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BolOST1, an ortholog of Open Stomata 1 with alternative splicing products in *Brassica oleracea*, positively modulates drought responses in plants

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ABSTRACT

Open Stomata 1 (OST1), an ABA-activated sucrose non-fermenting 1 (SNF1)-related protein kinase, is critical for plant drought responses. We investigated the functions of two splicing isoforms of the OST1 ortholog in *Brassica oleracea* (BolOST1). *BolOST1* expression was found to be dramatically induced by drought and high-salt stress, and the ectopic expression of *BolOST1* restored the drought-sensitive phenotype of *ost1*. Subcellular localization revealed that BolOST1 is localized in both the nucleus and cytoplasm. BolOST1 was also demonstrated to phosphorylate the N-terminal fragment of ABI5 (ABA Insensitive 5, ABI5-N). A firefly luciferase complementation assay revealed that BolOST1 interacts with both BolABI5 and an ABI1 ortholog in *B. oleracea* (BolABI1). Overall, these results suggest that *BolOST1* is a functional SnRK2-type protein kinase and that the early ABA signaling network may be conserved between Arabidopsis and cabbage.

1. Introduction

Plants have developed complex signaling networks to alleviate environmental stresses and ensure sustainable growth and development (reviewed by Cutler et al. [1]). When challenged by environmental stresses, especially drought and high-salt conditions, endogenous ABA significantly increases, which is a requirement for the establishment of adaptive responses in plants [1]. The stimulation of regulators, such as sucrose non-fermenting related kinase (SnRK)-2 subfamily members, is central to this physiological response [1].

Open Stomata 1 (OST1)/SnRK2.6/SnRK2E, the best-known member of the SnRK2 family, is involved in the sophisticated orchestration of both the "short-term" (stomata closure) and "long-term" (gene expression) ABA responses in plants [2–5]. Two other SnRK2-type kinases, SnRK2.2 and SnRK2.3, also play key roles in ABA signaling [6]. Studies of mutants that are deficient in these three SnRK2s have shown that they are crucial for plant ABA sensitivity and ABA-dependent drought tolerance [7].

The recently reported double-negative regulatory mechanism in the core module of early ABA signaling denotes that an OST1-type protein kinase is activated via the ABA receptor (PYR/RCAR) and protein phosphatase type-2C (PP2C) proteins [1]. Upon activation, OST1 is able to immediately activate ion channels in guard cells, including SLAC1 and QUAC1, which is essential for stomata closure [8,9]. To establish the long-term response, activated OST1 can phosphorylate the ABI5 subfamily of bZIP transcription factors [4].

Orthologs of the SnRK2-type protein kinases have been identified in various plant species [10–13]. For example, 10 SnRK2-type protein kinases have been reported in rice and are differentially activated by hyperosmotic stress and abscisic acid [10]. In addition to the first cloned SnRK2 gene *PKABA1* (ABA-induced protein kinase 1), three other *SnRK2* genes have been recently characterized in wheat [12–14]. Functional studies have revealed that ZmOST1 (an ortholog of OST1 in maize) initiates the closure of stomata possibly via the phosphorylation of ZmSNAC1 [11].

RNA processing and alternative splicing in particular plays an important role in the regulation of post-transcriptional gene expression in eukaryotes [15]. Recently, evidence of a functional link between ABA signaling and RNA processing has been reported [15]. In Arabidopsis, several SnRK2 alternative splicing isoforms have been identified, including OST1 [16].

Cabbage (*Brassica oleracea* var. capitata L.) is an important vegetable for economic and oil resources worldwide. We recently identified an ABA Insensitive 5 ortholog that functions as ABI5 in the positive modulation of ABA responses in *oleracea* (BolABI5)

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[17]. More recently, we also isolated an ABA-insensitive 1 ortholog in *B. oleracea* (BolABI1) that is possibly involved in the negative modulation of plant ABA responses as ABI1 [18]. However, the early molecular mechanism of ABA signaling in cabbage remains elusive. Here, we describe the identification and characterization of two alternative splicing products of BolOST1, an ortholog of OST1 in *B. oleracea*. Our data indicate that BolOST1 could compensate for OST1 in the modulation of plant drought stress responses and that the early ABA signaling network may be conserved between Arabidopsis and cabbage.

2. Materials and methods

2.1. Plant materials, growth conditions and stress treatments

Plants of wild-type Col-0, *ost1* (SALK_008068) and transgenic plants harboring *Myc-BolOST1* (*ost1*::*Myc-BolOST1*) were grown as previously described [17]. For protoplast isolation, plants were grown in a growth chamber with 45% humidity under a 12-h daylight cycle at 20 °C (night) and 23 °C (day). For the abiotic stress and exogenous hormone treatments, 11-day-old seedlings of cabbage (*B. oleracea* var. capitata L.) were treated with abiotic stresses (300 mM NaCl, −1.7 MPa PEG 8000 and drought) and hormones (0.1 mM ABA), followed by sampling at 0, 4, 8, 12, 16, 20 and 24 h as previously described [17].

The responses of plants to drought stress were measured as previously described [19]. Briefly, three-week-old plants were subjected to drought stress by withholding water, and photos were taken when many of the *ost1* plants of were wilted (approximately 21 days).

2.2. Plasmid construction

To produce Myc-tagged BolOST1-1 and BolOST1-2, the full-length coding sequences (CDSs) were amplified with the primers BolOST1-BamF, 5′-CGCGGATCCATGGACCGACCAGCAGTGAGTG-3′, and BolOST1-woSalR, 5′-ACGCGTCGACCATTGCGTACACAATCTCTC-3′. The sequence-confirmed CDSs were then cloned into the *Bam*HI and *Sal*I sites of the binary expression vector p1307-Myc vector [17].

To construct GFP-tagged BolOST1-1 and BolOST1-2, the full-length CDSs were cut from p1307-Myc-BolOST1-1 and p1307-Myc-BolOST1-2 and inserted into Cam-35S-GFP vector at the *Bam*HI and *Sal*I sites, respectively.

To produce GST-tagged BolOST1-1 and BolOST1-2, the full-length CDSs were removed from p1307-Myc-BolOST1-1 and p1307-Myc-BolOST1-2 and cloned into the pGEX-6p-1 vector (GE Healthcare) at the *Bam*HI and *Sal*I sites, respectively. To produce GST-tagged ABI5-N, the CDS of the N-terminal 248 amino acids of ABI5 was inserted into the pGEX-6p-1 vector at the *Bam*HI and *Sal*I sites with the primers ABI5-10BamF, 5′-CGGGATCCTCAGAGCG AGAAGTAGAGTCGTC-3′, and ABI5-N-SalR, 5′-ACGCGTCGACATCAC CGGTTCCTTGAAACAC-3′.

To detect the interactions between ABI5, BolABI5, BolABI1 and BolOST1 using the firefly luciferase complementation technique, the CDSs of ABI5, BolABI5 and BolABI1 were cloned into the p2305-cLUC vector at the *Kpn*I and *Sal*I sites using following primers: for ABI5, ABI5-KpnF: 5′-CGGGGTACCATGGTAACTAGAGAAACG AAGTTG-3′ and ABI5-SalR: 5′-ACGCGTCGACTTAGAGTGGACAACTC GGGTTC-3′; for BolABI5, BolABI5-KpnF: 5′-GGGGTACCATGATGTCT GGACGAGAAGTAG-3′ and BolABI5-woSalR: 5′-ACGCGTCGACGA-GAGGGCAACTAGGGTTCCTC-3′; and for BolABI1, BolABI1-KpnF: 5′-GGGGTACCATGGAGGAAGTATCACCGGCGGTTTC-3′ and BolABI1-woSalR: 5′-ACGCGTCGACAGGCTTTTTGATCTTGAGTTTC-3′. The CDS fragments of BolOST1-1 and BolOST1-2 were removed from p1307-Myc-BolOST1-1 and p1307-Myc-BolOST1-2 and cloned into the p2305-nLUC vector at the *Bam*HI and *Sal*I sites, respectively.

2.3. Real-time quantitative reverse transcription PCR (qRT-PCR) analysis

The total RNA of the samples was extracted using RNAiso Plus reagent (TaKaRa). A total of 5 μg of treated RNA was used for reverse transcription with the PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa), according to the manufacturer's instructions. qRT-PCR was performed using a CFX 96 real-time PCR machine (Bio-Rad, Hercules, CA, USA) and SYBR Premix Ex Taq kit (TaKaRa) to monitor the double-stranded DNA products [17]. The thermal cycling consisted of a hold at 95 °C for 3 min followed by 40 cycles of 95 °C for 10 s, 55 °C for 10 s and 72 °C for 30 s. After amplification, the samples were held at 95 °C for 10 s, 85 °C for 5 s and 95 °C for 10 s to perform a melt-curve analysis. The relative expression of *BolOST1* was normalized to the expression of cabbage *ACTIN2* and expressed relative to that in mock-treated seedlings or young leaves. The following primer pairs were used for amplification: *BolOST1*, 5′-TGCAGAAGCGACTGTTCC-3′ and 5′-CCGCTACTGTCGATGTCAAG-3′, and for cabbage *ACTIN2*, 5′-GCAGACCGTATGAGCAAAG-3′, and 5′-GCTGAGGGAAGCAAGAATG-3′.

2.4. Subcellular localization and firefly luciferase complementation assay

The plasmids Cam-BolOST1-1-GFP and Cam-BolOST1-2-GFP and the firefly luciferase complementation assay-related cLUC/nLUC vectors were isolated and purified via a Plasmid Maxiprep Kit (Vigorous Biotechnology) and introduced into Arabidopsis leaf mesophyll protoplasts according to the PEG-Ca^{2+} protocol provided by the Sheen lab [20]. Briefly, leaf mesophyll protoplasts were isolated via the digestion of 0.5–1-mm leaf strips in a cellulase/macerozyme solution (1.5% cellulase R10 (Yakult Honsha, Tokyo, Japan), 0.4% macerozyme R10 (Yakult Honsha, Tokyo, Japan), 0.4 M mannitol, 20 mM KCl, 20 mM MES pH 5.7, 10 mM $CaCl_2$, 5 mM mercaptoethanol and 0.1% BSA). The protoplasts was washed twice with W5 solution (154 mM NaCl, 125 mM $CaCl_2$, 5 mM KCl and 2 mM MES pH 5.7) and resuspended in MMg solution (0.4 M mannitol, 15 mM $MgCl_2$ and 4 mM MES pH 5.7) at 1–2 $\times 10^5$/ml immediately prior to transfection. For transfection, 20 μg of plasmid was added to 200 μl of protoplasts in a microfuge tube and mixed well with 220 μl of PEG-Ca^{2+} solution (4 g of PEG 4000, 3 ml of H_2O, 2.5 ml of 0.8 M mannitol and 1 ml of 1 M $CaCl_2$). After 15 min of transfection, the protoplasts were washed twice with W5 solution, resuspended at approximately 2–4 $\times 10^4$/ml in W5 solution and incubated in a growth chamber for 12–18 h.

For the subcellular localization analysis, the fluorescence of BolOST1-1-GFP and BolOST1-2-GFP was detected, and images were collected using an inverted fluorescence Zeiss LSM 510 META confocal microscope after 12–18 h of incubation as previously described [21].

The firefly luciferase complementation assay was performed as previously described [22]. Briefly, 10 μl of 3.3 μM luciferin (Sigma–Aldrich) solution was added to transfected protoplasts prior to the luciferase (LUC) activity measurement. A Tecan Infinite M200 microplate reader (Tecan Austria GmbH) was used to measure the relative LUC activity according to the protocol of Magellan standard 7.1 provided by the manufacturer.

2.5. Recombinant protein purification and kinase assay

The recombinant protein purification and kinase assay were performed as previously described [21]. Briefly, the expression of recombinant proteins was induced with 0.3 mM IPTG, and these proteins were purified with Glutathione Sepharose™ 4B (GE Health-

care), according to manufacturer's instructions. The reactions were initiated by mixing 10 μg of BolOST1-1 or BolOST1-2 and 15 μg of ABI5-N or 2 μg of MBP (myelin basic protein) in a 25-μl system with kinase assay reaction buffer (20 mM Tris–HCl pH 7.2, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 10 μM ATP and 2 mM DTT) supplemented with 1 μCi [γ-^{32}P]-labeled ATP. The kinase assay was terminated after incubation at 30 °C for 30 min. The reaction products were separated via 12% SDS–PAGE analysis, stained with Coomassie brilliant blue (CBB) R-250 and exposed on a storage phosphor screen. The phosphorylation activities were visualized using a Typhoon 9410 Phosphorimager (Amersham Biosciences, GE Healthcare).

3. Results and discussion

3.1. Isolation and sequence analysis of two alternative transcripts of BolOST1 in cabbage

Two fragments of *BolOST1*, designated *BolOST1-1* and *BolOST1-2* (GenBank Accession Nos. KF577724 and KF577725), were isolated from cabbage (*B. oleracea* var. capitata L.) and encode predicted proteins with 362 and 319 amino acids, respectively. The calculated molecular masses of *BolOST1-1* and *BolOST1-2* are 40.6 and 35.6 kDa, and the predicted pI values are 4.72 and 4.49, respectively. The sequence analysis showed that the two *BolOST1* cDNAs are transcribed from the same initiation site. An exon skip was present in the splicing variants, resulting in a deletion of 43 amino acids in BolOST1-2 (Fig. 1A). The sequence analysis revealed that BolOST1-1 and BolOST1-2 contain nearly all of the conserved regions of OST1 [23], including the kinase domain at the N-terminus and the ABA box at the C-terminus (Fig. 1A). A phylogenetic analysis using the neighbor-joining method indicated that BolOST1-1 shares an even deeper evolutionary homology with Arabidopsis OST1 than with BolOST1-2 (Fig. 1C). OST1 is involved in orchestrating ABA-dependent drought responses in plants [2,5,8,9]. A high degree of amino acid identity was observed among BolOST1-1, BolOST1-2 and Arabidopsis OST1 (Fig. 1A and C), which prompted us to further investigate the role of BolOST1-1 and BolOST1-2 in plants.

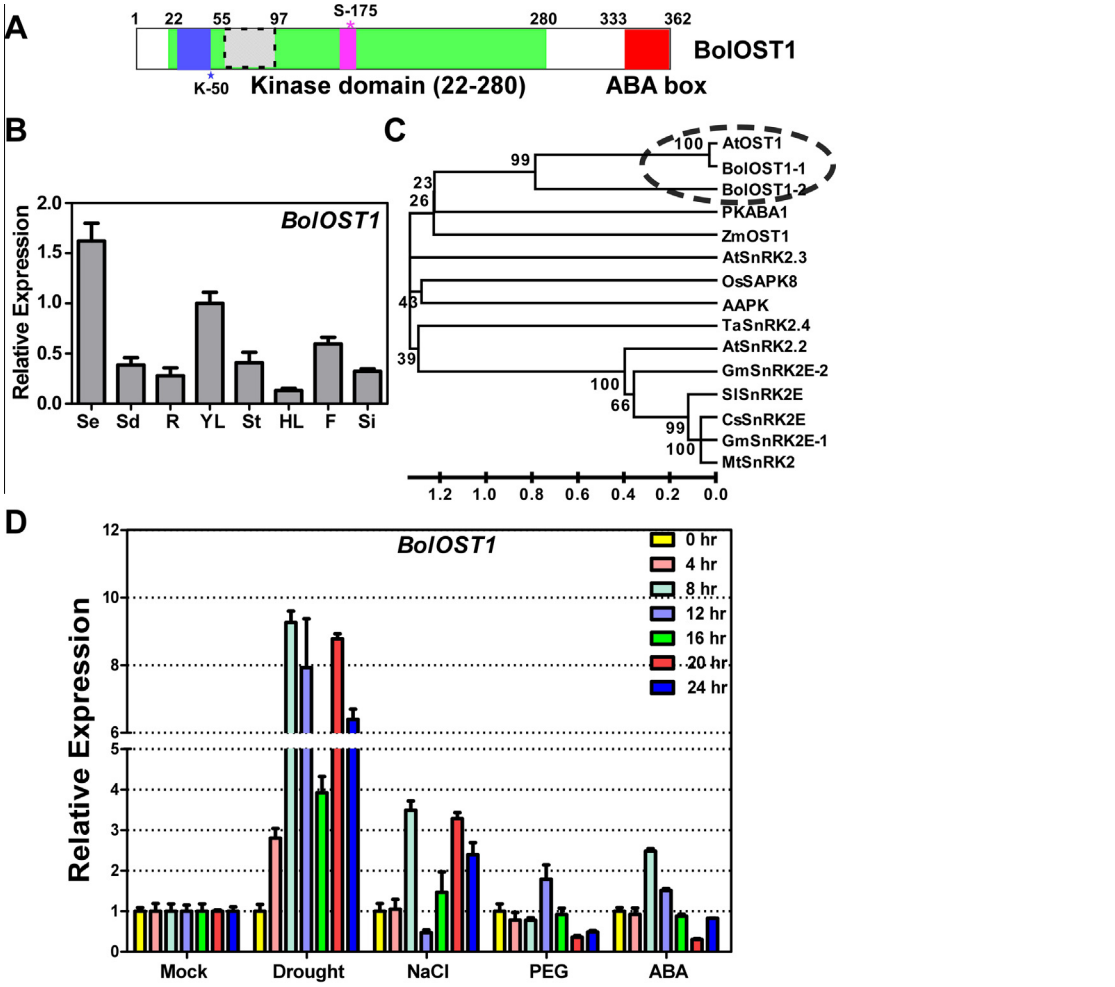

Fig. 1. Domain organization, sequence analysis and expression patterns of *BolOST1*. (A) Schematic diagram of domains of the BolOST1 protein. The ATP-binding motif and site (K-50) are shown by the blue box and blue star, respectively; the kinase domain is shown in the green box; the kinase activation loop and site (S-175) are shown by the pink box and pink star, respectively; the ABA box is shown in the red box; and the spliced amino acid residues in BolOST1-2 are shown in the gray box with the marginal dotted line. (B) Sequence relationships between BolOST1-1, BolOST1-2 and their orthologs. The dendrogram was generated using the neighbor-joining method with MEGA 5.1 (http://www.megasoftware.net/). BolOST1-1, BolOST1-2, *Brassica oleracea* OST1-like protein isoform 1 and 2; AtOST1 (NP_567945.1), AtSnRK2.2 (F4J0N1), AtSnRK2.3 (NP_201489.1); PKABA1 (AAM75356.1); ZmOST1 (ACG36261.1); OsSAPK8 (NP_001050653.1); AAPK (AAF27340.1); TaSnRK2.4 (ACU65228.1); GmSnRK2E-1 (XP_003555707.1); GmSnRK2E-2 (XP_003555708.1); SlSnRK2E (XP_004230794.1); CsSnRK2E (XP_004136995.1); MtSnRK2 (AFK33688.1). (C) RT-PCR analysis of *BolOST1* expression in various tissues. Se, seeds; Sd, seedling; R, root; YL, young leaves; St, stem; HL, heading leaves; F, flower; Si, silique. The relative expression of *BolOST1* was normalized to the expression of cabbage *ACTIN2* and expressed relative to the level in the leaves. (D) qRT-PCR analysis of the expression pattern of *BolOST1* under various environmental stress conditions and after treatment with exogenous hormones. The relative expression of *BolOST1* was normalized to the expression of the cabbage *ACTIN2* gene and expressed relative to the level in mock-treated seedlings. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. BolOST1 is mainly expressed in young leaves and induced by drought and salt

It is well known that gene expression patterns may be associated with specific functions. The expression of *TaSnRK2.7* and *TaSnRK2.8* is strongly induced by PEG, NaCl and cold [12,13]. Therefore, we determined the expression patterns of *BolOST1* in different tissues and in response to different abiotic stresses. As there are only a few differences between *BolOST1-1* and *BolOST1-2*, different primer pairs flanking the skipped exon were designed to detect the expression of these fragments. However, more than 10 tested primer pairs in qRT-PCR analyses failed to identify a difference in their expression (data not shown). Therefore, the combined expression of *BolOST1-1* and *BolOST1-2* was analyzed using a primer pair that covers the identical 3′ regions of *BolOST1-1* and *BolOST1-2*. As shown in Fig. 1B, the *BolOST1* transcripts were highly expressed in the seeds and leaves and were significantly induced within 8 h of the drought and NaCl treatments (Fig. 1D). The inducible expression of *BolOST1* by drought increased until it plateaued at 8 h followed by a minor decrease at 16 h and increase after 20 h (Fig. 1D). *BolOST1* was significantly induced by high-salt conditions at 8 h followed by a rapid decrease at 12 h and increase after 20 h (Fig. 1D). The expression of *BolOST1* was not obviously induced by the PEG and ABA treatments within the tested time course (Fig. 1D). As mentioned above, gene expression patterns often provide insight into gene function; therefore, the inducible expression pattern of *BolOST1* suggests that it participates in plant drought responses.

3.3. BolOST1 rescues the lack of OST1 function in drought stress signaling

In Arabidopsis, OST1 prevents water loss in leaves via the modulation of stomatal closure [2,8]. To address whether *BolOST1* is also important for plant drought stress signaling, we generated transgenic lines carrying *BolOST1-1* (*ost1*::*Myc-BolOST1-1*) and examined their responses to drought. Two independent transgenic lines of the T3 generation were chosen to test the sensitivity of plants to drought. As previously reported [3], *ost1* plants are sensitive to drought; many plants began to wilt after approximately 12 d of water withholding, and many leaves turned white or yellow after approximately 20 d (Fig. 2A and B). In contrast with *ost1*, transgenic plants harboring *Myc-BolOST1-1* displayed a tolerance to drought similar to that of Col-0 plants (Fig. 2A).

To compare the biological functions of *BolOST1-1* and *BolOST1-2* in drought stress signaling, we also constructed *ost1* transgenic lines harboring *BolOST1-2* (*ost1*::*Myc-BolOST1-2*). Interestingly, the *BolOST1-2* overexpression lines also complemented the drought stress-sensitive phenotype of *ost1* (Fig. 2B). The number (relative to the total number tested) and fresh weights of the

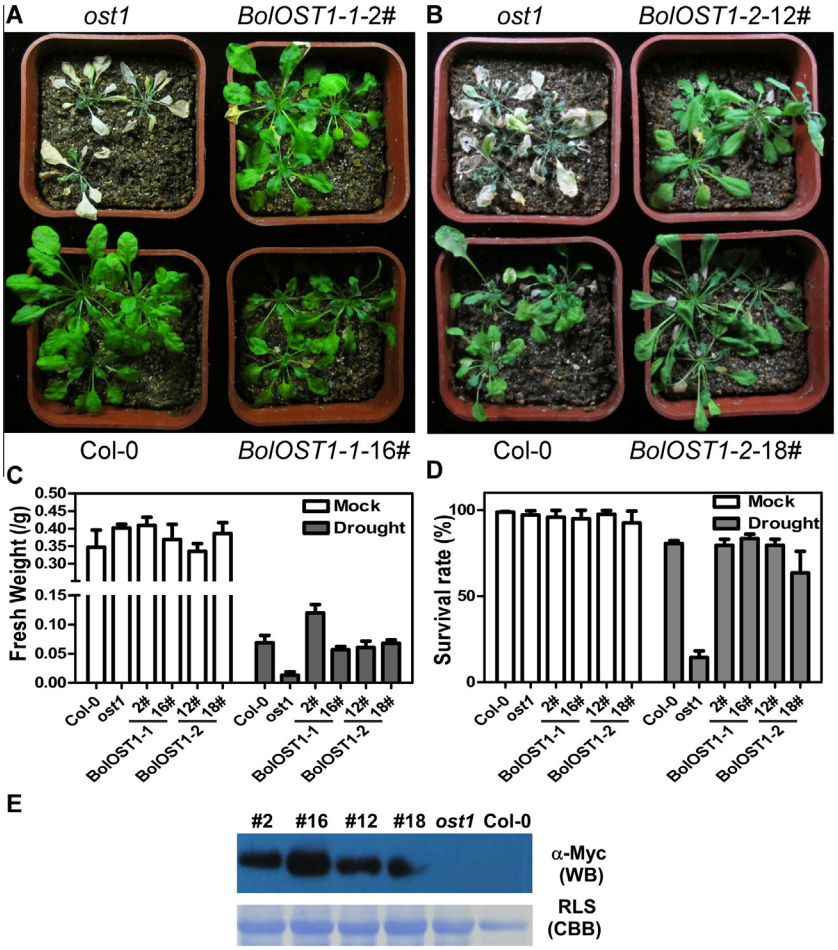

Fig. 2. Heterogeneous expression of *BolOST1-1* and *BolOST1-2* rescues the drought-sensitive phenotype of *ost1*. (A, B) Responses of plants to drought stress. Three-week-old plants were subjected to drought stress by withholding water and were photographed after 21 days. (C, D) Quantification of the fresh weight and the number of surviving plants (out of the total number tested) for each treatment. Approximately 60 plants were used in each experiment. The error bars represent the standard deviation (plant number > 50). (E) Western blots of Myc-BolOST1-1 and Myc-BolOST1-2 protein levels in the transgenic *ost1* lines. CBB R250-stained Rubisco large subunit (RLS) served as a loading control.

surviving plants were measured concurrently. As shown in Fig. 2C and D, *ost1*::*Myc-BolOST1-1* and *ost1*::*Myc-BolOST1-2* transgenic plants exhibited a tolerance to drought similar to that of Col-0 plants. These results indicate that BolOST1-1 and BolOST1-2 are also positive regulators of plant drought stress signaling.

3.4. Kinase activity of BolOST1-1 and BolOST1-2 in vitro

The kinase activity of OST1-like SnRK2-type protein kinases is fundamental for the biological significance of OST1, and thus, we sought to determine the kinase activity of BolOST1-1 and BolOST1-2. To measure this activity, GST-BolOST1-1 and GST-BolOST1-2 were mixed with MBP in a kinase assay system. As shown in Fig. 3A, both BolOST1-1 and BolOST1-2 phosphorylated MBP. Moreover, autophosphorylation activity was detected in both BolOST1-1 and BolOST1-2 (Fig. 3A, right panel). In terms of the core regulatory network of early ABA signaling, ABI5-like bZIP transcription factors are major downstream targets of OST1-like SnRK2-type protein kinases. To ascertain whether the N-terminal region of ABI5 is phosphorylated by BolOST1-1 and BolOST1-2, GST-ABI5-N was added to the kinase assay system with recombinant BolOST1-1 and BolOST1-2 kinase, respectively. Both of these fragments could phosphorylate ABI5-N (Fig. 3B). The relative kinase activity of MBP and ABI5-N was also determined (Fig. 3C and D). These assays indicate that the recombinants BolOST1-1 and BolOST1-2 are active protein kinases.

3.5. Subcellular localization of the BolOST1-GFP fusion protein

To examine the subcellular localization of BolOST1-1 and BolOST1-2, we monitored the GFP fluorescence of Cam-BolOST1-1-GFP- and Cam-BolOST1-2-GFP-transfected protoplasts as previously described [21]. As shown in Fig. 4A–F, both BolOST1-1 and BolOST1-2 are localized in the cytoplasm and nucleus of protoplasts. Interestingly, GFP fluorescence presented as spots in the cell (Fig. 4A–F), which is very similar to the fluorescence pattern of OST1, SnRK2.2 and SnRK2.3 [24]. These data suggest that BolOST1-1 and BolOST1-2 are also nuclear- and cytoplasmic-localized proteins that may be associated with plant ABA signaling.

3.6. BolOST1 interacts with homologs of the cabbage bZIP transcription factor BolABI5 and Group A PP2C BolABI1

According to the core module of early ABA signaling, group A PP2C phosphatases and ABI5-like bZIP transcription factors are upstream regulators and downstream targets of OST1 [1]. Recently, direct specific interactions among SnRK2 type kinases, group A PP2C phosphatases and ABI5-like bZIP transcription factors have been observed in Arabidopsis [25]. To date, we have characterized the role in ABA signaling and plant drought responses of BolABI1 and BolABI5 [17,18]. We used a firefly luciferase complementation assay to determine whether these interactions also exist among BolOST1, BolABI1 and BolABI5. As shown in Fig. 4H, a high level of luciferase activity was detected when BolOST1-1 or BolOST1-2

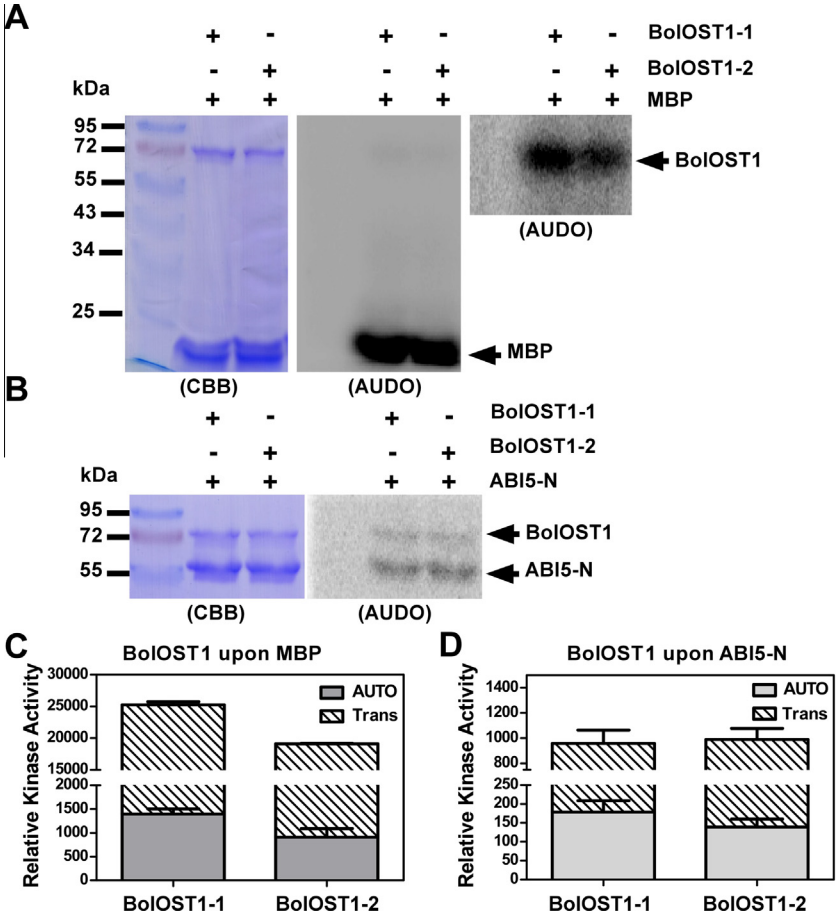

Fig. 3. Kinase assay of BolOST1-1 and BolOST1-2 *in vitro*. Phosphorylation of MBP (A) and ABI5-N (B) by BolOST1-1 and BolOST1-2, respectively. The proteins are indicated by arrows. Left panels, CBB R250-stained 12% SDS–PAGE gel; middle panels, phosphorylation activities of proteins (AUDO); right panel, autophosphorylation activities of kinase in a new scan window excluded MBP band (AUDO). (C, D) Quantification of data shown in (A) and (B), respectively.

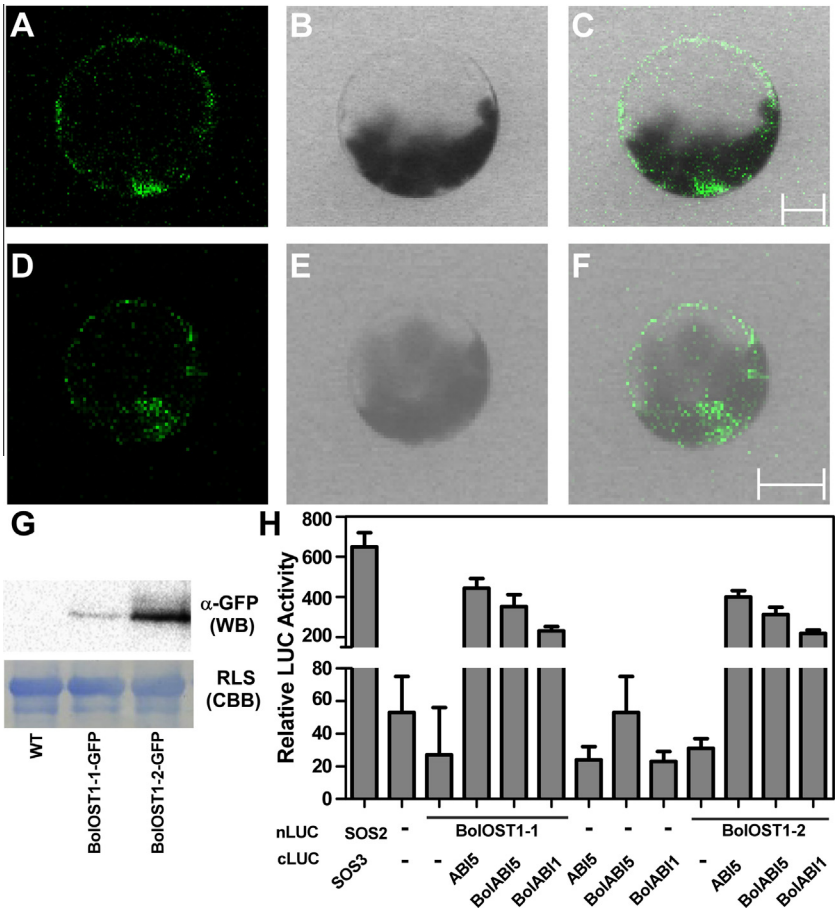

Fig. 4. Subcellular localization of BolOST1-1 and BolOST1-2 and the protein–protein interactions among BolOST1-1, BolOST1-2, ABI5, BolABI5 and BolABI1. (A–C) The GFP fluorescence signal of BolOST1-1-GFP. (D–F) The GFP fluorescence signal of BolOST1-2-GFP. (A, D) Green fluorescence under a dark field. (B, E) Cell morphology of the protoplasts under a bright field. (C, F) Overlay of bright-field and green-fluorescence signals. Bars = 10 μm. (G) Western blots of BolOST1-1-GFP and BolOST1-2-GFP protein levels in the transfected protoplasts. (H) Protein–protein interactions among BolOST1-1, BolOST1-2, ABI5, BolABI5 and BolABI1. The indicated combinations of nLUC/cLUC vectors were co-expressed in protoplasts, and the relative LUC activity was measured. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

was co-expressed with BolABI1 or BolABI5, respectively. Nearly no activity was detected when BolOST1-1 or BolOST1-2 was co-expressed with the empty cLUC vector or BolABI1 or BolABI5 with the empty nLUC vector (Fig. 4H). Interestingly, there was a minor difference in the luciferase activity in BolOST1-1 and BolOST1-2 when co-expressed with BolABI1 or BolABI5 (Fig. 4H).

In this study, we characterized two alternative splicing products of BolOST1, which is an OST1 ortholog in *B. oleracea* var. capitata L. Taken together, our data suggest that BolOST1-1 and BolOST1-2 are functional SnRK2-type protein kinases that compensate for OST1 in plant drought responses. Moreover, BolOST1 interacts with BolABI1 and BolABI5 and phosphorylates ABI5-N (Figs. 3 and 4H). Therefore, the activation mechanism of SnRK2-related kinases via the inhibition of ABI1-like PP2C proteins and the phosphorylation-dependent activation of ABI5-like bZIP transcription factors by SnRK2-related kinases is possibly conserved between Arabidopsis and cabbage. Further comprehensive investigations of BolOST1 and the reconstitution of the regulatory network of early ABA signaling in cabbage are required to further understand this relationship and its mechanism.

Acknowledgments

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