

## Research Article

# Basal Transcription Factor 3 Plays an Important Role in Seed Germination and Seedling Growth of Rice

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BTF3 has been recognized to be involved in plant growth and development. But its function remains mostly unknown during seed germination and seedling stage. Here, we have analyzed OsBTF3-related sequences in *Oryza sativa* L. subspecies, japonica, which resembles with the conserved domain of a nascent polypeptide associated complex (NAC) with different homologs of OsBTF3 and human BTF3. Inhibition of *Osj10gBTF3* has led to considerable morphological changes during seed germination and seedling growth. Germination percentage was not influenced by the application of GA<sub>3</sub>, ABA, and NaCl but all concentrations caused wild-type (WT) seeds to germinate more rapidly than the RNAi (*Osj10gBTF3<sup>Ri</sup>*) transgenic lines. Seedling inhibition was more severe in the *Osj10gBTF3<sup>Ri</sup>* seedlings compared with their WT especially when treated with 100 or 200 μM GA<sub>3</sub>; 50% reduction in shoots was observed in *Osj10gBTF3<sup>Ri</sup>* seedlings. The expression of *Osj3g1BTF3*, *Osj3g2BTF3* and *Osj10gBTF3* was primarily constitutive and generally modulated by NaCl, ABA, and GA<sub>3</sub> stresses in both *Osj10gBTF3<sup>Ri</sup>* lines and WT at the early seedling stage, suggesting that *Osj3g1BTF3* and *Osj10gBTF3* are much similar but different from *Osj3g2BTF3* in biological function. These results show that OsBTF3 plays an important role in seed germination and seedling growth gives a new perception demonstrating that more multifaceted regulatory functions are linked with BTF3 in plants.

## 1. Introduction

Different transcription factors (TFs) play an important role in multiple physiological mechanisms such as cell cycle progression, metabolism, growth, development, and reproduction [1, 2]. Among these TFs, the basal transcription factors 3 (BTF3) is one of the extremely important transcription factors due to their role in various biotic and abiotic stress processes [3–6] and different physiological and developmental mechanisms such as ionic homeostasis [7], photosynthetic rate [8], and pollen development [6] in plants.

BTF3 was basically found in HeLa cell that is essential for RNA polymerase II dependent transcription [9]. The BTF3 codes two isoforms, BTF3a and BTF3b, in the human, as an outcome of alternative splicing. BTF3a encodes for a protein with complete characteristics of BTF3 and stimulates transcription, while BTF3b encodes a shortened form of BTF3a, as it is missing the first 44 amino-terminal extension.

Afterward, BTF3b was documented as a component of the nascent polypeptide associated complex (NAC). It consists of two subunits, that is, αNAC and βNAC [10]. BTF3 is a β-subunit of the NAC that has been participating in regulating protein localization during translation [11].

BTF3 in plant lacks the N-terminals which exist in the human BTF3a form. But, the N-terminals extension of the plant proteins interacts with that of the transcriptionally dormant BTF3b isoform [12]. In *Arabidopsis*, AtBTF3 coincides with the translation initiation factor (iso) 4E (eIFiso4E) and formed the translation initiation complex eIF(iso)4F [12]. *NbBTF3* silenced plants show an abnormally developed phenotype in *Nicotiana benthamiana*. Similarly, in *TaBTF3*-silenced wheat transgenic plants, Ma et al. [13] demonstrated that the transcripts of the mitochondrial and chloroplast encoded genes were noticeably decreased. In *Capsicum annuum*, BTF3 gene was observed to play a role in hypersensitive response (HR) cell death and might function

as TFs in the nucleus via transcriptional regulation of HR-related gene expression [14].

Recently, in rice, Wang et al. [6] have demonstrated that BTF3 plays important role in plant growth and development. However, the function of different variants of OsBTF3 in seed germination and seedling growth is not yet documented. In the present work, a cDNA sequence of a BTF3-like gene (*Osj10gBTF3*) was extracted, and its function examined via transgenic strategies to recognize the role it played in seed germination and seedling growth in rice. We also attempt to investigate that how the OsBTF3s were transcriptionally regulated by salt, GA<sub>3</sub>, and ABA stresses during seed germination and seedling stage in *Osj10gBTF3*<sup>Ri</sup> lines and wild type.

## 2. Materials and Methods

**2.1. Isolation of *Osj10gBTF3* and Generation RNAi Repression Lines.** RNAs extractions, the full length of cDNA synthesis, were carried out using the BTF3 gene-specific primers via procedures as described by Wang et al. [6]. The amplified products of cDNA were cloned into a pMD18-T vector (TaKaRa). RNAi repression vector was constructed as suggested by Wang et al. [6]. The resultant vector was incorporated into the EHA105 (*A. tumefaciens* strain), which was used to infect rice embryogenic calli raised from mature seeds of Nipponbare to produce transformed calli. Transformants were screened using primers specific for the hygromycin B phosphotransferase gene (*Hpt*) by PCR amplification as demonstrated by Wang et al. [6].

**2.2. Seed Germination and Seedling Growth.** Seeds of T<sub>1</sub> *Osj10gBTF3*<sup>Ri</sup> and wild-type (WT) of *Oryza sativa* L. subspecies *japonica* line Nipponbare were germinated on double filter paper in Petri plates treated with 0 (distilled water), 50 and 100 mM NaCl, 100 and 200 μM GA<sub>3</sub>, and 5 and 10 μM ABA. The seeds were incubated at 30 ± 2 °C in dark condition in growth chamber. Seed germination was counted at an interval of 12 hours up to 7 days, up to emergence of ~2 mm radical and germination percentage was calculated when there was 50% of seed germination. Seedlings after 15 days were taken and separated into shoots and roots for measuring root and shoot lengths.

**2.3. RNA Isolation from Different Tissues.** RNA was extracted from the fresh roots and shoots of *Osj10gBTF3*<sup>Ri</sup> lines and WT under normal and stress conditions with Trizol reagent (Invitrogen) by following the manufacturer's instructions. Full length of cDNA synthesis of first-strand was done with the M-MLV first-strand synthesis system.

**2.4. Expression Analysis Using Real-Time PCR.** To check the expression of *Osj3g1BTF3*, *Osj3g2BTF3*, and *Osj10gBTF3*, real-time quantitative PCR (q-PCR) was used. The target gene names and their primers for the q-PCR are listed in Supplementary Table 2 (see Supplementary Material available online at <http://dx.doi.org/10.1155/2014/465739>). Q-PCR was done using the method of Wang et al. [6]. The relative expression levels of *Osj3g1BTF3*, *Osj3g2BTF3*, and *Osj10gBTF3* in

root and shoot were calculated using the  $2^{-\Delta\Delta C_T}$  method [15]. Here,  $\Delta\Delta C_T = (C_{T(\text{Target,test})} - C_{T(\text{ubiquitine,test})}) - (C_{T(\text{Target,calibrator})} - C_{T(\text{ubiquitine,calibrator})})$ . The  $C_T$  (cycle threshold) value was the average value of the three different independent samples for both target and reference genes.

**2.5. Statistical Analysis.** The data were analyzed by one-way analysis of variance (ANOVA), and mean values were separated by least significant difference (LSD) at the 5% and 1% probability level using Statistical software (SigmaPlot 10.0.). Sequences of different variants of OsBTF3 were aligned by using ClustalX, while MEGA 5.0 was used for construction of phylogenetic tree.

## 3. Results

**3.1. *OsBTF3* Sequence and Phylogenetic Tree.** The protein sequences of different variants of OsBTF3 were aligned with the related sequences from *Arabidopsis* (AtBTF3, AEE29647), wheat (TaBTF3, AFV31408), sorghum (SbBTF3, EER93008), maize (ZmBTF3, ACG28870), *Ricinus communis* (RcBTF3, EEF34688), *Capsicumvannum* (CaBTF3, ABM55742), *Solanum lycopersicum* (SlBTF3, NP\_001234229), and *Nicotiana benthamiana* (NbBTF3, ABE01085) (Supplementary Table 1). Protein sequence analysis showed that different variants of OsBTF3 such as *Osj10gBTF3* and *Osj3g1BTF3* contained a putative mitochondrial matrix targeting sequence (MMTS) (residues 1–15, MNVDKLLKMA-GAVRT), a putative nuclear localization signal (NLS) (residues 22–25, RRKK), a conserved NAC domain (residues 33–97), and a putative ER-retention/retrieval signal (ERRS) (residues 161–164, AEEK), while *Osj3g2BTF3* lack MMTS or ERRS (Figure 1(a)). It means that these two genes having different genomic loci are either cytosolic or a nucleus proteins. These results show that *Osj10gBTF3* and *Osj3g1BTF3* are probably differing from *Osj3g2BTF3* in subcellular localization and other biological function.

Phylogenetic analysis (Figure 1(b)) was performed between different variants of OsBTF3 and BTF3-like members from other plant species. The analysis showed that *Osj10gBTF3* is more strongly associated to *Osj3g1BTF3*, followed by other BTF3 homologs of the plant species. The BTF3 homologs of monocot and dicot species form different clades. Additionally, *Osj3g2BTF3* has a closer association to the homologs of dicot species AtBTF3 and RcBTF3 than *Osj3g1BTF3* and *Osj10gBTF3*. These results indicate that there may be functional variation between *Osj3g1BTF3*, *Osj10gBTF3*, and *Osj3g2BTF3* of BTF3 proteins.

**3.2. Inhibition of *Osj10gBTF3* Causes Phenotypic Changes.** The functional analysis of BTF3 was investigated using repression and overexpression strategies. Different independent *Osj10gBTF3* overexpressed lines were produced before the application of the RNAi construct for functional analysis of *Osj10gBTF3* using the overexpression constructs. But most of these lines did not show any significant change in phenotype; therefore, we mainly focused on RNAi inhibition analysis. *Osj10gBTF3*<sup>Ri</sup> transgenic seeds were investigated

	10	20	30	40	50	60	70	80
		-----		=====				
TaBTF3	MNV	DKLKKMAGAVRTGGKGSVRRKKKAVHKT	TTTDDKRLQSTLKR	VGVNTIPGIEEVNIFKDDVVIQFQNP	K-VQASIAA			
<i>Os03g1BTF3</i>	MNV	DKLKKMAGAVRTGGKGSRRKKKAVHKT	TTTDDKRLQSTLKR	VGVNNIPGIEEVNIFKDDVVIQFQNP	KAVQASIGA			
<i>Os10gBTF3</i>	MNV	DKLKKMAGAVRTGGKGSVRRKKKAVHKT	TTTDDKRLQSTLKR	VGVNTIPGIEEVNIFKDDVVIQFQNP	K-VQASIGA			
ZmBTF3	MNV	EKLKKMAGAVRTGGKGSRRKKKAVHKT	ATDDDKRLQSTLKR	IGVNTIPGIEEVNIFKDDI	VIQFQNP	K-VQASIPA		
SbBTF3	MNV	DKLKKMAGAVRTGGKGSRRKKKAVHKT	TTTDDKRLQSTLKR	IGVNTIPGIEEVNIFKDDVVIQFQNP	K-VQASIPA			
<i>Os03g2BTF3</i>	MNK	ERLMKMAGAVRTGGKGTVRRKKKAVHKT	TTDDKRLQSTLKR	VGVNTIPAIEEVNIFKDDLVIQFVNP	K-VQASIAA			
AtBTF3	MN	REKLMKMANVTRTGGKGTVRRKKKAVHKT	TTTDDKRLQSTLKR	VGVNSIPAIEEVNIFKDDVVIQFINP	K-VQASIAA			
RcBTF3	MN	REKLMKMGAVRTGGKGSVRRKKKAVHKT	TTTDDKRLQSTLKR	IGVNTIPAIEEVNIFKDDI	VIQFVNP	K-VQASIAA		
CaBTF3	MNV	EKLQKMGASVTRTGGKGTMRKKKAVHKT	TTTDDKRLQSTLKR	IGVNAIPAIEEVNIFKEDVVIQFINP	K-VQASIAA			
SlBTF3	MNV	EKLQKMGASVTRTGGKGTMRKKKAVHKT	TTTDDKRLQSTLKR	IGVNGIPAIEEVNIFKEDVVIQFVNP	K-VQASIAA			
NbBTF3	MNV	EKLKMGASVTRTGGKGTMRKKKAVHKT	TTTDDKRLQSTLKR	IGVNAIPAIEEVNIFKEDVVIQFINP	K-VQASIAA			
	90	100	110	120	130	140	150	160
								=====
TaBTF3	NTWV	SGTPQTKKLQDLLPTIINQLGPDNLGNLRR	LAEQFQKQMPGG	---EAGGASIGAAQDDDDVPELVPGET	FEEA			
<i>Os03g1BTF3</i>	NTWV	SGTPQTKKLQDLLPTIINQLGPDNLGNLRR	LAEQFQKQVPGA	---EAG-ASAGNAQDDDDVPELVPGET	FEEA			
<i>Os10gBTF3</i>	NTWV	SGTPQTKKLQDLLPSIINQLGPDNLGNLRR	LAEQFQKQAPGASG	-EEAG-ASAGAAQDDDDVPELVPGET	FEEA			
ZmBTF3	NTWV	SGVPQTKSLQDLLPTIINQLGPDNLENL	KRLAEHFQKQVPGA	-----EASASAAAQDDDDVPELVPGET	FEEA			
SbBTF3	NTWV	SGVPQTKSLQDLLPSIINQLGPDNLGNLRR	LAEHFQKQVPGA	-----EAG-AAAQDDDDVPELVPGET	FEEA			
<i>Os03g2BTF3</i>	NTWV	SGSPQTKKLQDVLPGIINQLGPDNMEHL	KRIAEEMQKVAAAG	-----ATAQAKEENDDVPELVPGEN	FEEV			
AtBTF3	NTWV	SGTPQTKKLQDILPQIISQLGPDNLGNL	KKLAEQFQKQAPGAG	----DVPATIQEEDDDVDPDLVGET	FETP			
RcBTF3	NTWV	SGTPQTKKLQDILPQVLGHLGPDNLGNL	KKLAEQIQKQTPSAG	----AAATTEQVEDDDEVPDLVGET	FEA-			
CaBTF3	NTWV	SGTPQTKKLQDILPQIIHQLGPDNLENL	KKLAEQFQKQAPGAAAGTDAAGAVAAQ	EDDD-VPELVAGET	FEA-			
NbBTF3	NTWV	SGSPQTKKLQDILPQIIHQLGPDNLENL	KKLAEQFQKQAAAAG	-----TGEAAAQEDDDEVPDLVAGET	FEG-			
	170	180						
TaBTF3	AEEK	KEPEAKKEPEPEEKKESS						
<i>Os03g1BTF3</i>	AEEK	-EPEEKKEAEVEEKKESS						
<i>Os10gBTF3</i>	AEEK	-----K-ES-						
ZmBTF3	AEV	KEVPE---AEPEEKKESS-						
SbBTF3	AEEK	KVPE---PEPEEKKESS-						
<i>Os03g2BTF3</i>	AQET	-----KA---						
AtBTF3	ATEE	APKAAAAS-----						
RcBTF3	AAEE	GKTAS-----						
CaBTF3	AAEE	GHTS-----						
SlBTF3	AAEE	NHAS-----						
NbBTF3	AAEE	GHTS-----						

(a)

FIGURE 1: Continued.

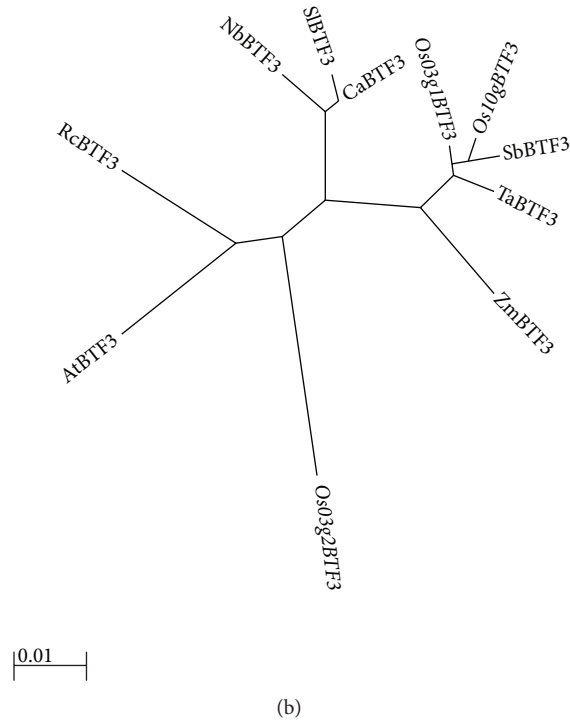


FIGURE 1: Amino acid sequence comparison (a) and phylogenetic tree (b) of different variants of OsBTF3. A deduced amino acid sequence of different variants of OsBTF3 and alignment with related sequences of different plants. The residues conserved among the compared sequences are in red colour. The overline (---) indicates a putative nuclear localization signal sequence, while the double dashed line (==) indicates the NAC domain. Gene Bank accession numbers for the proteins in the alignment are as follows: *Arabidopsis thaliana* BTF3 (AEE29647), *Nicotiana benthamiana* BTF3 (ABE01085), *Oryza sativa 10gBTF3* (NP\_001064883), *Oryza sativa 3g1BTF3* (NP\_001048709), *Oryza sativa 3g2BTF3* (NP\_001051911), *Capsicum annuum* BTF3 (ABM55742), *Ricinus communis* BTF3 (EEF34688), *Solanum lycopersicum* BTF3 (NP\_001234229), *Triticum aestivum* BTF3 (AFV31408), *Zea mays* BTF3 (ACG28870), and *Sorghum bicolor* BTF3 (EER93008). The tree was constructed using MEGA5 software.

for their capability to germinate, mainly in the presence of different concentrations of GA<sub>3</sub>, ABA, and NaCl. Though the final germination percentage was not affected by the application of GA<sub>3</sub>, ABA, and NaCl but all the levels of GA<sub>3</sub>, ABA, and NaCl caused WT seeds to germinate more rapidly than the *Osj10gBTF3*<sup>Ri</sup> transgenic lines and their mean germination rate reduced with increased GA<sub>3</sub>, ABA, and NaCl concentrations (Figure 2). It is worth mentioning here that the decrease in germination rate was more severe in the GA<sub>3</sub> treated seeds as compared to ABA and NaCl. These results demonstrate that *Osj10gBTF3*<sup>Ri</sup> transgenic lines are more sensitive to GA<sub>3</sub> than ABA and NaCl.

The seedling growth of WT and *Osj10gBTF3*<sup>Ri</sup> lines showed a different trend of variations in response to GA<sub>3</sub>, ABA, and salt treatments (Figure 3). The root and shoot lengths decreased in response to increasing concentrations of ABA and NaCl (Figure 4). However, NaCl had a more negative effect on root and shoot than ABA. On the other hand, GA<sub>3</sub> did not induce significant changes in root with increasing concentration of GA<sub>3</sub>; however, a considerable increase in the shoot was observed with the increasing level of GA<sub>3</sub>. It was worth observing that the repression was more serious in the *Osj10gBTF3*<sup>Ri</sup> seedlings compared with their WT. When treated with 100 or 200 μM GA<sub>3</sub>, 50% reduction

in shoots was observed in *Osj10gBTF3*<sup>Ri</sup> seedlings compared to their WT (Figure 4). However, shoot length was increased in *Osj10gBTF3*<sup>Ri</sup> seedlings when treated with 50 mM NaCl. These results demonstrate that inhibition of BTF3 caused significant phenotypic changes during seed germination and seedling growth; however, these changes were more serious when treated with GA<sub>3</sub> as compared to ABA and NaCl.

To assess whether these phenotypic changes in *Osj10gBTF3*<sup>Ri</sup> seedling were caused by RNAi inhibition, real-time quantitative PCR analyses were carried out using the OsBTF3-specific primers (Supplementary Table 2) to check the regulation of different variants of OsBTF3 in root and shoot at seedling stage under ABA (5 and 10 μM), GA<sub>3</sub> (100 and 200 μM), and (50 and 100 mM) salt stresses. The ubiquitin mRNA level was used as a positive control.

The results showed that the expression of *Osj3g1BTF3* was induced by low and high concentrations of GA<sub>3</sub> in root and shoot but was repressed by high concentrations of GA<sub>3</sub> in shoot (Figure 5). In contrast, the number of transcripts increased with GA<sub>3</sub> in *Osj10gBTF3*<sup>Ri</sup> seedling at 100 μM but decreased at higher concentrations. In *Osj3g2BTF3*, samples treated with 100 μM GA<sub>3</sub> had less transcripts compared with controls in root. However, the transcript level significantly increased in root and shoot at 100 and 200 μM

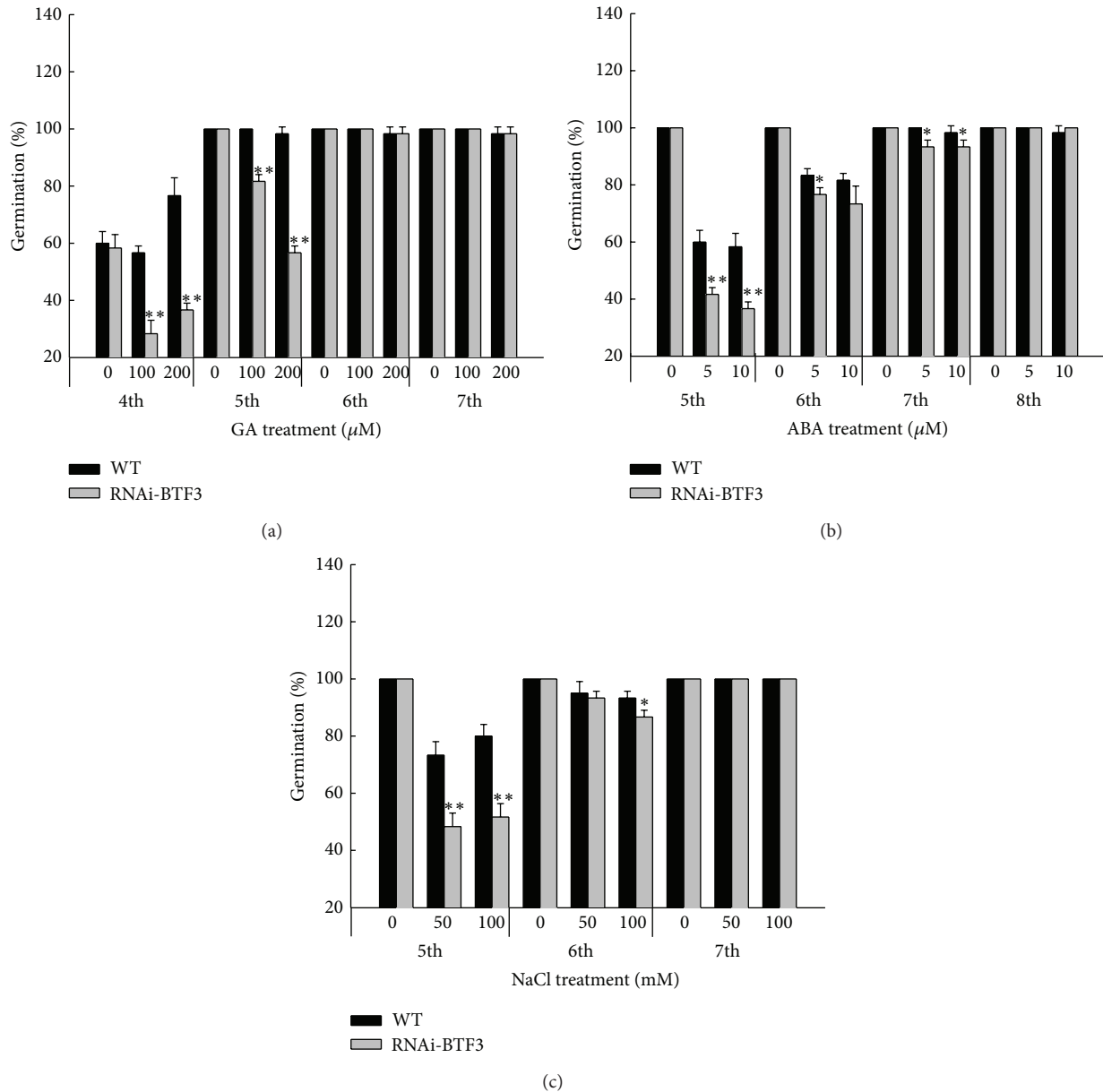


FIGURE 2: Germination of *Osj10gBTF3<sup>Ri</sup>* lines and wild-type (WT) rice seeds treated with different concentrations of GA<sub>3</sub>, ABA, and NaCl. Germination readings were taken after 4th, 5th, 6th, 7th, and 8th days. Error bars indicate the S.D. (*n* = 30). \**P* ≤ 0.05, \*\**P* ≤ 0.01 (Student's *t*-test).

GA<sub>3</sub> (Figure 5). For *Osj10gBTF3<sup>Ri</sup>* lines, the transcript level increased with GA<sub>3</sub> concentration both in root and in shoot but the transcript increased by about 50% in shoot and 100% in root treated with 100 μM GA<sub>3</sub>, respectively, as compared to controls (Figure 5) and the expression of transcript increased with the increasing concentration of GA<sub>3</sub> in both root and shoot (Figure 5). In contrast, the transcript level decreased in both root and shoot with GA<sub>3</sub> in *Osj10gBTF3<sup>Ri</sup>* seedling particularly in root at 200 μM (Figure 5).

For the ABA treatment, the number of *Osj3g1BTF3* transcripts increased with increasing level from 5 to 10 μM in the root. However, the level appeared to be significantly

decreased in shoot at 5 μM, after which the expression level increased at 10 μM (Figure 6). However, the transcript level decreased in root and shoot of *Osj10gBTF3<sup>Ri</sup>* lines after treatment with 5 μM ABA, but the expression level significantly increased in root and shoot at 10 μM ABA (Figure 6). In *Osj3g2BTF3*, the expression decreased with increasing level of ABA in the root and the transcript level increased significantly in shoot at 10 μM ABA. In *Osj10gBTF3<sup>Ri</sup>* seedling, the expression level significantly decreased in root and shoot with increasing ABA concentration (Figure 6). Similarly, in *Osj10gBTF3*, the expression of transcript increased with increasing concentration of ABA in the root. However,

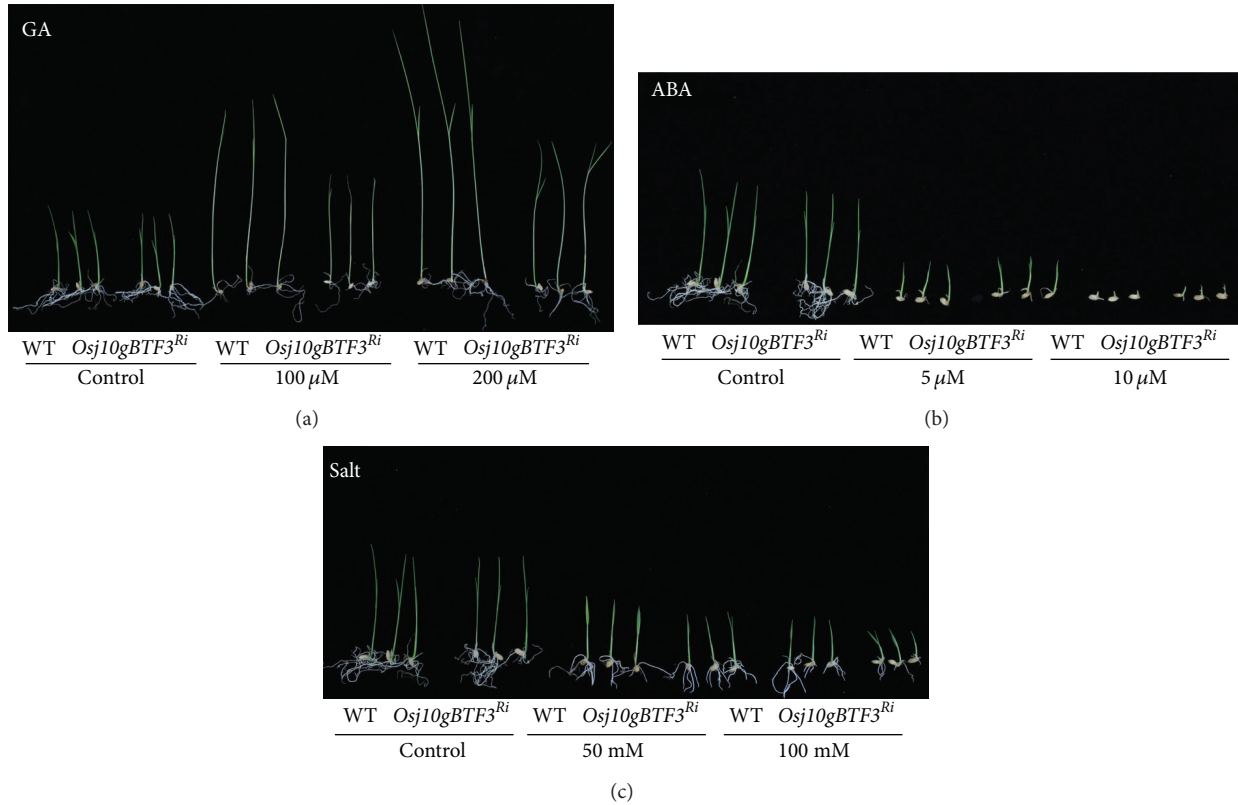


FIGURE 3: Comparison of *Osj10gBTF3<sup>Ri</sup>* lines and wild-type (WT) rice seeds grown under various concentrations of GA<sub>3</sub>, ABA, and NaCl.

the transcript level did not change significantly in shoot after treatment with ABA. In contrast, the number of transcripts decreased about 50% with ABA in *Osj10gBTF3<sup>Ri</sup>* seedling as compared to WT. These results show simply that OsBTF3 expression is mostly constitutive but slightly modulated by ABA in shoot at higher concentrations.

For salt treatment, the expression level of *Osj3g1BTF3* increased with increasing level of salt in the root and shoot particularly at 50 mM. In contrast, the transcript level decreased significantly in *Osj10gBTF3<sup>Ri</sup>* seedling at 100 mM NaCl (Figure 7), but the level of transcript was dramatically decreased about 80% in root at 50 and 100 mM as compared to control (Figure 7). In *Osj3g2BTF3*, the transcript level increased in both root and shoot with salt particularly at 50 mM; however, in *Osj10gBTF3<sup>Ri</sup>* lines, the level of expression gradually decreased in both root and shoot with increasing concentration as compared with WT (Figure 7). The samples treated with salt significantly upregulated the transcript level of *Osj10gBTF3* at 50 mM both in root and in shoot, after which the expression level decreased at 100 mM. In *Osj10gBTF3<sup>Ri</sup>* seedling, the number of transcripts decreased mainly in roots with salt as compared to control (Figure 7). These results show that the downregulation of the BTF3 gene in *Osj10gBTF3<sup>Ri</sup>* seedling may reflect the compromised protein translation and importing activity under salt stress. For hormone treatment, the induction effect showed by ABA was much more effective than the repression effect caused

by GA<sub>3</sub>. There was a difference between the two hormones, indicating that BTF3 is more sensitive to GA<sub>3</sub> than ABA.

#### 4. Discussion

Recently, it has been documented that BTF3 plays a vital role in plant growth and development [6]. However, there are no reports available about the role of OsBTF3 and how they are regulated by GA<sub>3</sub>, ABA, and NaCl during seed germination and seedling growth. In the present study, BTF3-like gene *Osj10gBTF3* was cloned and functionally analyzed and the results may help to understand OsBTF3 protein function more comprehensively.

There are numerous BTF3 genes in rice genomes and in other plant species that are highly similar to each other (Figure 1(a)). The amino acid sequences of different variants of OsBTF3 are similar to other plant species excluding variations in the lengths of the exon and intron. We have observed three signal systems which include important MMTS, NLS, and ERRS in *Osj3g1BTF3* and *Osj10gBTF3*, used to target the correct destination of new synthesized proteins [6]. It has been reported that these specific addressing systems such as MMTS [16], NLS [17], and ERRS [18] have been used by the new synthesized proteins to specifically target the correct destination [19]. A NLS “RRKKK” is putatively positioned between amino acid residues 22–26 in the N-terminal (Figure 5(a)), demonstrating that the OsBTF3

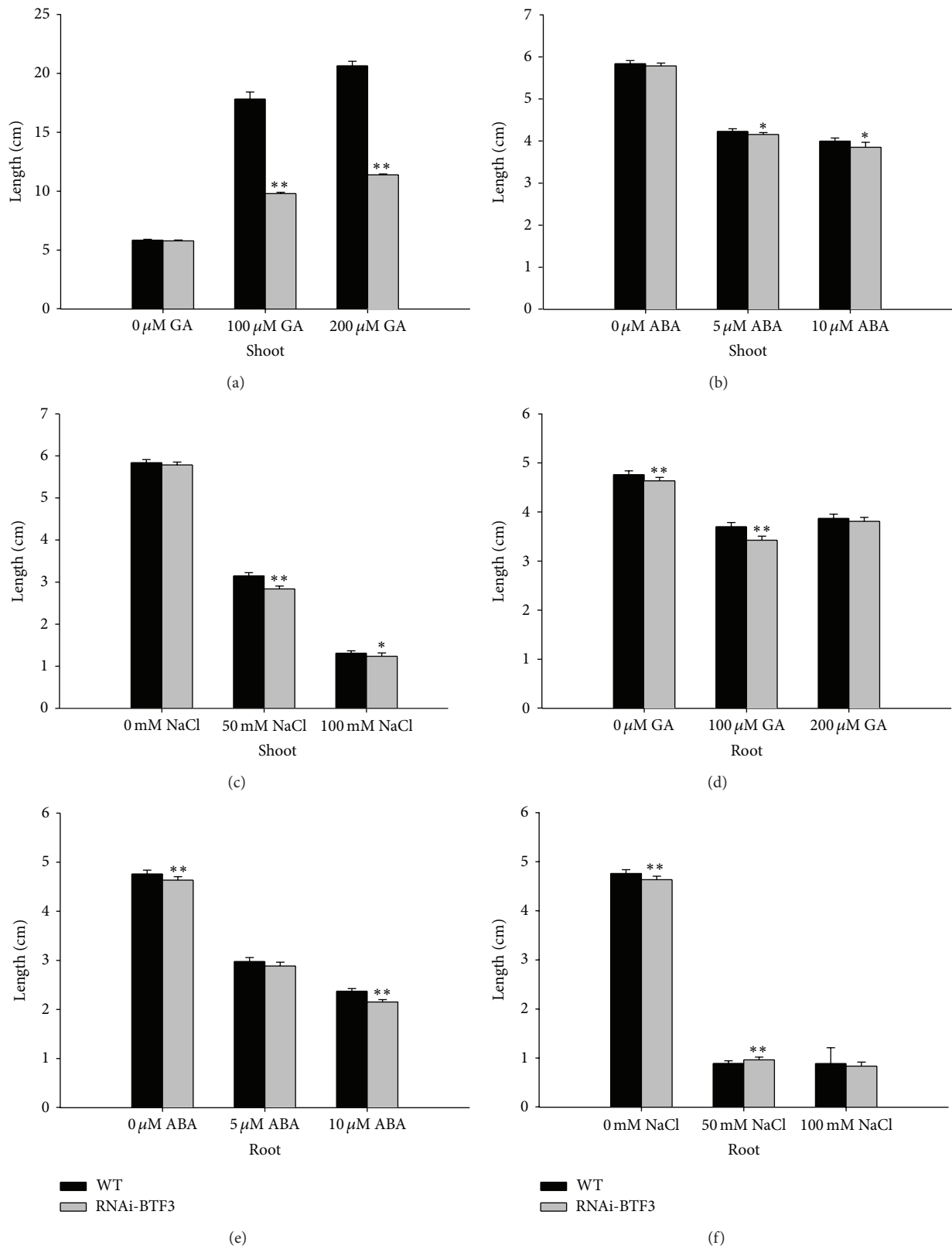
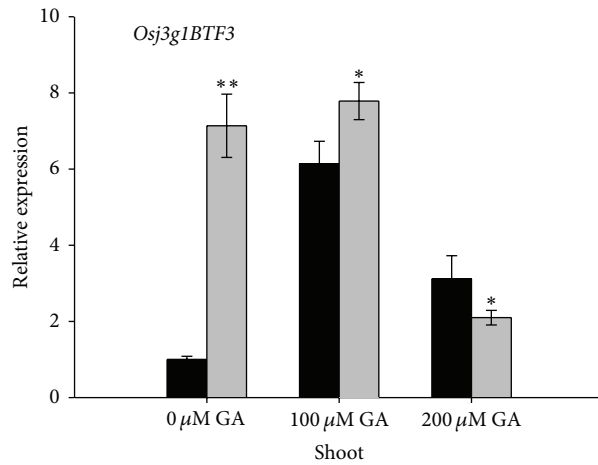
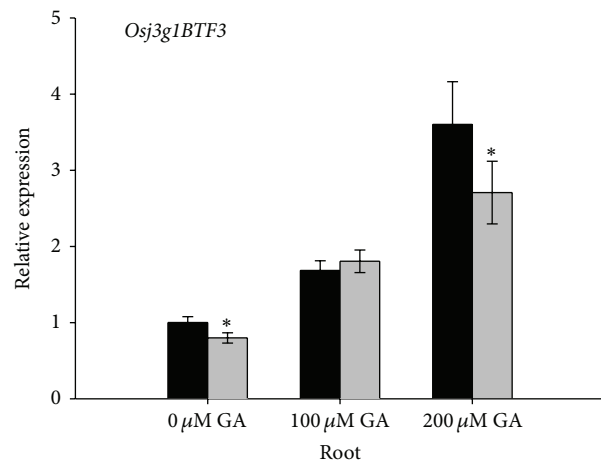


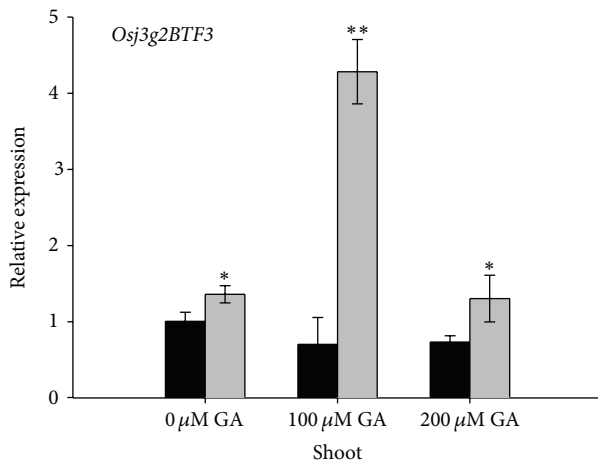
FIGURE 4: Response of *Osj10gBTF3<sup>Ri</sup>* lines and wild-type (WT) seedlings to different concentrations of GA<sub>3</sub>, ABA, and NaCl after 15 d of germination. Error bars indicate the S.D. ( $n = 30$ ). \* $P \leq 0.05$ , \*\* $P \leq 0.01$  (Student's  $t$ -test).



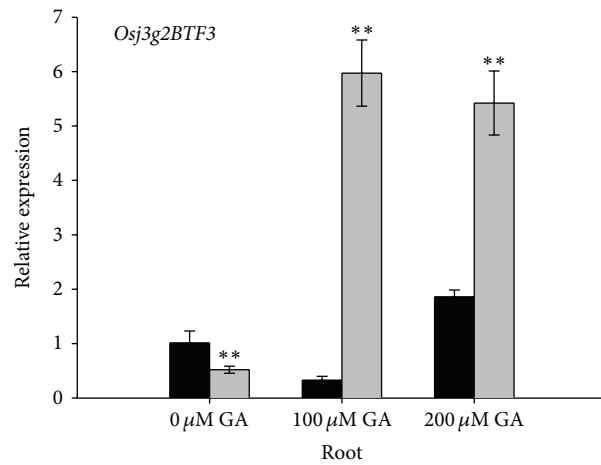
(a)



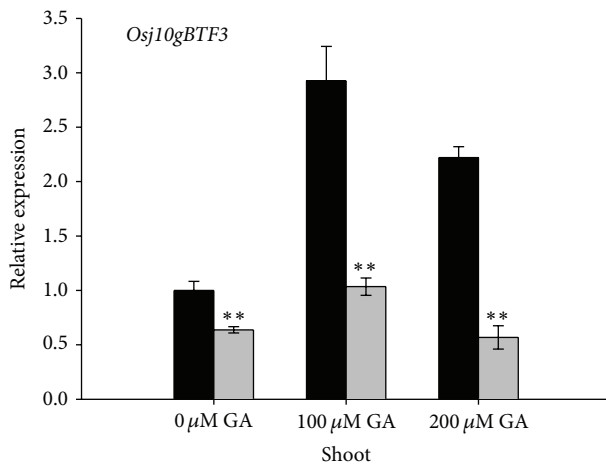
(b)



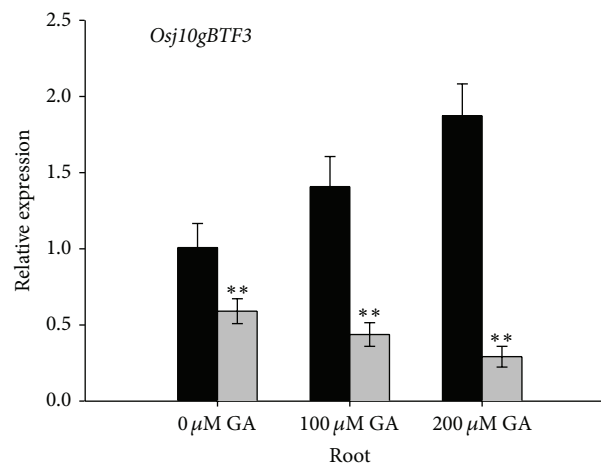
(c)



(d)



(e)



(f)

■ WT  
 ■ RNAi-BTF3

■ WT  
 ■ RNAi-BTF3

FIGURE 5: The expression analyses of *Osj10gBTF3* (LOC\_Os10g34180) and its two homologous genes *Osj3g1BTF3* (LOC\_Os03g01910) and *Osj3g2BTF3* (LOC\_Os03g63400) in root and shoot of *Osj10gBTF3*<sup>RI</sup> lines and wild-type (WT) under different concentrations of GA<sub>3</sub>. The detection was done based on three independent samples and ubiquitine was used as internal control.



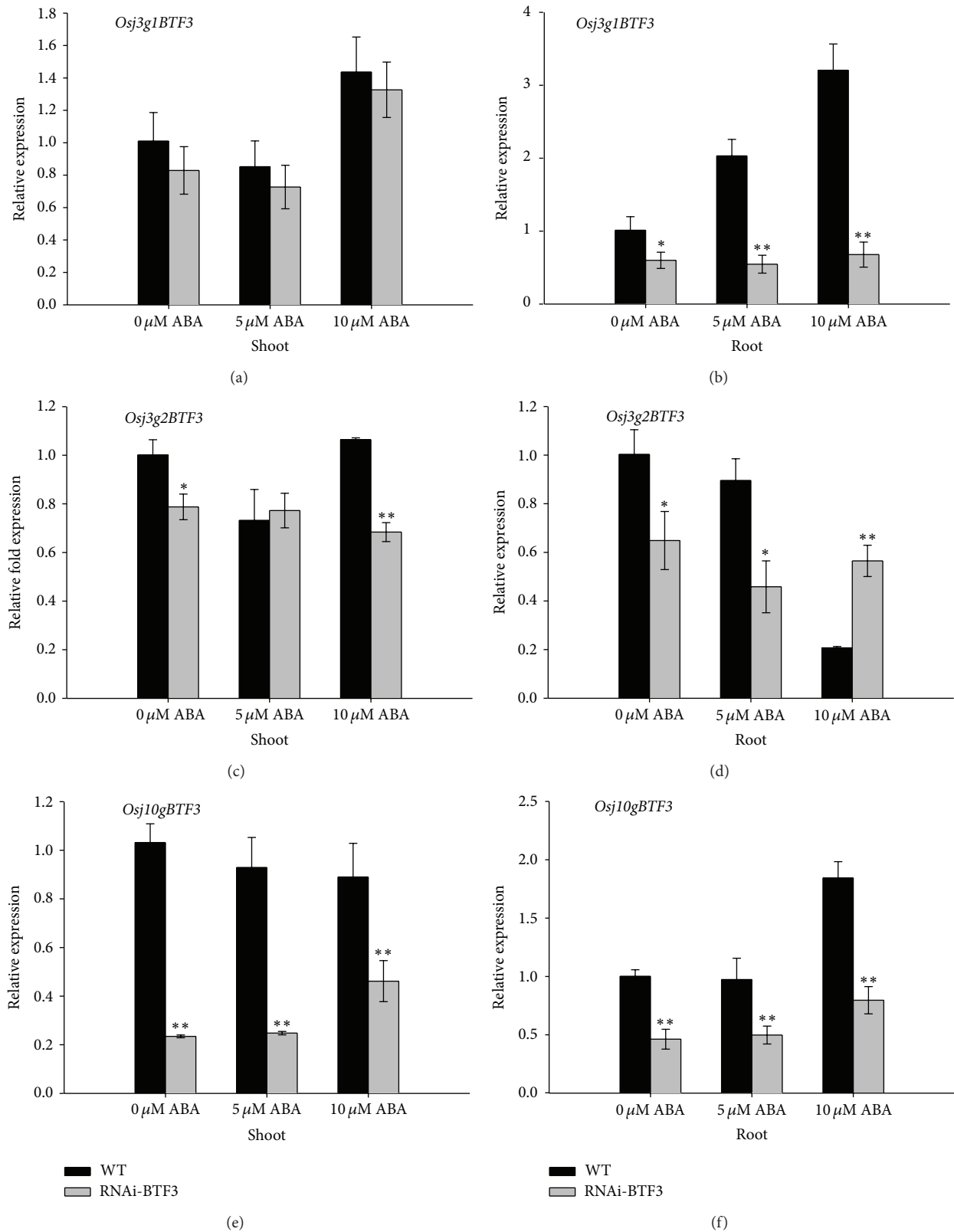
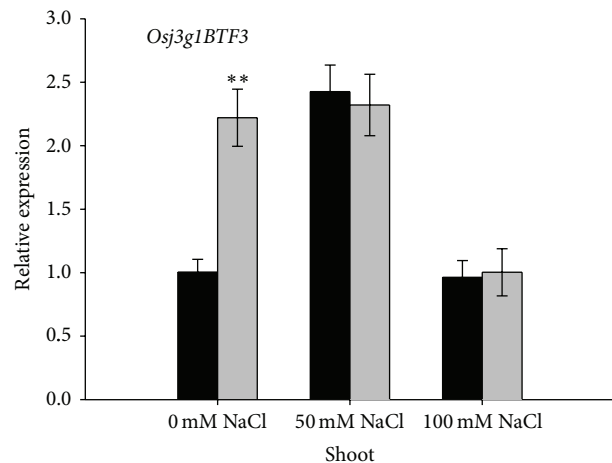
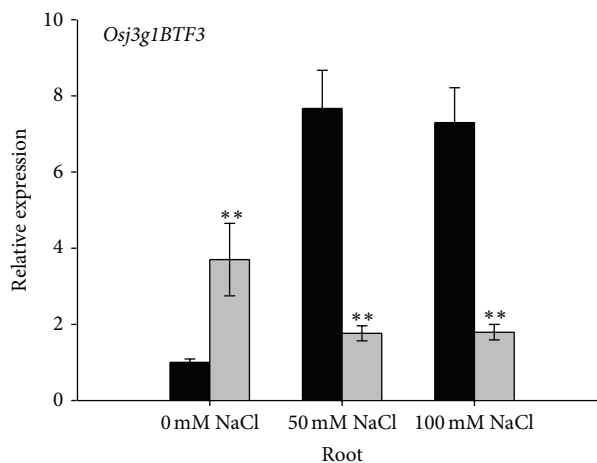


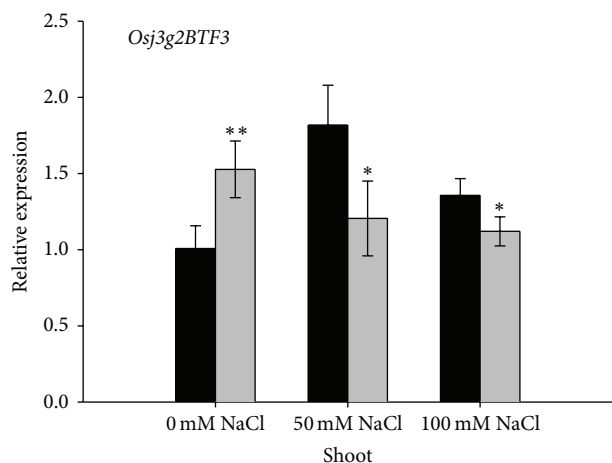
FIGURE 6: The expression analyses of *Osj10gBTF3* (LOC\_Os10g34180) and its two homologous genes *Osj3g1BTF3* (LOC\_Os03g01910) and *Osj3g2BTF3* (LOC\_Os03g63400) in root and shoot of *Osj10gBTF3*<sup>Ri</sup> lines and wild-type (WT) under different concentrations of ABA. The detection was done based on three independent samples and ubiquitine was used as internal control.



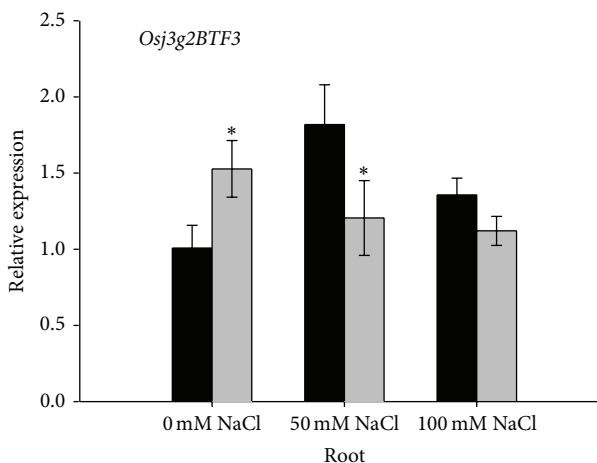
(a)



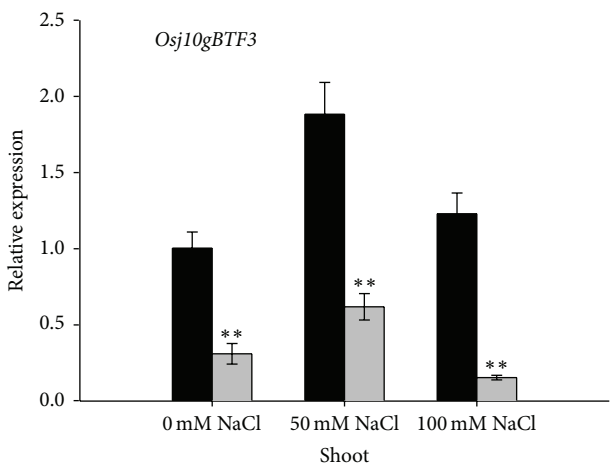
(b)



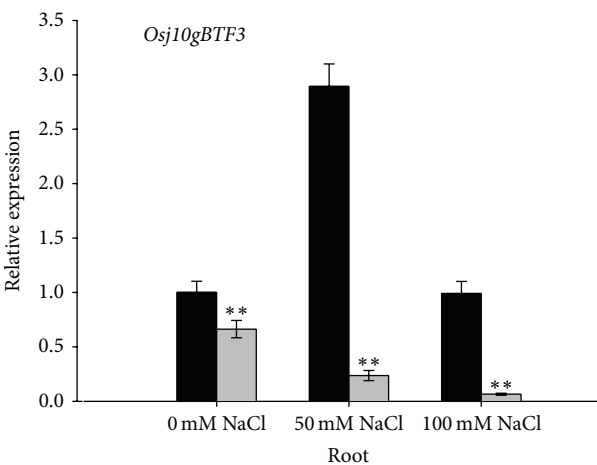
(c)



(d)



(e)



(f)

■ WT  
 ■ RNAi-BTF3

■ WT  
 ■ RNAi-BTF3

FIGURE 7: The expression analyses of *Osj10gBTF3* (LOC\_Os10g34180) and its two homologous genes *Osj3g1BTF3* (LOC\_Os03g01910) and *Osj3g2BTF3* (LOC\_Os03g63400) in root and shoot of *Osj10gBTF3<sup>Ri</sup>* lines and wild-type (WT) under different concentrations of salt. The detection was done based on three independent samples and ubiquitine was used as internal control.

protein might be located in both cytoplasmic membrane system and nucleus [6]. It is well established that BTF3 is not only a cytoplasmic membrane protein that functions in the targeting and translocation of nascent polypeptides [20, 21] but may also play an important role in the nucleus, affecting the transcription levels of some genes in rice [22–24].

It was observed that *Osj10gBTF3* and *Osj3g1BTF3* are probably similar but different from *Osj3g2BTF3* in biological function. To verify this, BTF3-like gene *Osj10gBTF3* was cloned and functionally analyzed during seed germination and seedling growth. In the present study, it was observed that the *Osj10gBTF3<sup>Ri</sup>* lines were sensitive to GA<sub>3</sub>, ABA, and NaCl treatment, particularly to GA<sub>3</sub> since their applications did not affect final germination percentage but caused WT seeds to germinate more rapidly than the *Osj10gBTF3<sup>Ri</sup>* transgenic lines, and their mean germination rate decreased with increased concentrations of GA<sub>3</sub>, ABA, and NaCl. Similarly, seedling growth was inhibited compared with that of WT control, particularly when treated with 100 or 200 μM GA<sub>3</sub>; 50% reduction in shoots were observed in *Osj10gBTF3<sup>Ri</sup>* seedlings (Figure 5). RNAi inhibition of *Osj10gBTF3* reduced the expression of *Osj10gBTF3* transcripts, as a result reducing the manufacturing of the essential amount of NAC complex for normal plant growth. Hence, once the transcript level of *Osj10gBTF3* is decreased, it disturbed the function of this monomeric subunit plus the complete NAC, resulting in severe vegetative defects in *Osj10gBTF3<sup>Ri</sup>* lines [6]. It has been reported that the repression of *Osj10gBTF3* caused serious plant growth and development defects [6]. These results are similar to some other studies demonstrating that BTF3 take part in cell cycle regulation via interaction with cyclins [25]. Mutants lacking NAC also showed visible growth and morphological problem in yeast and tobacco [20, 26]. Furthermore, the inhibition of BTF3 participated in the repression of transcription and protein synthesis in apoptotic K562 cells [27], while change in BTF3 expression is linked with apoptosis in BL60 Burkitt lymphoma cells [28]. On the basis of these results, we can conclude that BTF3 plays an important role in seed germination and seedling growth.

To further verify these results, we observed expression of different variants of OsBTF3 in root and shoot in *Osj10gBTF3<sup>Ri</sup>* and WT seedlings. We observed that the expression of different variants of OsBTF3 was primarily constitutive, being generally modulated by different GA<sub>3</sub>, ABA, and salt stress treatments in *Osj10gBTF3<sup>Ri</sup>* and WT seedlings (Figures 5, 6, and 7). The expression of *Osj3g1BTF3* and *Osj10gBTF3* was induced by low and high concentrations of GA<sub>3</sub> and ABA in root and shoot (Figures 5 and 6), but the transcript level decreased in shoot at high concentrations (Figures 5 and 6). In *Osj10gBTF3<sup>Ri</sup>* seedling, the transcript level increased both in root and in shoot with increasing GA<sub>3</sub> and ABA concentration, whereas the transcript level of *Osj10gBTF3* decreased with increasing GA<sub>3</sub> concentration (Figures 5 and 6). In *Osj3g2BTF3*, seedling treated with 100 μM GA<sub>3</sub> had less transcripts, but the level significantly increases in root at 100 μM GA<sub>3</sub> (Figure 5), whereas, in *Osj10gBTF3<sup>Ri</sup>* lines, the transcript level increased both in root and shoot with GA<sub>3</sub> concentration (Figure 5).

In *Osj3g2BTF3* the transcript levels decreased with increasing level of ABA in the root but increased in shoot at 10 μM ABA. In *Osj10gBTF3<sup>Ri</sup>* seedling, the expression level significantly decreased in root and shoot with increasing ABA concentration (Figure 6). Wang et al. [6] observed a 62% increase in *Osj10gBTF3* expression level with ABA, compared to a 22% reduction with GA<sub>3</sub> after treatment with the same concentration for 12 h. In previous reports, it has been observed that the transcript level of *NbBTF3* remained unaffected when exposed to 100 μM of ABA and GA<sub>3</sub> [26]. Similarly, He et al. [29] did not find any significant change in *SmBTF3*, when treated with ABA for 24 hrs. By using 100 μM of ABA to treat maize (*Zea mays* L.) plants, Zhang et al. [30] found that *ZmBTF3* (*Zea mays* BTF3) expressions were significantly decreased. The salt concentration upregulated *Osj3g1BTF3*, *Osj3g2BTF3*, and *Osj10gBTF3* expression at 50 mM in both root and shoot, but, in *Osj10gBTF3<sup>Ri</sup>* seedling, the number of transcripts decreased mainly in roots with salt as compared to control (Figure 7). Wang et al. [6] have observed that the expression of *Osj10gBTF3* was downregulated under high salt concentration, while low salt concentrations upregulated *Osj10gBTF3* expression in rice. Similarly, Li et al. [4] demonstrated that transcript level of OsBTF3 was significantly reduced in rice plants under salt stress and the salt resistance was improved in the seedlings of OsBTF3 transgenic lines. In maize, Zhang et al. [30] observed that expression of BTF3 was downregulated at 250 mM NaCl. In contrast, BTF3 transcript level was significantly increased by salt stress in *Suaeda asparagoides* [31], while expression level of *SabBTF3* was differentially regulated by various abiotic stresses, such as drought, salinity, ABA, and temperature in roots [7]. These results also suggested that *Osj3g1BTF3* and *Osj10gBTF3* are much similar in their response to GA<sub>3</sub>, ABA, and NaCl during seedling stage.

This is a first report demonstrating the involvement of different variants of BTF3 in seed germination and seedling stage. It was observed that *Osj3g1BTF3* and *Osj10gBTF3* are a similar type of genes which plays an important role in plant growth, especially in seed germination and seedling growth. Functional analysis revealed that both of these genes are much similar in biological functions. Further investigation is required to provide the insight into the functions of BTF3 and the connected mechanisms responsible for improving abiotic stress tolerance in plants.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Authors' Contribution

Wenyi Wang and Muhammad Jamil contributed equally to this work.

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