Cloning and Characterization of TaPP2AbB”-α, a Member of the PP2A Regulatory Subunit in Wheat

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Abstract

Protein phosphatase 2A (PP2A), a major Serine/Threonine protein phosphatase, consists of three subunits; a highly conserved structural subunit A, a catalytic subunit C, and a highly variable regulatory subunit B which determines the substrate specificity. Although the functional mechanism of PP2A in signaling transduction in Arabidopsis is known, their physiological roles in wheat remain to be characterized. In this study, we identified a novel regulatory subunit B, TaPP2ABB”-α, in wheat (Triticum aestivum L.). Subcellular localization indicated that TaPP2ABB”-α is located in the cell membrane, cytoplasm and nucleus. It interacts with both TaPP2Aa and TaPP2Ac. Expression pattern analyses revealed that TaPP2ABB”-α is strongly expressed in roots, and responds to NaCl, polyethylene glycol (PEG), cold and abscisic acid (ABA) stresses at the transcription level. Transgenic Arabidopsis plants overexpressing TaPP2ABB”-α developed more lateral roots, especially when treated with mannitol or NaCl. These results suggest that TaPP2ABB”-α, in conjunction with the other two PP2A subunits, is involved in multi-stress response, and positively regulates lateral root development under osmotic stress.

Introduction

Environmental stresses, such as water deficit and high salinity, are major challenges for plant growth and development. Plants adapt to severe environments by regulating morphogenesis and physiological reactions. Increased lateral root formation is a strategy for plants to survive in unfavorable conditions [1,2]. More lateral roots permit plants to absorb nutrients and water more easily in order to overcome stress damage, especially under osmotic stress [3–5]. Many genes are involved in plant stress response [6]; among them, protein kinases and protein phosphatases are basic components of stress signal transduction [7]. For example, ABA-activated SNF1-related protein kinases (SnRK) phosphorylate downstream substrates to enhance drought tolerance [8–10]. Protein kinases have been well described in wheat, but the roles of protein phosphatases (PPs) have not been investigated as widely [11,12].

PP2A is one of the most important Serine/Threonine (Ser/Thr) protein phosphatases that regulate many cellular processes, such as transcription, translation, the cell cycle, metabolism, and apoptosis [13,14]. PP2A positively regulates salt stress response through modulating polar auxin transport (PAT). It dephosphorylates auxin efflux carrier PIN proteins, which can be regulated by Ser/Thr kinase PINOID, and influences the distribution of auxin [15–17].

As a heterotrimeric phosphatase, PP2A consists of a scaffolding subunit A (PP2Aa), a regulatory subunit B (PP2Ab), and a catalytic subunit C (PP2Ac). In Arabidopsis, PP2Aa has three isoforms, PP2Aa1, PP2Aa2 and PP2Aa3, which are composed of tandem HEAT repeats that form a hook-like structure for binding PP2Ac and PP2Ab [18]. Auxin transportation is affected in the mutant xra1 (root curl in naphthylphthalamic acid1), which exhibits a root coiling phenotype and belongs to the PP2Aa group [19]. Two single mutants (pp2aa2 and pp2aa3) and one double mutant (pp2aa2 pp2aa3) in Arabidopsis develop normally, but plants exhibit serious developmental aberrations when PP2Aa1 is absent [15]. PP2Ac has five isoforms grouped into two subfamilies based on their conserved sequences. Subfamily I (PP2Ac1, PP2Ac2, and PP2Ac5) is involved in stress response, and the ABA and brassinosteroid (BR) signaling pathways. Subfamily II (PP2Ac3 and PP2Ac4) participates in dephosphorylation of PIN proteins in the auxin signaling pathway [20]. Methylated PP2Ac dephosphorylates receptor BRI1 to attenuate BR signaling [21]. Regulatory subunit B of PP2A (PP2Ab) determines multiple roles of PP2A in different signaling pathways.

Most researches on PP2Abs have been performed on animals where four gene families encode PP2Abs, including B (B55, PR5, PP2B2), B’ (B56, PR61, PP2PR3), B”(PR93/PR110) [22,23]. Down-regulation of PR5/B5 resulted in a diminished eye and enlarged trunk in gold fish [24]. Knockdown of PR61/B’ in mice led to decreased glycogen synthase kinase 3β (GSK3β) activity, increased cyclin-dependent kinase 5 (CDK5) activity in the brain, and reduced ability to perform beam walking [25]. PP2Ab” containing two calcium binding sites formed by EF-hands is involved in the adenosine 5’-monophosphate (AMP)-activated protein kinase (AMPK) signaling pathway through which it regulates the activity of PP2A in pig [26]. In Arabidopsis, at least 17 PP2Ab have been reported,
including two PP2AbB, nine PP2AbB* and TAP46 [27]. Only a few PP2Ab are well studied. The expression of TAP46 is induced by low temperature suggesting a role in cold stress signaling in Arabidopsis [28]. TON2, a member of the Arabidopsis PP2AbB subfamily, has a role in determining the geometry of microtubule nucleation and organization of the cortex [29]. Some members of B regulatory subunits of PP2A dephosphorylate BZR1 thus promoting plant growth [30]. Although PP2Abs are restricted to TAP46 and TON2, PP2Ab in wheat has not been well characterized. TaB-PR74 (AAK98641), detailed studies in Arabidopsis thus promoting plant growth [30], although PP2Abs and sequence information for a dehydration-inducible cDNA

Subcellular localization of TaPP2AbB*-¿ protein

The ORF of TaPP2AbB*-¿ without the termination codon was amplified using forward primer: 5′ CCAAGCTTGGAGTGAGGGGGGCAGC 3′ (Hind III site in bold italics), and reverse primer: 5′ ACATCTGGAGCATGACATCAGTGC 3′ (Sal I site in bold italics), then inserted into the pJIT163-GFP vector using T4 ligase.

The 10-day-old wheat seedling was used for protoplast isolation. The leaf strips of 0.5–1 mm length were cut from the middle part of leaves, and digested in cellulase R10 and macerozyme R10 enzyme solution buffer for 3 h to obtain wheat mesophyll protoplast cell. The fusion construct (TaPP2AbB*-¿-GFP) and control (GFP) were transformed into wheat mesophyll protoplast cells by the PEG-mediated method [37]. After incubation in a 500–570 nm wavelength. For chloroplast auto-fluorescence, the leaf strips of 0.5–1 mm length were cut from the middle part of leaves, and digested in cellulase R10 and macerozyme R10 enzyme solution buffer for 3 h to obtain wheat mesophyll protoplast cell. The fusion construct (TaPP2AbB*-¿-GFP) and control (GFP) were transformed into wheat mesophyll protoplast cells by the PEG-mediated method [37]. After incubation in a nutrient solution at 23°C for 16 h, the protoplast cells were examined using a laser scanning confocal microscope (Leica TCS-NT; Germany). GFP auto-fluorescence was collected in the range of 500–570 nm wavelength. For chloroplast auto-fluorescence, the wavelength range measured was 630–700 nm. Under which wavelength, the chloroplasts were shown in red color.

Interactions of TaPP2AbB*-¿ with TaPP2Aa and TaPP2Ac

Yeast two-hybrid and firefly luciferase complementation imaging assays were carried out to identify physical interaction of TaPP2AbB*-¿ with both TaPP2Aa and TaPP2Ac. The TaPP2AbB*-¿ coding region was amplified using primer pair: Forward: 5′ CCAAGCTTGGAGTGAGGGGGGCAGC 3′ (Hind III site in bold italics) Reverse: 5′ ACATCTGGAGCATGACATCAGTGC 3′ (Sal I site in bold italics) for the pGBK7 vector construct. Full length cDNAs of TaPP2Aa and TaPP2Ac were separately sub-cloned into the pGADT7 vector. Two pairs of vectors, TaPP2AbB*-¿-pGBK7/TaPP2Aa-pGADT7, and TaPP2AbB*-¿-pGBK7/TaPP2Ac-pGADT7 were transformed into yeast strain AH109. Transformations were selected on SD/-Trp/-Leu screening medium with X-gal (5-bromo-4-chloro-3-indolyl-B-D-galactopyranoside). The interaction was tested on SD/-Trp/-Leu/-His plates containing 5 mM 3-AT (3-amino-1,2,4-triazole). Yeast cells on SD/-Trp/-Leu/-His plates were used as controls for co-transformation.

For the firefly luciferase complementation imaging assay, which is an effective method to detect protein-protein interaction [38], full length cDNAs of TaPP2AbB*-¿ and TaPP2Aa were separately PCR-amplified and ligated into the pCAMBIA-cLuc and pCAMBIA-nLuc vectors. The constructed vectors were transformed into Agrobacterium tumefaciens strain GV3101, and further transformed into Nicotiana benthamiana leaves. After 3 days, the leaves were sprayed with 1 μM luciferin, and kept in darkness for 10 min. A camera fitted with a low-light cooled charge-coupled device was employed to capture the LUC (luciferase) image (Nikon-L936, Andor Tech; UK). The exposure time for LUC images was 15 min. ABA-insensitive 1 (ABI1) and its substrate 001154834.1), M. palustris PP2AbB* isoform 2 (NP_002709.2), Homo sapiens PP2AbB* isoform 1 (NP_031453.1) and TaPP2AbB*-¿ were used to construct the tree.

Database (nucleotide and protein blasts) searches were performed through the NCBI website. Sequence alignments and comparisons were implemented by the MegAlign program in DNASTar and DNAman. Protein predictions were performing Softberry (http://www.softberry.com).

Materials and Methods

Plant materials, growth conditions and stress treatments

Drought-tolerant common wheat (Triticum aestivum L.) cultivar Hanxuan 10 was used as the plant material. Ten-day-old seedlings (two-leaf stage) grown in 1/2 MS liquid culture at 23°C in a 12 h light/12 h darkness photoperiod were subjected to the following four treatments: 16.1% PEG (–0.5 MPa), 250 mM NaCl, 4°C, and 50 μM ABA. The leaf (seedlings sprayed with ABA) and root (sampled from 0.5–1 cm) and 50 μM ABA. The leaf (seedlings sprayed with ABA) and root (sampled from 0.5–1 cm) samples were separately sub-cloned into the pCAMBIA-cLuc and pCAMBIA-nLuc vectors. The constructed vectors were transformed into Agrobacterium tumefaciens strain GV3101, and further transformed into Nicotiana benthamiana leaves. After 3 days, the leaves were sprayed with 1 μM luciferin, and kept in darkness for 10 min. A camera fitted with a low-light cooled charge-coupled device was employed to capture the LUC (luciferase) image (Nikon-L936, Andor Tech; UK). The exposure time for LUC images was 15 min. ABA-insensitive 1 (ABI1) and its substrate 001154834.1), M. palustris PP2AbB* isoform 2 (NP_002709.2), Homo sapiens PP2AbB* isoform 1 (NP_031453.1) and TaPP2AbB*-¿ were used to construct the tree.

Cloning and sequencing of full-length TaPP2AbB*-¿ cDNA

The putative full-length cDNA of TaPP2AbB*-¿ was obtained using the candidate sequence from Oryza sativa (NM_001071385.1), with sequence information for a dehydration-inducible cDNA library of wheat D genome sequence and the candidate sequence from Oryza sativa. We found that TaPP2AbB*-¿ interacted with PP2Ac, which was isolated from wheat. Expression of TaPP2AbB*-¿ was up-regulated by multiple stresses. Arabidopsis lines overexpressing TaPP2AbB*-¿ developed more lateral roots and grew better than the wild type under the medium osmotic stress, presenting a possibility of using TaPP2AbB*-¿ in transgenic breeding to improve abiotic stress tolerance in crop plants.

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Figure 1. Alignment of the predicted amino acid sequences of PP2AbB"-α from different plant species. Alignment was performed according to DNAman. Common identical amino acid residues are shown in black background. The FRQ1 domain is marked in red rectangles, and the EF-hand-5 domain is marked in green rectangles. Abbreviations on the left side of each sequence: Bd, Brachypodium distachyon; Si, Setaria italica; Os, Oryza sativa; Ta, Triticum aestivum.

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Figure 2. Phylogenetic tree of TaPP2AbB" from wheat and PP2Ab from other species. This phylogenetic tree performed by MEGA 5.05 is based on putative amino acid sequences. Bootstrap values are in percentages. There are three distinct isoform groups in the phylogenetic tree. At, Arabidopsis thaliana; Gm, Glycine max; Vv, Vitis vinifera; Ta, Triticum aestivum; Si, Setaria italica; Os, Oryza sativa; Mt, Medicago truncatula; Bd, Brachypodium distachyon.

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Quantitative real-time PCR (qRT-PCR) was performed to determine the expression pattern of TaPP2AbB"-α. Actin transcript was used as an internal control to quantify the relative transcript level. The qRT-PCR was performed in triplicate with an ABI PRISM H 7900 system (Applied Biosystems, USA) using the SYBR Green PCR master mix kit (Takara, Japan). The specific primers (F: 5’ CTGGCTCTCCCCGTGTTATG 3’, R: 5’ AGAGGA-GATCCCAAGGATGATG 3’) were designed according to the full-length of the TaPP2AbB"-α cDNA sequence. The relative level of gene expression was detected using the 2^(-△△CТ) method [40].

$$\Delta\Delta C_T = (C_T, \text{Target} - C_T, \text{Actin})_{\text{Time} \times} - (C_T, \text{Target} - C_T, \text{Actin})_{\text{Time} 0}.$$ 

The C_T (cycle threshold) values for both the target and internal control genes were the means of triplicate independent qRT-PCRs. Time x represented the treatment time point (0.5, 1, 1.5, 2, 3, 6, 12, 24, 48 and 72 h), and time 0 h represented the time immediately prior to treatment. Expression of TaPP2AbB"-α in seedling leaves was regarded as standard due to its lowest expression level in that tissue. The corresponding formula was modified to $\Delta\Delta C_T = (C_T, \text{Target} - C_T, \text{Actin})_{\text{leaf}} - (C_T, \text{Target} - C_T, \text{Actin})_{\text{root}}$ to identify the expression pattern of TaPP2AbB"-α in leaf and root tissues from wheat seedlings.

Generation of transgenic plants in Arabidopsis
TaPP2AbB"-α cDNA containing the entire ORF was inserted into pCHF3 under control of the CaMV 35S promoter and NOS terminator, using primers 5’ GG GGTACC ATGGAGGTG-GAAGCGGC 3’ (Kpn I site in bold italics) and 5’ GG GATCC T-CAGAATGGAGCTTCAAGTGAC 3’ (BamHI site in bold italics). The p35S-TaPP2AbB"-α-NOS construct and the p35S-NOS vector were separately transformed into Agrobacterium strain GV3101 and then infected into wild type Arabidopsis plants by floral infiltration. Positive T3 generation transgenic Arabidopsis plants overexpressing TaPP2AbB"-α were screened by kanamycin plates and PCR amplification.

Morphological characterization of transgenic Arabidopsis
Arabidopsis seeds were sown on MS medium solidified with 0.8% agar. Seeds were vernalized for 36 h at 4°C before being transferred to a growth chamber. To examine root morphology, the 8-day-old seedlings vertically grown on normal MS solid medium were replanted to MS medium plates containing NaCl (50, 100 and 150 mM), and mannitol (100, 200 and 300 mM). Root system morphologies were examined 3 days after replanting. The root length of Arabidopsis was measured with a ruler. The number of lateral root was captured with EXPSON EXTRESSION 10000XL (EXPSON, Japan) and counted with winRHIZO software.

Results
Isolation and sequence analysis of TaPP2AbB"-α
TaPP2AbB"-α is 1,648 bp in length, consisting of an 8 bp 5’ untranslated region, a 1,626 bp ORF, and a 14 bp 3’ untranslated region. The ORF encodes 541 amino acid residues (AAR) with a calculated molecular mass of 62 kDa and predicted pI of 4.92. TaPP2AbB"-α has two domain structures, characterized by the
Figure 5. Overexpression of TaPP2AbB"-a promotes root growth in Arabidopsis as exhibited by more lateral roots and longer primary roots. (A) Lateral root density (number of lateral roots/cm primary root) of TaPP2AbB"-a overexpression lines and control. (B) Primary root length of TaPP2AbB"-a overexpression lines and control. *, significantly different at $P = 0.05$; **, significantly different at $P = 0.01$. Data represent mean ± SE (n = 9). doi:10.1371/journal.pone.0094430.g005
EF-Hand-5 (330–422 AAR) and FRQ1 (272-391 AAR) domains that belong to the EF-hand superfamily. The deduced amino acid sequence shows high homology with counterpart PP2AbB“-α family members from other plant species, viz. Oryza sativa (NP_001064850.1), Setaria italica (XP_004982973.1) and Brachypodium distachyon (XP_003574057.1) (Figure 1). We also performed protein blast in the website of TAIR (http://www.arabidopsis.org/Blast/index.jsp). Several amino acid sequences were identified, sharing about 70% similarity with TaPP2AbB“-α. But, the classification of these amino acid sequences from Arabidopsis was unclear.

Phylogenetic analysis

A phylogenetic tree was constructed with the putative amino acid sequences of TaPP2AbB“-α and members of the PP2AbB” family in other species. PP2AbB“-α from animals and plants were in two distinct clades (Figure 2). TaPP2AbB“-α and its counterparts from plants were placed into Group 1; PP2AbB“-γ in Group 2 were in one clade, and PP2AbB“-α from animals were in another (Group 3).

Subcellular localization of TaPP2AbB“-α protein

Wheat protoplast cells were used to check subcellular localization of TaPP2AbB“-α. The deduced amino acid sequence contains no putative nuclear localization sequence (NLS) site or transmembrane region. The fusion construct TaPP2AbB“-α::GFP driven by the CaMV 35S promoter was transiently expressed in living wheat protoplast cells. As predicted, TaPP2AbB“-α-GFP was present in the cell membrane, cytoplasm and nucleus (Figure 3).

Phenotypes of overexpression lines of TaPP2AbB“-α in Arabidopsis

To evaluate the possible function of TaPP2AbB“-α, transgenic plants were assessed under different abiotic stress conditions. Morphological assays indicated differences in root systems between transgenic and control plants (the p35S-NOS vector plants) (Figure 4). Overexpression lines grew better, and developed more lateral roots, especially under the stresses of mannitol and NaCl (Figure 5A). Primary roots of overexpression lines were significantly longer than those of control plants under osmotic stress (Figure 5B). These results suggested that TaPP2AbB“-α may be involved in stress response and regulation of root development, especially lateral root densities under 100 mM NaCl and 200 mM mannitol treatments.

Expression pattern of TaPP2AbB“-α in wheat

TaPP2AbB“-α was expressed strongly in roots, and weakly in leaves of wheat seedlings (Figure 6A). Various up-regulated expression patterns occurred under diverse abiotic stresses (Figure 6B). Under NaCl stress, the expression of TaPP2AbB“-α decreased during the first 2 hours, the transcription level increased and peaked at 12 h, and then decreased. Under PEG stress, the transcription level decreased during the first hour, increased from 12 h, slightly decreased at 48 h, and increased again at 72 h. Under cold stress, expression increased rapidly in the first hour, and the level peaked at 3 h, 24 h and 48 h. With ABA treatment, the expression level of TaPP2AbB“-α increased rapidly and reached a maximum at 3 h, then quickly decreased to the 0 h level.

TaPP2AbB“-α interacts with both TaPP2Aa and TaPP2Ac

Yeast cells co-transformed with the TaPP2AbB“-α-pGBK7/TaPP2Aa-pGADT7, TaPP2AbB“-α-pGBK7/TaPP2Ac-pGADT7,
and TaPP2AbB'-'-pGBK7/pGADT7 vector, and with TaPP2Ac-pGBK7/pGADT7 grew well on SD/-Trp/-Leu plates. Yeast cells co-transformed with the TaPP2AbB'-'-pGBK7/pGADT7 vector, TaPP2Aa-pGBK7/pGADT7 and TaPP2Ac-pGBK7/pGADT7, could not grow on SD/-Trp/-Leu/-His with 5 mM 3-AT (Figure 7A). These results indicated that TaPP2AbB'-' interacted with both TaPP2Aa and TaPP2Ac. In the firefly luciferase complementation imaging assay, the image of LUC was captured during co-transformation of TaPP2AbB'-cLuc/TaPP2Aa-nLuc as well as the positive control in Nicotiana benthamiana leaves (Figure 7B). Different fluorescence intensity resulted in the color-mixed spots. These results confirmed that TaPP2AbB'- interacts with TaPP2Aa.

**Discussion**

The PP2A regulatory subunit is responsible for substrate specificity and localization of PP2A [41]. Approximately fifteen and seventeen B subunits have been identified in vertebrates and plants, respectively. All B subunit members are derived from four diverse gene families, and little sequence similarity exists between families while maintaining high sequence similarity within families [22]. In this study, the identified wheat PP2Aβ, designated as TaPP2AbB'-'-α, has the same EF-hand domain as PP2AbB'-' from animals and plants.

We used the cDNA sequence of TaPP2AbB'-a to blast in wheat A genome and D genome. There are 12 regions in TaPP2AbB'-a matching with scaffold59526 of A genome and scaffold72594 of D genome, respectively. The 12 regions of TaPP2AbB'-a almost covered the whole coding region. Most part of the 12 regions have 97% identity with scaffold59526 and scaffold72594. We presumed that TaPP2AbB'-a was exist in A genome and D genome. What's more, we did not find other scaffolds similar to TaPP2AbB'-a. This phenomenon may imply that the TaPP2AbB'-a has no homologue in wheat.

Our data indicates that transgenic Arabidopsis plants overexpressing TaPP2AbB'-α have a more developed root system than control plants under normal conditions. Previous studies showed that root growth can be affected by environmental conditions and genetic characters [42], such as moderate soil drying which promotes the elongation of lateral roots [43]. Overexpression of TaSnRK2.3-GFP in Arabidopsis also generated an improved root system, expressed by longer primary root and more lateral roots [12]. In this study, TaPP2AbB'-α-overexpressing Arabidopsis lines developed more lateral roots and stronger primary roots than the wild type.
control under normal conditions, suggesting that TaPP2AbB"-α is involved in regulation of root growth and development. Under medium stresses, i.e. 100 mM NaCl and 200 mM mannitol, both transgenic lines developed more lateral roots (Figure 5), implying that the plants can spontaneously adapt to certain environmental stress, and that this phenotype may also represent a common adaptive response to diverse stresses among plants. As in rice, water deficit also results in increased lateral root formation [44]. In addition, more lateral roots were produced under salt stress in wild-type Arabidopsis [45]. Previous studies showed that Arabidopsis developed more lateral roots under 100 mM NaCl, but less lateral roots under 125 mM NaCl [17]. A more developed root system helps plants to improve the efficiency of water and nutrient uptake, especially under abiotic stress [46]. With more lateral roots and longer primary roots, more water and nutrient uptake may be available in transgenic Arabidopsis. This could be the reason why overexpression lines of TaPP2AbB"-α grew better than control plants, even under NaCl and mannitol stresses.

The transcript expression level of TaPP2AbB"-α is stronger in roots than in leaves of wheat seedlings, suggesting that TaPP2AbB"-α mainly functions in the root system. This inference also matches the phenotypes of overexpression lines of TaPP2AbB"-α in Arabidopsis. The expression of TaPP2AbB"-α is up-regulated by multiple stresses (Figure 6). Previous studies showed that PP2A, or specific PP2A subunits, function as transducers of osmotic stress and in low temperature signaling [47]. In Saccharomyces cerevisiae, PP2AbB' is an essential activating factor for stress gene transcription, such as CTT1 (encoding cytosolic catalase T), HSP12 (encoding a small heat shock protein), and PGM2 (encoding phosphoglucomutase 2) [48]. Moreover, expression of genes encoding PP2Ac subfamily I is induced by drought and NaCl stress [49,50]. TaPP2Ac1, which clades in PP2Ac subfamily II, is induced by NaCl, PEG, low temperature and ABA [51]. Considering these findings, the role of PP2Ac in stress signaling matches with the expression of TaPP2AbB"-α under multiple environmental stresses. Together with the phenotypic data for TaPP2AbB"-α overexpression lines, we surmise that transgenic plants develop more lateral roots when the transcript level of TaPP2AbB"-α is induced by osmotic stress.

PP2A is highly conserved and the core enzyme interacts with a variable regulatory subunit to form a PP2A holoenzyme. Human PP2Ac interacts with PP2Ac to form a stable AC core dimer through its intra-repeat loops and inner helices, and B56 (PP2AbB') interacts with both PP2Ac and PP2Ac [52]. Here, TaPP2AbB"-α exhibits the same subcellular localization pattern with TaPP2Ac (unpublished data) and TaPP2Ac [51]. As PP2A is a heterotrimetric protein, we detected interactions of TaPP2AbB"-α with PP2Ac and PP2Ac. As shown in Figure 7, the interacting relationships of TaPP2AbB"-α with TaPP2Ac and TaPP2Ac were verified in both yeast and tobacco leaves. Moreover, we found that TaPP2Ac has high homology with RCN 1 in Arabidopsis (Figure S1). The mutant line of rcn1 (pp2ac3) is sensitive to osmotic stress and developed less lateral root than the normal plants [53]. RCN1 modulates polar auxin transport (PAT), and dephosphorylates auxin efflux carrier PIN proteins to influence the distribution of auxin accompanied with Ser/Thr kinase PINOID [15,16]. TaPP2Ac is in the same clade with PP2Ac subfamily II (PP2Ac3 and PP2Ac4) in Arabidopsis (Figure S2), and a double mutant of PP2Ac, pp2ac3/pp2ac4 exhibits defective root development in Arabidopsis. PP2Ac subfamily II is also involved in the auxin signaling pathway [20]. Interaction and the phenotype of PP2Ac and PP2Ac suggest that positive regulation of TaPP2AbB"-α in root development may be associated with auxin signaling.

In this study, TaPP2AbB"-α enhanced root development in overexpressing Arabidopsis under normal conditions. With onset of environmental stress, TaPP2AbB"-α is induced to promote increased root growth, and thus enhanced absorption efficiency in the uptake of water and nutrients for survival. In this process, PP2Aα and PP2Ac may interact with PP2AbB"-α and then function as a holoenzyme to act on certain substrates. More evidence needs to be obtained to uncover the molecular mechanism by which TaPP2AbB"-α enhances osmotic-stress tolerance.

Supporting Information

Figure S1 Alignment of the amino acid sequences of PP2AbB"-α from wheat and Arabidopsis. Alignment was performed according to DNAnan. The accession number of AtPP2Aa1 is NP_173920.1, the accession number of TaPP2Aa is AEB40165.1. Common identical amino acid residues are shown in black background. The HEAT motif is marked in red rectangles. Abbreviations on the left side of the sequence are: At, Arabidopsis thaliana; Ta, Triticum aestivum. (TIF)

Figure S2 Phylogenetic tree of TaPP2Ac from wheat and PP2Ac from Arabidopsis. This phylogenetic tree is performed by MEGA 5.05. TaPP2Ac, accession number: ABO16371.1; AtPP2Ac-1, NP 176192.1; AtPP2Ac-2, NP 172514.1; AtPP2Ac-3, NP 567066.1; AtPP2Ac-4, NP 567067.1; AtPP2Ac-5, NP 172514.1. Bootstrap values are in percentages. Abbreviations on the right side of the tree are: At, Arabidopsis thaliana; Ta, Triticum aestivum. (TIF)

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Author Contributions

Conceived and designed the experiments: DL RJ. Performed the experiments: DL. Analyzed the data: DL. Contributed reagents/materials/analysis tools: XM AL. Wrote the paper: DL.

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Cloning and Characterization of TaPP2AbB+ allele