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In silico promoter and expression analyses of rice *Auxin Binding Protein 57* (*ABP57*)

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ABSTRACT

Auxin Binding Protein 57 (*ABP57*) is one of the molecular components involved in rice response to abiotic stress. The *ABP57* gene encodes an auxin receptor which functions in activating the plasma membrane H^+-ATPase. Biochemical properties of *ABP57* have been characterized; however, the function of *ABP57*, particularly on stress and hormone responses is still limited. This study was conducted to understand the regulation of *ABP57* expression under abiotic stress. Thus, *in silico* identification of *cis*-acting regulatory elements (CAREs) in the promoter region of *ABP57* was performed. Several motifs and transcription factor binding site (TFBS) that are involved in abiotic stress such as ABRE, DRE, AP2/EREBP, WRKY and NAC were identified. Next, expression analysis of *ABP57* under drought, salt, auxin (IAA) and abscisic acid (ABA) was conducted by reverse transcription-PCR (RT-PCR) to verify the effect of these treatments on *ABP57* transcript level. *ABP57* was expressed at different levels in the shoot and root under drought conditions, and its expression was increased under IAA and ABA treatments. Moreover, our results showed that *ABP57* expression in the root was more responsive to drought, auxin and ABA treatments compared to its transcript in the shoot. This finding suggests that *ABP57* is a drought-responsive gene and possibly regulated by IAA and ABA.

Introduction

As sessile organisms, plants adapt to the environment by maximizing the growth potential to escape stressful conditions or undergo growth reduction for survival (1). Drought promotes abscisic acid (ABA) synthesis, which subsequently initiates signal transduction and affects cellular responses to stress (2). ABA is involved in plant adaptation to abiotic stress, especially in regulating physiological responses such as root elongation and stomatal aperture (3). Additionally, ABA modulates root architecture under water scarcity by regulating root meristem function and root cell length (4). Plant physiological changes during stress conditions are regulated by ABA interactions with other hormones such as auxin, ethylene and jasmonate (3).

Gene expression is diverse among plant stages of development, tissues and response to external stimulus. A stress-responsive gene expression is regulated by specific sequences present at the promoter known as *cis*-acting regulatory elements (CAREs). These regulatory elements served as motifs or binding sites for proteins called transcription factors (TFs), activating or repressing the expression of stress-responsive genes (5-7). For example, abscisic acid responsive element (ABRE) is involved in transcriptional responses induced by phytohormone ABA (5, 8) and this element is recognized by ABA-RESPONSIVE ELEMENT BINDING PROTEIN (AREB) and ABRE BINDING FACTOR (ABF) (9-11). These TFs play a role in regulating plant response to osmotic stress such as drought and high salinity (9, 10).

Auxin is a key phytohormone that regulates various processes in plant growth, development and stress response (12, 13). Together with ABA and ethylene, auxins act as communication signals between roots and shoots in rice (14, 15). Auxin signaling is facilitated by AUXIN RESPONSE FACTORS (ARFs), which bind to the Aux/IAA motif and activate the transcription of auxin-inducible genes (16, 17). However, not all auxin responses employ this pathway, especially some rapid responses at the plasma membrane (18). This response involved another candidate of the auxin receptor, which is AUXIN BINDING PROTEIN 1 (ABP1). *ABP*1 localizes mainly at the endoplasmic reticulum (ER), and it is involved in regulating the membrane potential at the plasma membrane (18, 19).

Other than ABP1, another putative auxin receptor known as AUXIN BINDING PROTEIN 57 (ABP57) has been isolated from the shoot of rice (20). Apparently, this protein exists only in monocots such as *Oryza sativa*, *Zea mays*, *Sorghum bicolor* and *Setaria viridis*, but not in the dicot plant (21, 22). The binding of *ABP57* to indole-3-acetic acid (IAA) leads to the activation of the plasma membrane H$^+$-ATPase by direct interaction (21). Previously, a study showed that the *ABP57* overexpression had increased the resistance of transgenic rice to drought stress (23). Moreover, the *35S::ABP57* transgenic *Arabidopsis* possessed root elongation changes and also restoration of root cells under drought and submergence conditions (24-26).

These previous studies suggest that *ABP57* is involved in abiotic stress and root development. However, more studies are needed to uncover the role of *ABP57* in plant development and stress response. To date, no information regarding *ABP57* transcriptional regulation is available, particularly on the characterization of its promoter. Nevertheless, the effect of stress and hormone treatments on *ABP57* expression has yet to be determined. Hence, the objectives of this study were to characterize the CAREs present at the promoter region of *ABP57* via *in silico* analysis and to determine the effect of osmotic stresses (drought and salt) and hormones (IAA and ABA) on the transcript level of *ABP57* in the rice at various time points.

By performing *in silico* promoter analysis, CAREs including transcription factor binding sites (TFBS) involved in stress response were identified. In addition, our expression analysis showed that *ABP57* responds to drought, high salinity, auxin and ABA. It confirms that the presence of several motifs is required to activate *ABP57* expression under these treatments. Taken together, our study provided insight into the transcriptional regulation of *ABP57* under stress conditions.

Materials and Methods

In silico promoter analysis

The *ABP57* (Gene ID: LOC_Os07g02970) promoter sequence (upstream of -1000 and 220 bp 5'-UTR) was obtained from Phytozome v12.1 (https://www.phytozome.net) (27). This promoter region was then analyzed in PLACE (https://dna.affrc.go.jp/PLACE/?action=newplace) (28), PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) (29) and PlantPAN 3.0 (http://plantpan.itps.ncku.edu.tw/) (30) to identify *cis*-acting regulatory elements (CAREs) and transcription factor binding sites (TFBS). The function of TFs identified in these analyses was further examined in Oryzabase (https://shigen.nig.ac.jp/rice/oryzabase/). Only TFs involved in biotic and/or abiotic stress response were selected in this study.

Plant materials and growth conditions

The *indica* rice cv. IR64 and MR219 were obtained from the Malaysian Agricultural Research and Development Institute (MARDI). Drought treatment was conducted in a greenhouse with relative humidity was 85%, and the maximum and minimum temperatures were 30 ºC and 25 ºC respectively. Salt, auxin and ABA treatments were conducted at 25 ºC under 16 hr light/8 hr dark in a temperature-controlled growth room. All experiments were performed at Plant Biotechnology Centre, Universiti Kebangsaan Malaysia (UKM).

Drought treatments

Drought treatment was conducted as per standard procedure (31). The seeds (cv. IR64) were soaked in distilled water for two days to break seed dormancy before they were sowed in a petri dish with wet cotton to allow germination. The 10-days-old rice seedlings were transferred into pots (D 12 cm x H 13 cm) that contain mixed soils (topsoil: organic matter: sand with ratio 3: 2: 1). Five pots were prepared for each control and treatment, and each pot contained three seedlings. All seedlings were watered daily for three weeks before drought treatment begins. Control plants were watered continuously, whereas water withholding was imposed on treatment plants for four weeks. The mature leaf (L5) and root samples from three seedlings were collected weekly (Week 1, 2, 3 and 4). All samples were flash-frozen before total RNA extraction.

Salt, auxin and abscisic acid treatments

Seeds of rice (cv. MR219) were surface-sterilized with sodium hypochlorite for 15 min before they were soaked in distilled water for two days. Seeds were planted on moist cotton and they were grown for 10 days. These 10-days-old rice seedlings were used for salt (NaCl), IAA and ABA treatments. The seedlings were transferred separately into 50 ml centrifuge tubes containing 35 ml of 250 mM NaCl, 100 µM IAA and 100 µM ABA solutions respectively. In each tube, three seedlings were transferred and whole seedlings were soaked for 0, 6 and 24 hrs. Three replicates were prepared in each treatment. The root and leaf (L3) samples were cut and flash-frozen in liquid nitrogen prior to total RNA isolation.

Semi-quantitative expression analysis of ABP57

To determine the expression pattern of *ABP57* during osmotic stress and hormone treatments, RT-PCR was carried out. Total RNAs from the leaf and root were extracted using Trizol® Reagents (Thermo Scientific, USA) following the manufacturer's instruction. The quality and quantity of the total RNA were determined by a spectrophotometer (NanoDrop 1000, USA). DNase treatment was performed on 1 mg of total RNA with RQ1 RNase-Free DNase (Promega Corp, USA) to ensure that RNA samples were free from genomic DNA contaminations. The cDNA was synthesized by reverse transcription on the total RNA using the SuperScript cDNA Synthesis kit (Invitrogen, Life Technologies, USA) following the manufacturer's instruction. The synthesized cDNA was then used as a template for PCR.

Two pairs of primers were used for PCR which are *ABP57*-specific (Forward: 5'-ATGGCAGAGATTGTTAGTTC-3'; Reverse: 5'-

CTAAAATTTCAGGCGCAGTA-3') and a housekeeping gene, *U6 small nuclear RNA* (*U6 snRNA*) that acts as an internal control (Forward: 5'-TACAGATAAGATTAGCATGGCCC-3'; Reverse: 5'-GGACCATTTCTCGATTTGTACGTG-3') (24, 25). The amplicon sizes were 1550 bp and 60 bp, respectively. Amplification of *ABP57* was performed using the GoTaq® Green Master Mix kit (Promega Corp. USA) with the following program: 95 °C for 2 min followed by 35 cycles of (95 °C, 1 min; 58 °C, 1 min; 72 °C, 2.5 min) and 72 °C for 5 min. Meanwhile, the following programme was performed for amplification of *U6*: 95 °C for 2 min followed by 25 cycles of (95 °C, 1 min; 56 °C, 1 min; 72 °C, 1 min) and 72 °C for 5 min. The PCR products were analyzed by 1.0% agarose gel electrophoresis, whereas the gel image was analyzed by ImageJ (https://imagej.nih.gov/ij) (32) for quantification of band densities (densitometry).

Statistical analysis

The mean data was recorded by taking average value and subjected to analysis of variance (ANOVA) by using the SAS programme version 9.4. The ANOVA was used to determine the variation for all parameters such as type of organ (leaf and root), genotypes and treatments. Mean comparisons were performed using Duncan's New Multiple Range Test (DNMRT). Differences among the treatments mean were considered statistically significant when the p-value is less than 0.01 (33). All data represent an average of three replicates and the standard error.

Results

In silico analysis of the *ABP57* promoter

Cis-acting regulatory elements (CAREs) of the promoter regions play a significant role in the transcriptional regulation of stress-inducible genes. To obtain an insight into the regulation of *ABP57* expression, *in silico* promoter analysis was conducted. Many CAREs were identified at the promoter of *ABP57* (Table 1). Based on PLACE and PlantCARE databases, several motifs that are related to stress responses were found in the *ABP57* promoter, which include ABRE (ABA-responsive element), DRE (dehydration responsive element) and LTR (low temperature-responsive element). Besides that, motifs involved in hormone response such as EREBP (ethylene-responsive binding protein) and GARE (gibberellin-responsive element) were also present.

Several transcription factor binding sites (TFBS) available in the promoter region of *ABP57* were listed in Table 1. This TFBS belong to TFs such as WRKY, MYB, MYC, bZIP (Basic Leucine Zipper), HD (Homeodomain) and NAC (NAM, ATAF, CUC). Based on PlantPAN database search, binding sites for other TF families were also discovered, such as B3, bHLH (Basic Helix-Loop-Helix), C2H2 Zinc Finger, GATA Zinc Finger, NF-YB (Nuclear Factor-Y Subunit B) and TCP (TEOSINTE BRANCHED 1, CYCLOIDEA, PCF1) (Table 2). The listed TFs in Table 2 are involved in stress response and this result suggests that they could be the regulator for *ABP57* during adverse conditions.

ABP57 expression pattern under drought and salt treatments

Water withheld of the 4-weeks-old seedlings were carried out for four weeks, and expression patterns of *ABP57* under prolonged drought stress were determined (Fig. 1). The *ABP57* transcript was slightly expressed in the leaf and root under normal conditions (Fig. 1A). During drought treatment, *ABP57* was lowly expressed in the leaf during the first and second week, but its expression was increased dramatically at Week 3 and slightly decreased at Week 4 (Fig. 1B). *ABP57* transcript was also observed in the untreated root; however, its expression level was significantly increased (p≤0.01) at Week 2 of drought treatment and noticeably decreased at Week 3 and Week 4 (Fig. 1B). These results indicate that *ABP57* is expressed under normal conditions, but prolonged drought treatment has much induced *ABP57* expression in both leaf and root at different time points. *ABP57* was highly expressed in the leaf at Week 3, whereas the highest expression in the root can be seen at Week 2 of drought treatment.

On the other hand, *ABP57* expression in the seedlings treated with high salinity (250 mM NaCl) was examined for 6 and 24 hrs. Our results showed that *ABP57* was expressed in the control but significantly reduced (p≤0.01) in the shoot and root after 6 hrs of NaCl treatment (Fig. 2). After 24 hrs, the expression of *ABP57* in both of leaf and root was significantly lowered than its expression at 6 hrs. This result suggests that the expression of *ABP57* in the seedlings is suppressed by high salt concentration.

Auxin and abscisic acid treatments induced ABP57 expression

ABP57 acts as an auxin receptor and binds to IAA to activate the plasma membrane H^+-ATPase by regulating proton (H^+) translocation activity (21). To examine the effect of IAA treatment on *ABP57* expression, we analyzed the *ABP57* transcript in the shoot and root of rice treated with this hormone. Our results showed that *ABP57* was induced after 6 hrs of IAA treatment in both shoot and root (Fig. 3A). The highest expression of *ABP57* in the leaf and root can be seen at 6 hrs and 24 hrs of IAA treatment respectively. Although *ABP57* expression in the root was gradually increased from 6 to 24 hrs, its transcript in the shoot was reduced at 24 hrs of IAA treatment (Fig. 3A). These results showed that although *ABP57* is responsive to exogenous auxin, it is preferably expressed in the root.

In this study, ABRE was found at the *ABP57* promoter (Table 1) and *ABP57* expression was increased under drought (Fig. 1). Thus, to determine whether *ABP57* is affected by ABA, we examined its transcript in the rice seedling treated with a high ABA concentration (100 μM). The expression of *ABP57* was significantly reduced (p≤0.01) in both shoot and root after 6 hrs of ABA treatment but was only increased after 24 hrs (Fig. 3B). Additionally, *ABP57* expression in the root was significantly higher (p<0.01) than in the shoot at both time points (Fig. 3B). This result showed that *ABP57* is responsive to exogenous ABA but only after 24 hrs of treatment and preferably expressed in the root.

Table 1. Identification of *cis* acting regulatory elements (CAREs) and transcription factor binding sites (TFBS) at the *ABP57* promoter through PLACE (28) and PlantCARE (29) databases.

Binding site name	Position	Motif Sequence	Function
CGCG box	+14, -14, +66, -66, +68, -68, +181, -181	VCGCGB	involved in multiple signaling transduction pathways (light signal perception, ethylene- and ABA-signaling)
ABRE (ABA Responsive Element)	+586	ACGTG	ABA and dehydration response
	+465, -465, -586	ACGT	
DRE (Dehydration Responsive Element)	+22	ACCGAGA	ABA and drought response
	+170	ACCGAC	
AS-1 (Activation Sequence-1) element	-259, -441	TGACG	Induced by abiotic and biotic stresses, involved in activation of genes by auxin and/or salicylic acid
LTRE (Low Temperature Responsive Element)	+171	CCGAC	Involved in cold and ABA response
GARE (GA Responsive Element)	+767	TAACAAR	GA response
GCC core	-34, -44, +50, +53, +62, -76	GCCGCC	Ethylene responsive element
EREBP	+1105	AWTTCAAA	Ethylene responsive element, related to senescence
W-box	-395, +805	TTGACC	WRKY recognition site
	-396, -442, +656, +802	TTGAC	
	+20, -260, -396, -442, +646, +657, -665, +686, -698, +806	TGAC	
MYB motif	+227, +238, -776	CNGTTR	Response to dehydration stress MYB recognition site
	-618	AACGG	
	+795	CTAACCA	
MYC motif	+262, -262, +360, -360, +682, +726, -726, +737, -737, +776, -776, +909, -909	CANNTG	MYC recognition site, involved in drought stress and ABA response
	-1060	CACATG	
bZIP motif	+315	ACACNNG	bZIP recognition site, ABA response
HD (Homeodomain) motif	-657, -686	TGTCA	Binding site of OsBIHD1
NAC	+1060	CATGTG	Dehydration stress

Table 2. Putative transcription factors (TFs) that may regulate *ABP57* expression. Their binding site was found at the *ABP57* promoter.

Transcription factor family	Hit sequence	Inferred transcription factor
AP2/ERF AP2/EREBP	tggCGGCG	OsERF67, OsERF3, SUB1B, OsERF106, OsERF92, OsERF53, OsERF99, OsERF91, OsERF88, OsERF95, OsERF101, OsERF77, OsERF76
	cGGCGGcg cgCCGCCg	OsERF123, OsERF91, OsERF88, OsERF96, OsERF84
	cCGCCGgc gcCGGCGt	SUB1B, OsERF106, OsERF76, OsERF50, OsERF72, OsERF3, OsERF68, OsERF99, OsERF91, OsERF95, OsERF103, OsERF62, OsERF101, OsERF71, OsERF66, OsERF77
	cCACCGact tCACCGcct	DREB2C, DREB2A, DREB2E, OsERF42
	aCCGAC	DREB2C, DREB2A, DREB1G, DREB2E, OsERF42, DREB1C, DREB1F, DREB1E, DREB2D, DREB1D, OsERF28, DREB1B, DREB1H, DREB1A
	AGCCGcc	EREBP1
B3	GCATG	IDEF1 (IRON DEFICIENCY BINDING FACTOR 1)
bHLH	cccACTTGa	OsbHLH110
C2H2 zinc finger	cACACTa	ZFP252 (ZINC FINGER PROTEIN 252), BSRD1 (BROAD SPECTRUM RESISTANCE DIGU 1), DRZ1 (DROUGHT RESPONSIVE ZINC FINGER PROTEIN 1)
	tTCACTc	ZFP182, ZFP179,
GATA zinc finger	tcTGATCac ctGATCAct	CGA1 (CYTOKININ GATA TRANSCRIPTION FACTOR 1)
	acAGATCagg actGATCTgt	OsGATA4
Homeodomain TALE	TGACC CGTCA GGTCA TGACA AGTCA	HOMEOBOX1
Myb/SANT	taGATTCtta cagGAATCa	OsDLN196
	TAACAaa	GAMYB (GIBERELLIN-MYB)
NAC/NAM	ggGTCAAtt ttTTGACcc	ONAC10, ONAC88, NAC4, ONAC58, ONAC122, ONAC103, ONAC131, NAC5
WRKY	ggGTCAAt tTTGACcc	WRKY9, WRKY1, WRKY102, WRKY107, WRKY77, WRKY10, WRKY11, WRKY12, WRKY14, WRKY27, WRKY26, WRKY16, WRKY17, WRKY39, WRKY66, WRKY42, WRKY71, WRKY20, WRKY13, WRKY51, WRKY3, WRKY32, WRKY36, WRKY37, WRKY80, WRKY121, WRKY6, WRKY11, WRKY43, WRKY49, WRKY67, WRKY68, WRKY8, WRKY5, WRKY7, WRKY29, WRKY31, WRKY73, WRKY28, WRKY76, WRKY25, WRKY47, WRKY94, WRKY2, WRKY57, WRKY90, WRKY62, WRKY72
	atcGTCAAca	WRKY111, WRKY9, WRKY94, WRKY51, WRKY1, WRKY11, WRKY12, WRKY14, WRKY15, WRKY17, WRKY19, WRKY2, WRKY20, WRKY22, WRKY25, WRKY44, WRKY27, WRKY29, WRKY3, WRKY31, WRKY32, WRKY36, WRKY37S, WRKY39, WRKY43, WRKY46, WRKY47, WRKY48, WRKY49, WRKY52, WRKY54, WRKY57, WRKY66, WRKY67, WRKY68, WRKY69, WRKY73, WRKY74, WRKY102, WRKY8, WRKY90, WRKY116, WRKY80, WRKY84, WRKY113, WRKY115, WRKY97, WRKY121, WRKY26, WRKY6, WRKY107
TCP	GGTCC GGGCC GGCCC	PCF5 (PROLIFERATING CELL FACTOR 5), PCF8, PCF3
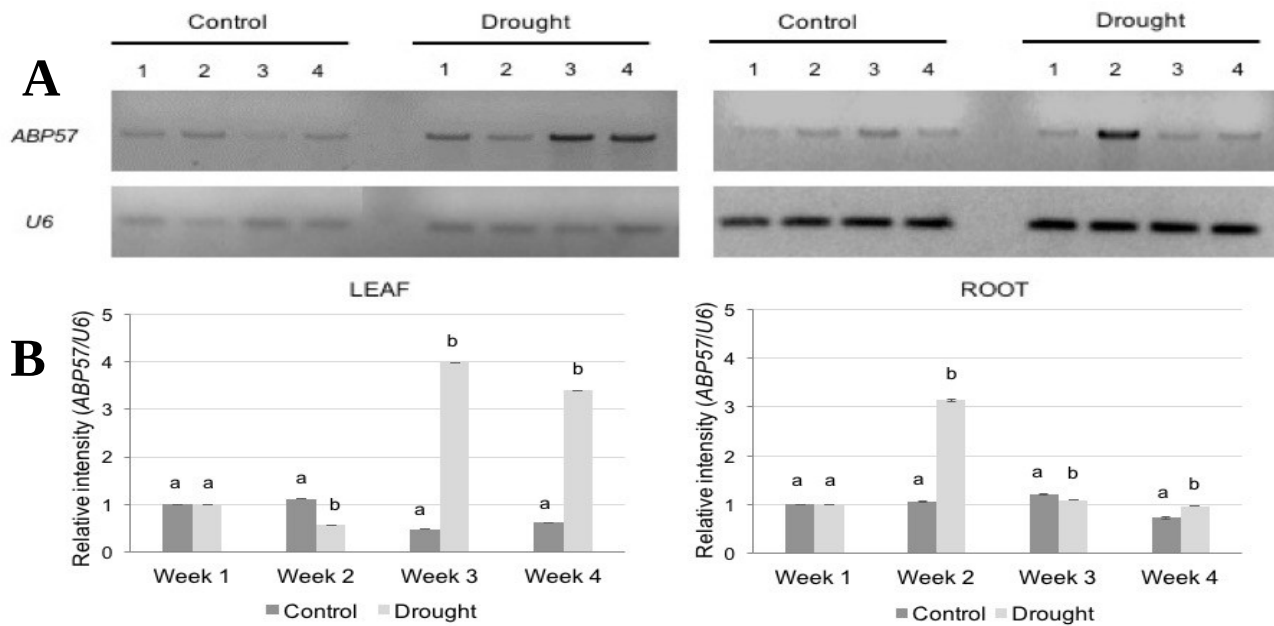
Fig. 1. (A) Semi-quantitative RT-PCR analysis in the leaf and root under drought conditions. Numbers 1 to 4 indicated the week of drought treatment. **(B)** The expression level of *ABP57* in the leaf (left) and the root (right) was normalized to *U6*. Different letters indicate significant difference based on comparison using DNMRT at p ≤ 0.01.

Discussion

The biochemical properties of ABP57 have been characterized before (20, 21), but the molecular characterization of this gene is still limited. The overexpression of *ABP57* in *Arabidopsis* and rice had increased their tolerance to high salt and drought, respectively (23, 25). These studies suggested that *ABP57* is required during the osmotic stress response. Therefore, to obtain more insight into the regulation of *ABP57* expression during abiotic stress, we analyzed the *ABP57* promoter *in silico* and its transcript changes under drought, salt, IAA and ABA treatments.

Promoter manipulation is one of the tools used in crop improvement, including stress response (34). It is known that different TFs attach to the promoter of stress-inducible genes in response to abiotic or biotic stresses. Indeed, several types of CAREs are essential in regulating the expression of drought-responsive genes (35). Thus, identifying CAREs at the promoter of a stress-related gene will help in understanding its expression regulation. In this study, several CAREs involved in stress response were identified at the *ABP57* promoter region, such as ABRE, DRE, LTR and others. ABRE contains an ACGT core, which is crucial for *DREB2A* expression in *Arabidopsis* (8, 35, 36) and a recognition site for plant bZIP transcription factors during drought conditions (37). The DRE motif consists of a core sequence A/GCCGAC, which is a conserved sequence in the promoter region of DREBs' direct target genes (38). Meanwhile, the LTR element (CCGAC) is necessary for the activation of low temperature-responsive genes during cold stress (39, 40). These results suggest that *ABP57* might respond to various environmental stimulus such as water deficit and low temperature.

Besides that, many TF binding sites related to abiotic stress response present at the *ABP57* promoter. Osmotic stress activates ABA-independent and ABA-dependent signaling pathways, including TFs like DREB, WRKY, bZIP, MYC, MYB and NAC (3, 8, 41). These TFs attach to the *cis*-acting elements in the promoter and activate the transcription of secondary responsive genes, thus, increasing plant tolerance to stress (41, 42). The binding site of AP2/EREBP and WRKY was mainly found in the upstream region of *ABP57*. AP2/EREBPs regulate numerous abiotic stress
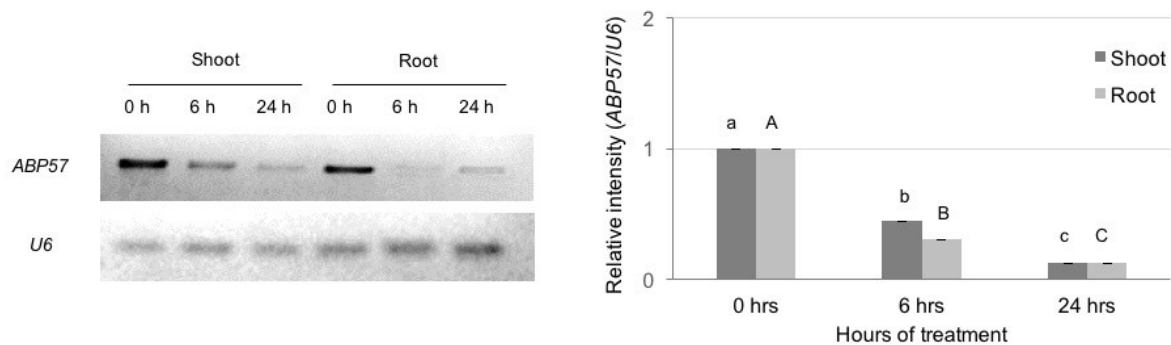
Fig. 2. Semi-quantitative RT-PCR analysis in the shoot and the root under salt treatment. The expression level of ABP57 was normalized to U6. Different letters indicate significant difference based on comparison using DNMRT at p ≤ 0.01. Capital letters and small letters indicate differences among root and shoot samples, respectively.
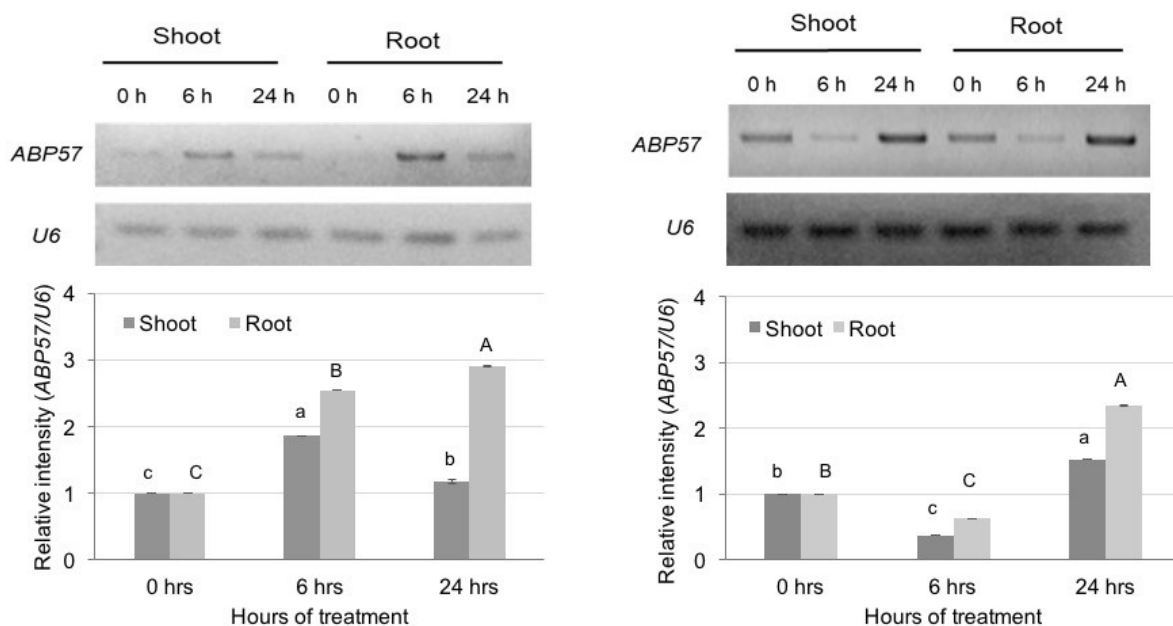
Fig. 3. Semi-quantitative RT-PCR analysis in the shoot and the root under **(A)** auxin and **(B)** abscisic acid. The expression level of ABP57 was normalized to U6. Different letters indicate significant difference based on comparison using DNMRT at p ≤ 0.01. Capital letters and small letters indicate differences among root and shoot samples, respectively.

and hormone responses, and one of its family members, DREB, is a major regulator in plant response to cold, drought, heat and salt (42). Meanwhile, WRKY proteins recognize and bind to W-box, which is also essential in activating stress-responsive genes (43). With these findings, we suggest that *ABP57* might be regulated by ABA, and could be the direct or indirect target gene for TFs such as DREB, WRKY and bZIP during stress response. Therefore, further studies need to be carried out to determine the interaction between *ABP57* and its putative transcriptional regulator.

Both ABRE and DRE were found at the *ABP57* promoter, and these elements regulate the expression of ABA-, osmotic- and drought-responsive genes (36, 38). It suggests that *ABP57* could be involved in ABA and osmotic stress response. Therefore, to confirm this hypothesis, *ABP57* transcript levels under drought, high salinity and ABA treatments were analyzed. Our results showed that *ABP57* expression is induced by drought and ABA preferably in the root, but suppressed by salt treatment, indicating that ABA could regulate *ABP57* involvement in drought response. Based on Rice eFP Browser, *ABP57* expression is not highly induced by drought, salt, nor cold conditions (http://bar.utoronto.ca/eplant_rice/). Although our result contradicted the expression profile in the database, it might happen because different types of samples were used. In addition, the difference maybe because of the time point of the study, as results showed differential expression at different time point. The expression of *ABP57* in this study might be affected by the stage of rice development, types of tissues, duration of drought treatment and concentrations of NaCl and ABA.

Although the AuxRE motif was absent at the promoter region of *ABP57*, we speculated that auxin still could affect *ABP57* expression. It is because other than ARF-mediated transcription, auxin response can also be modulated by an additional signaling

pathway (44). Under IAA treatment, the *ABP57* transcript level was higher in the root compared to the shoot. This result suggests that *ABP57* is induced by auxin and preferentially expressed in the root. The high concentration of exogenous IAA may accelerate the binding of *ABP57* to auxin and consequently, may elicit an auxin response of rice plasma membrane (21). Furthermore, the *ABP57* response to auxin might be distinct from ARF-mediated transcription and might involve different regulation of gene transcriptional activation and post-transcription (17, 45, 46). Besides, plant response to auxin also depending on auxin concentrations and the types of cells and tissues (46).

Conclusion

In this study, we reported the *cis*-acting regulatory elements available at the promoter region of *ABP57*, as well as the effect of drought, salt, auxin and ABA treatments on *ABP57* expression patterns at various time points. *In silico* promoter analysis showed several CAREs essential in stress response were identified, revealing the possible transcriptional regulation of *ABP57* under abiotic stress and hormone treatments. Additionally, our expression analysis confirmed that *ABP57* is a drought-responsive gene and may be involved in the root response to drought stress, possibly under auxin and ABA regulation. Drought response in plants involved regulation of gene expression and crosstalk among hormone signaling pathways. Therefore, the *ABP57* loss-of-function mutant can be studied to further characterize the role of *ABP57* in abiotic stress and hormone response signaling pathways.

Acknowledgements

Authors' contributions

FAB performed expression and statistical analyses, and wrote the manuscript. KZX performed expression analysis. ZZ designed the experiment and wrote the manuscript. NLS designed the experiment, performed *in silico* promoter analysis and wrote the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors do not have any conflict of interest to declare.

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