abiotic stress

The spatial and temporal expression pattern of *OsZFP350* gene was performed by using mRNA extracted from different tissues of rice at four growing stages. Fig. 2a shows that the *OsZFP350* gene is mainly expressed in the roots. In the seedling stage, there was no significant difference in the expression level of *OsZFP350* between the root (RO) and the leaf blade (LB). When

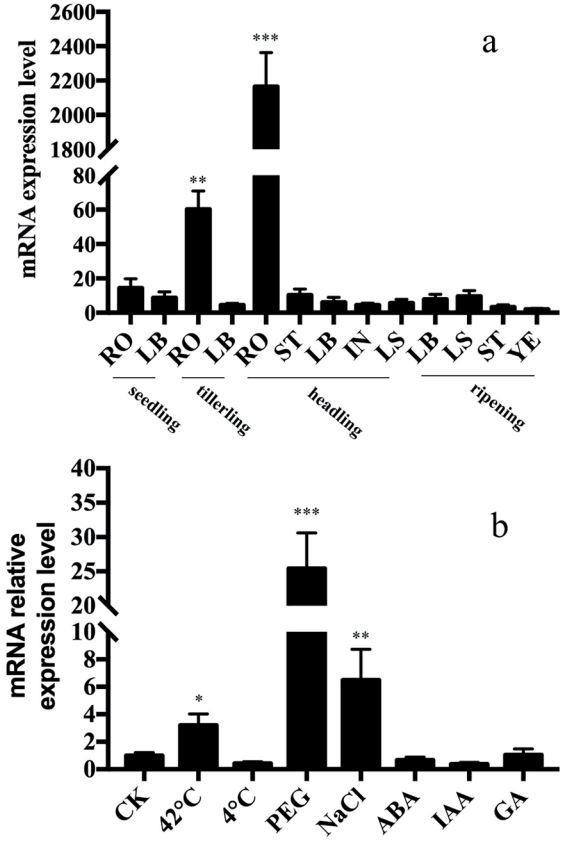

Figure 2. Spatial and temporal expression of the *OsZFP350* gene and its response to abiotic stress.
Expression of *OsZFP350* in various rice tissues and at the seedling, tillering, heading and ripening stage (**a**); expression of *OsZFP350* after treatment with different abiotic stresses of the wild-type plants (**b**). Each value is the mean of at least three biological replicates and the error bars indicate standard errors. Leaf blades (LB), leaf sheaths (LS), stem (ST), root (RO), inflorescence (IN) and young embryos (YE). Asterisk *, ** and *** stands for significant of difference at the level of 0.05, 0.01 and 0.005, respectively. Bar=1 cm.

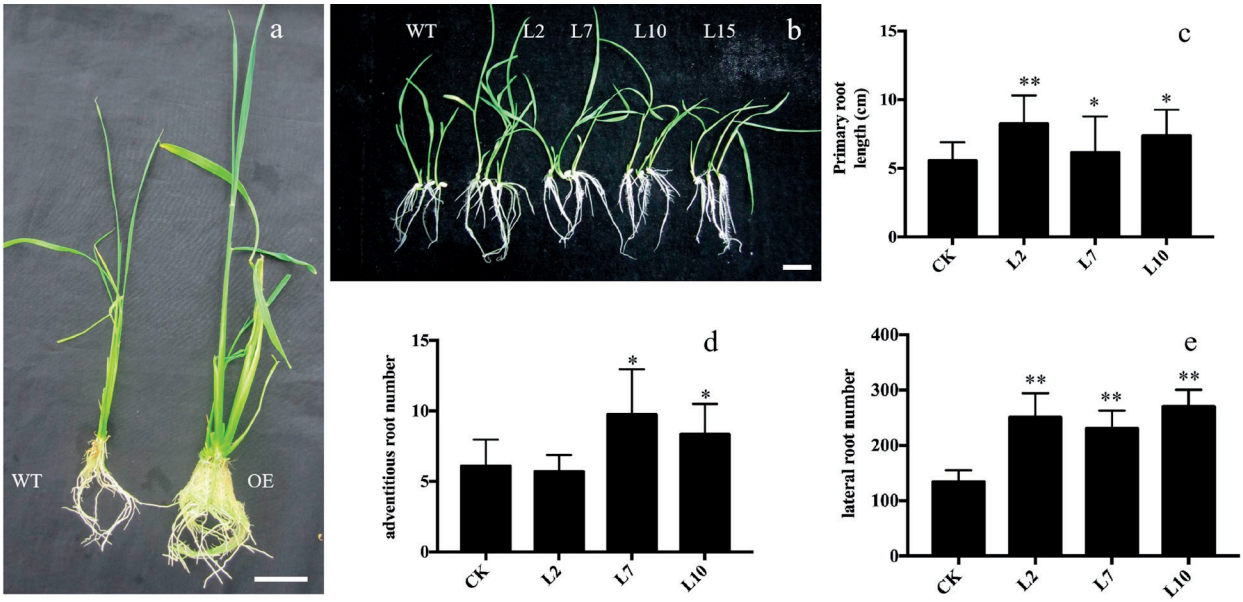

Figure 3. Overexpression effects of *OsZFP350* on morphology of the rice root.
The phenotype of wild-type and transgenic T0 generation plants (**a**); the phenotype of wild-type and four lines of T1 generation (**b**); primary root length (**c**), adventitious root number (**d**) and lateral root number (**e**) of T1 generation of *35S::OsZFP350* and wild-type seedlings of 7 DAG. Data from thirty independent transgenic seedlings were statistically analyzed. Asterisk * and ** stand for significant differences at the level of 0.05 and 0.01, respectively.

the rice enters into the tillering stage, the expression level of *OsZFP350* in the root increased significantly, and reached its peak at the heading stage. However, the expression in roots was significantly decreased after the heading stage. This indicates that *OsZFP350* has an obvious spatio-temporal and root-specific expression pattern. In a word, *OsZFP350* is a root specific expressing gene.

Next, the seedlings were treated with such series of stress as 0.15 M NaCl, 20% PEG6000, 100 mg L^{-1} IAA, 100 mg L^{-1} ABA, 100 μM GA, heat and cold stress for 36 h. The root mRNA was extracted and qPCR was used to analyze the expression of *OsZFP350* gene in roots under different stress conditions. As shown in Fig. 2b, the expression level of *OsZFP350* was significantly increased after treatment with PEG6000 and NaCl, as well as under heat stress. However, the expression level of *OsZFP350* after treatment with GA, IAA, ABA and cold stress was similar to that of control. These results indicate that high salt, drought and high temperature would up-regulate the expression of *OsZFP350*.

Effects of upregulated expression of *OsZFP350* on root growth and development

We transformed the *35S::OsZFP350* vector into rice calli by *Agrobacterium*-mediated transformation. Twenty-five positive transgenic lines were obtained and verified by PCR. It can be seen from Fig. 3a that the root volume and length of the T0 transgenic plants were significantly larger and longer than those of the wild-type at the seedling stage, and the biomass above ground was also significantly higher than the wild-type. Fifteen seperate seedlings of the T1 generation were randomly selected for OsZFP350 expression analysis by qPCR. It was found that the expression level of *OsZFP350* in the line of OE-2, OE-5, OE-7, OE-10, OE-15 was significantly increased by 5-20 times when compared to the wild-type (Fig. S1 at https://ojs.ptbioch.edu.pl/). We then selected OE-2, OE-7 and OE-10 for the following study. Firstly, seeds of the T1 generation of OE-2, OE-7 and OE-10 were germinated in 1/2 MS solid medium, and the root phenotype was quantified after the seedlings were cultivated vertically for 7 d. Then, positive transgenic seedlings (we renamed them as L2, L7 and L10, respectively, because of T1 generation) were verified again by PCR. We finally performed a statistical analysis of the primary root length, adventitious root and lateral root number of the seedlings (30 for the wild-type and 30 for each transgenic line in total). As shown in Fig. 3b, 3c and 3d respectively, the primary root length, the number of adventitious and lateral roots of *35S::OsZFP350* transgenic plants was significantly increased when compared to the wild type. These results reveled that *OsZFP350* might play a positive role in rice root morphology.

The adaptability of transgenic plants to abiotic stress

Since the expression of *OsZFP350* had a various degree of response to PEG, NaCl and heat stress, we therefore treated both, the seeds and transgenic plants of the T1 generation, with the identical stress to investigate the effects of *OsZFP350* overexpression on the growth and development of the root system in rice plants. Although all – NaCl, PEG and heat stress inhibited the growth of wild-type and transgenic seedlings when compared to the normal growth conditions, the stress resistance was significantly enhanced in *OsZFP350* overexpressing plants when compare to the wild-type. As shown in Fig. 4a, the germination rates of transgenic lines, when compared to wild-type, after treatment with PEG, NaCl, and heat stress were 91.3% to 85.7%, 90% to 65% and 100% to 50%, respectively. In addition, Fig. 4b shows that the stress treatment had inhibited the initial rooting of all of the sample plants. Although the length was shortened, the primary root of the transgenic plants was still significantly longer than that of the wild-type. As it is illustrated in Fig. 4c, under different stress conditions, the number of adventitious roots in the transgenic plants was significantly higher than in the case of the wild-type. What's more, it can be seen in Fig. 4d

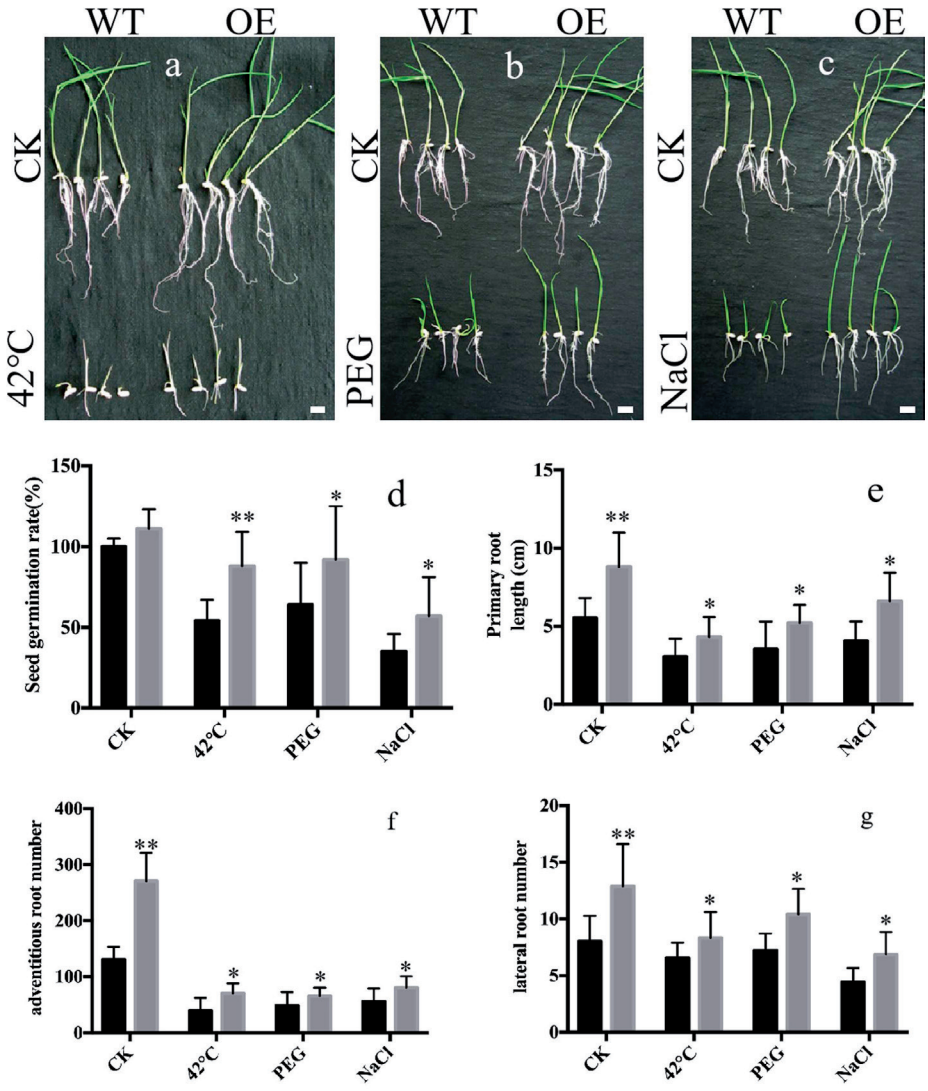

Figure 4. Response of *OsZFP350* overexpressing plants to abiotic stress.
The phenotype of wild-type and transgenic T2 plants (generation of L10) which were treated by 2 d with heat (**a**), PEG (**b**) and NaCl (**c**); seeds germination rates (**d**), primary root length (**e**), adventitious root number (**f**) and lateral root number (**g**) of T2 generation of *35S::OsZFP350* and wild-type seedlings of 7 DAG after 36 h of treatment with abiotic stress. Thirty independent transgenic seedlings were collected. The black (left) and gray (right) column represent the wild-type and overexpression line, respectively, under the same treatment. Asterisk * and ** stand for significant differences at the level of 0.05 and 0.01 in the same group, respectively. Bar=1 cm.

that the number of the lateral roots of the transgenic plants after different stress treatments was significantly higher than the wild-type. These results show that the up-regulated expression of *OsZFP350* could significantly increase the germination rate of seeds under abiotic stress, and weaken the influence of the heat, high salinity and drought stress on the growth and development of rice roots. In general, overexpression of *OsZFP350* significantly increased the adaptability of rice roots to high salt, drought and heat stress.

Stress-related genes in transgenic plants are up-regulated in regard to heat, drought and salinity stress

The morphological characteristics of transgenic rice roots had clearly revealed substantially improved resistance of *OsZFP350* overexpressing plants to drought and salt stress. It had been shown that overexpression of such genes could strengthen the tolerance of transgenic plants to abiotic stresses (Tang *et al.*, 2019). In order to elucidate the potential molecular mechanism underlying the increased stress tolerance, we finally compared the expression level of abiotic stress-related genes (*OsLEA3, OsDREB1A, OsDREB1B* and *OsHSP70*) of transgenic plants between normal growth and stress conditions. As is displayed in Fig. 5, when compared to normal growth conditions, the expression of *OsLEA3, OsDREB1A, OsDREB2A* and *OsHSP70* was constitutively elevated in the transgenic and wild-type plants under drought and salinity stress conditions. In addtion, the expression of these genes was significantly higher in the transgenic plants than the wild-type plants after treatment with heat, drought and salinity stress. Interestingly, we also found that under normal growth conditions, there was no significant difference in the expression of these stress-related genes between the transgenic and wild-type plants, which is consistant with the results listed by Tang and others (Tang *et al.*, 2019). These results show that stress-related genes in the *OsZFP350* transgenic plants are up-regulated in regards to abiotic stresses.

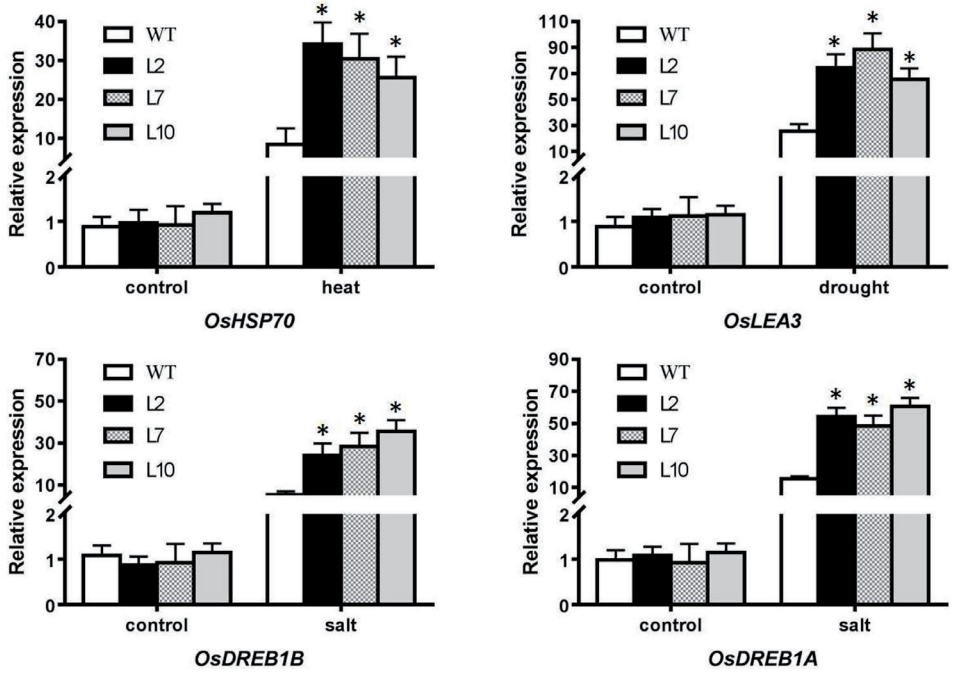

Figure 5. Overexpression of *OsZFP350* in rice enhances the expression level of stress-responsive genes in the transgenic plants. qRT-PCR analysis of *OsLEA3, OsDREB1A, OsDREB1B* and *OsHSP70* in the wild-type and transgenic rice plants between normal (control) and drought, salinity and heat stress. Actin was used as an internal control. Datas are means of n=6±S.D. from three biological replicates, and each with two technical replicates. The asterisks indicate significant differences at $P<0.01$.

DISCUSSION

To our knowledge, this study is the first to describe the biofunctional characterization of a rice zinc finger protein gene, *OsZFP350*, which plays a role in response to abiotic stress. During long period of interaction with environmental cues, rice plants have evolved series of antagonisms to cope with abiotic stresses. Increased soil salinity has a direct impact on the reduction of plant growth and crop yield, and it is therefore fundamental to understand the molecular mechanism underlying gene expression regulation under adverse environmental conditions. For example, RING E3 ligases were found to be involved in the transduction of abiotic stress signals, in which OsSIRP1 is a negative regulator of salinity stress tolerance mediated by ubiquitin-mediated protein degradation (Hwang *et al*., 2016). OsMADS25 confers rice salinity tolerance *via* ROS scavenging, and partially ABA signaling (Yu *et al*., 2015; G. Zhang *et al*., 2018; Xu *et al*., 2018). OsJAZ1 plays a role in regulating the drought resistance of rice partially *via* the ABA and JA pathways (Fu *et al*., 2017). WOX11 is involved in the control of crown root development through cytokinin signals and redox in rice (Zhao *et al*., 2015; Zhou *et al*., 2017; Cheng *et al*., 2018). Even in regard to ZFPs, OsZFP36 is shown to be an important regulator of the cross-talk between NADPH oxidase and ABA signaling (Zhang *et al*., 2014). Although our results had revealed that OsZFP350 plays a role in the anti-abiotic stress, the molecular mechanism remains elusive. As a transcription factor, OsZFP35 might directly regulate some certain genes being directly involved in a given abiotic stress (Fig. 5). Future work would aim to identify these genes as targets by yeast two-hybrid assay and/or cofactors of OsZFP350 through yeast single-hybrid or Chip-Seq methods.

The spatio-temporal expression pattern of *OsZFP350* indicated that this gene was mainly expressed in roots at the tillering and heading stages of rice, while the expression was extremely low in other periods and tissues. In view of the important roles of root-specific expressing genes, we speculated that *OsZFP350* may be closely related to the growth and morphogenesis of rice roots. In this study, we used 0.15 M NaCl and 20% PEG to mock the salt and drought stresses to rice seedlings. It was found that the expression of *OsZFP350* in roots of rice seedlings was significantly increased with regard to PEG and NaCl (Fig. 2b). Therefore, we propose that the up-regulated expression of *OsZFP350* might raise the resistant potential of rice plants to high salt, drought or even heat stress. In general, heat or cold stress is often coupled with drought and high salinity, so that *OsZFP350* expression was significantly increased under high temperature, but there was no induction at a low temperature. Thus, it cannot be ruled out that *OsZFP350* might also play a role under high temperature conditions in rice, as the heat stress marker gene *HSP70* is also up-regulated in transgenic plants.

Extensive publications have recently reviewed that ABA signaling is involved in the regulation of abiotic stress in plants (Julkowska & Testerink, 2015; de Zelicourt *et al*., 2016; Edel & Kudla, 2016). Given that *OsZFP350* was induced by the salt and drought stresses, it was possible that it might play a role in root morphogenesis through the ABA signaling. However, after treatment with IAA, ABA, and GA for 36 h respectively, the roots of rice seedlings showed inhibited expression of *OsZFP350* by both, IAA and ABA, but not GA (Fig. 2b). This suggested that OsZFP350 might participate in the development of rice roots through an ABA-independent signaling pathway, but the GA signaling.

It was also found in this work that after treatment with PEG, NaCl and heat stress, the transgenic plants showed stronger environmental adaptability and high-

er germination rate when compared to the wild-type (Fig. 4). It had been reported that overexpression of *OsNAC10* under the control of the root-specific promoter *RCc3* improves drought tolerance and grain yield in transgenic rice plants (*RCc3:OsNAC10*) under field drought conditions (Jeong *et al.*, 2010). The overexpression of *OsZFP350* resulted in a robust root system in transgenic rice plants under abiotic stress conditions, which would be beneficial to increase rice yield. In summary, our experiments have provided important application prospects for the development of a stress-tolerant rice genotype.

Conflict of interest

The authors declare no conflict of interest.

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