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Abstract: Sweet potato is one of the most important food crops with strong environmental adaptability. Protein phosphatase 2A (PP2A) is a major intracellular protein phosphatase that plays crucial roles in hormone signal transduction and abiotic stress response. Characterization of PP2A can elucidate the mechanisms of stress resistance in this crop. In this study, a total of 15 PP2A transcripts, including nine regulatory subunit-encoding sequences and six catalytic subunit-encoding sequences, were identified from sweet potato and found to be expressed at varying levels. Only one contained a complete open reading frame and encoded a B regulatory subunit (termed *IbPP2A1*). Ten polymorphic sites were distributed in the coding region of this gene, but most did not result in amino acid change. RNA sequencing-based digital gene expression profiling showed that this PP2A gene was primarily expressed in fibrous roots and developing tuberous roots under natural conditions. After drought, salinity, and alkaline stress treatment, the expression of *IbPP2A1* in leaves was only upregulated by drought stress at 2 days. However, heterogeneous expression of *IbPP2A1* did not enhance abiotic stress tolerance in recombinant *Saccharomyces cerevisiae*. The results described here indicate that *IbPP2A1* is an abiotic stress-responsive gene, but it could not work alone in vitro. This study provides a preliminary but global insight into PP2A proteins in sweet potato for further investigations on improving stress tolerance in this crop.

Key words: Sweet potato, PP2A, polymorphic site, expression pattern, stress resistance

1. Introduction

Sweet potato (*Ipomoea batatas* L. (Lam.)), one of the six most important food crops, is widely grown around the world due to its affinity for strong adaptability to varying environments (Low et al., 2009). It has much higher biomass yield per unit area per unit time than many other plants. Sweet potato has a complex genome (2n = 6x = 90) with a size between 2200 and 3000 Mb (Oziasakins and Jarret, 1994; Varshney et al., 2010). The genomic resources of this crop are much more deficient than those of other important crop species. Due to the development and usage of next-generation sequencing technology, several sweet potato transcriptomes have been released (Schafleitner et al., 2010; Wang et al., 2010; Tao et al., 2012; Xie et al., 2012; Tao et al., 2013). Although the complete genome sequence has not yet been published, it is encouraging that scientists have been attempting to construct a genetic map for sweet potato and have recently released the de novo whole-genome sequencing results of the diploid ancestor (Hirakawa et al., 2015). These data will facilitate the investigation of stress resistance in this crop and also the excavation of functional genes.

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As obligate partners, protein phosphatases can form a phosphorylation/dephosphorylation bilateral switch together with protein kinases to regulate the cellular control circuitry (DeLong, 2006). Protein phosphatase 2A (PP2A) is a ubiquitous serine/threonine protein phosphatase, which belongs to the protein phosphatase P (PPP) family. Studies indicate that PP2As usually occur as a heterotrimeric complex composed of a highly conserved regulatory subunit (A), a heterogeneous regulatory subunit (B), and a catalytic subunit (C) (Janssens and Goris, 2001; Sablina and Hahn, 2008; Strack et al., 2002). The A subunit is responsible for scaffolding B and C to form a trimeric holoenzyme. The C subunit is the catalytic subunit, whereas the B subunit selects substrates and regulates enzyme activity and subcellular localization (Strack et al., 2002; Janssens et al., 2008). Generally, there are several different subunits in each species. For example, three A subunits, 20 B subunits, and five C subunits exist in *Arabidopsis* (Farkas et al., 2007). These subunits may form different PP2A holoenzymes and recognize different substrates. PP2As have been characterized as major intracellular protein phosphatases that regulate multiple...
aspects of cell growth and metabolism in animal cells, such as DNA replication and transcription, mRNA translation, the cell cycle, development, and apoptosis (Janssens and Goris, 2001; Janssens et al., 2005; Mumby, 2007; Eichhorn et al., 2009). In plants, PP2As play crucial roles in hormone signal transduction and abiotic stress response (DeLong, 2006). Overexpression of some PP2As can enhance plant growth and increase drought tolerance in transgenic plants (Luo et al., 2006; Xu et al., 2007; Ahn et al., 2015). However, another study reported a contradictory result for the role of PP2A by overexpressing and knocking out the C1 subunit gene in Arabidopsis (Pernas et al., 2007). Since there are different PP2A holoenzymes in plants, the different roles of PP2As may be the result of different combinations.

In order to identify and characterize the stress response of PP2As in sweet potato, high-throughput RNA sequencing (RNA-Seq) and digital gene expression (DGE) sequencing data were carefully analyzed. Fifteen PP2A-encoding sequences were identified and one of these sequences was cloned and characterized in several ways, including sequence diversity, expression patterns, and yeast expression assays.

### 2. Materials and methods

#### 2.1. Sequence similarity analyses

PP2A sequences of several plant species were downloaded from GenBank (Benson et al., 2013). The local blast program was downloaded from NCBI (ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/2.2.25/) and installed in a Linux platform for sequence similarity analyses. Downloaded PP2A sequences were formatted as a database by using the following command: makeblastdb -in ncbi-blast-2.2.25+/bin/PP2A_NCBI.fasta -dbtype prot -input_type fasta -parse_seqids -out ncbi-blast-2.2.25+/db/PP2A_NCBI. Blast search was performed using the following command: ./blastx -db ncbi-blast-2.2.25+/db/PP2A_NCBI -query transcriptome.fasta -out PP2A-transcriptome.xml -evalue 0.001 -num_threads 12 -outfmt 5 -num_descriptions 5. High-quality hits were extracted by BlastParser (http://geneproject.altervista.org/) with an identity and positive threshold of ≥60%. Candidates were submitted to the BlastX platform of NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastx&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) for the further verification. Verified sequences were used for this study (Table 1).

### Table 1. PP2A-encoding transcripts identified from sweet potato transcriptome.

<table>
<thead>
<tr>
<th>Contig ID</th>
<th>Length (nt)</th>
<th>ORF (AA)</th>
<th>Annotation</th>
<th>Full length</th>
<th>Expression patterns (TPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>YL ML ST FR ITR ETR HTR</td>
<td></td>
</tr>
<tr>
<td>Contig_1240</td>
<td>2369</td>
<td>529</td>
<td>PP2A 57-kDa regulatory subunit B' iota isoform</td>
<td>Y</td>
<td>14.0 21.0 11.0 32.7 28.9 11.5 11.9</td>
</tr>
<tr>
<td>Contig_1513</td>
<td>2264</td>
<td>515</td>
<td>PP2A 57-kDa regulatory subunit B' theta isoform</td>
<td>N</td>
<td>7.5 5.8 76.7 13.4 32.8 8.4 18.6</td>
</tr>
<tr>
<td>Contig_6229</td>
<td>1446</td>
<td>267</td>
<td>PP2A 57-kDa regulatory subunit B' theta isoform</td>
<td>N</td>
<td>28.9 36.5 37.7 31.3 22.3 18.5 29.9</td>
</tr>
<tr>
<td>Contig_6836</td>
<td>1388</td>
<td>244</td>
<td>PP2A 57-kDa regulatory subunit B' beta isoform</td>
<td>N</td>
<td>17.3 4.4 4.3 3.6 4.4 4.2 16.7</td>
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<tr>
<td>Contig_8348</td>
<td>1257</td>
<td>300</td>
<td>PP2A 57-kDa regulatory subunit B' beta isoform</td>
<td>N</td>
<td>1.2 8.2 15.1 4.2 3.0 0.0 1.1</td>
</tr>
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<td>Contig_9483</td>
<td>1175</td>
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<td>1776</td>
<td>472</td>
<td>PP2A 59-kDa regulatory subunit B' gamma isoform</td>
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<tr>
<td>Contig_2901</td>
<td>1891</td>
<td>405</td>
<td>PP2A regulatory subunit TAP46</td>
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<td>7.8 9.9 16.8 6.1 9.4 12.3 12.4</td>
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<td>Contig_4602</td>
<td>1633</td>
<td>315</td>
<td>PP2A catalytic subunit</td>
<td>N</td>
<td>10.1 63.0 59.9 71.2 66.1 63.4 14.7</td>
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<tr>
<td>Contig_1816</td>
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<td>306</td>
<td>PP2A catalytic subunit</td>
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<td>21.2 82.0 39.1 87.9 59.2 18.8 20.0</td>
</tr>
<tr>
<td>Contig_7392</td>
<td>1339</td>
<td>306</td>
<td>PP2A catalytic subunit</td>
<td>N</td>
<td>16.4 2.9 12.5 18.4 10.2 1.7 33.9</td>
</tr>
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</tr>
<tr>
<td>Contig_3207</td>
<td>1837</td>
<td>312</td>
<td>PP2A catalytic subunit</td>
<td>N</td>
<td>40.0 35.0 36.5 71.4 57.0 18.5 33.6</td>
</tr>
<tr>
<td>Contig_6142</td>
<td>1456</td>
<td>311</td>
<td>PP2A-2 catalytic subunit</td>
<td>N</td>
<td>1.5 2.0 0.6 0.0 0.6 0.6 0.0</td>
</tr>
</tbody>
</table>

2.2. Plant materials
Stem cuts of sweet potato (*I. batatas* 'Xushu 18') were planted in the experimental field and grown for 3 months under natural conditions in Chengdu, Sichuan Province of China. After 3 months plants were divided into five groups and exposed to different abiotic stress treatments. Four groups were irrigated with either 10% PEG 6000, 200 mmol/L NaCl, 200 mmol/L Na₂CO₃, or 100 mmol/L NaCl + 100 mmol/L Na₂CO₃ every 3 days to simulate drought, salinity, alkaline, and salinity + alkaline stresses, while the remaining group was irrigated with an equal volume of water. Tissue samples were collected from five plants in each group, pooled together, and immediately snap-frozen in liquid nitrogen. Samples were stored at –80 °C until further processing.

2.3. Sequence cloning and nucleotide polymorphism analyses
Total RNA was extracted using the TRIZol reagent (Invitrogen, USA) according to the manufacturer’s instructions. The remaining genomic DNA was digested using DNase I (Fermentas, USA). Total cDNA was synthesized from 1 μg of total RNA with Moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen, USA) using oligo(dT) as a primer following the manufacturer’s instructions. The cDNAs were subjected to sequence cloning by using primers 2A-F1 and 2A-R1 (Table 2), which were designed according to the assembled transcript (Contig_1240). PCR amplification was carried out with 35 cycles of 2 min at 94 °C, 10 s at 98 °C, 30 s at 56 °C, and 45 s at 68 °C with KOD-Plus-Neo (TOYOBO, Japan). The PCR product was double digested using *Eco*RI and *Sal*I and subsequently subcloned into the *Saccharomyces cerevisiae/*Escherichia coli shuttle vector pEX-Tag (Meyer et al., 2000), which was also double-digested by *Eco*RI and *Sal*I. The recombinant plasmid was transformed into the *E. coli* strain and sequenced by the Sanger method. By using the RNA-Seq paired-end (PE) reads of this sweet potato cultivar (*I. batatas* 'Xushu 18') as a database (Tao et al., 2012, 2013), short sequence fragments of this sequenced PP2As served as bait to search for polymorphic sites.

2.4. Expression pattern analyses based on DGE profiling
By using Bowtie (v2.0.0-beta5) (Langmead et al., 2009) under default parameters, the 21-bp DGE sequencing tags of seven different tissues retrieved from NCBI’s Gene Expression Omnibus (GEO) (Barrett et al., 2011, 2013; Tao et al., 2012) (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE35929) were aligned to the assembled PP2As. The number of mapped tags were counted, and TPM values (transcripts per million clean tags) (Morrissy et al., 2009) were calculated and used for the quantification of each transcript in the different sweet potato samples.

2.5. Quantitative real-time polymerase chain reaction
Young leaf (YL), stem (ST), and expanding tuberous root (ETR) samples were collected at 0, 2, 11, and 20 days after stress treatment. Total RNA was extracted from each tissue sample following the method described above. Equal amounts of total RNA (500 ng) from tissues from each of the different treatment groups were reverse transcribed with M-MLV reverse transcriptase (Invitrogen, USA) using oligo(dT) and random hexamers as primers. The resulting cDNAs were subjected to quantitative real-time polymerase chain reaction (qRT-PCR) analyses. Primers used for qRT-PCR analyses were designed according to the Sanger sequencing results described above using Primer Premier 5.0 (Table 2). The qRT-PCR was performed following the manufacturer’s instructions with SsoFast EvaGreen Supermix (Bio-Rad, USA) on an iCycler MyiQ Real-Time PCR (Bio-Rad, USA). Sequence amplification was carried out with 40 cycles of 2 min at 95 °C, 10 s at 95 °C, 15 s at 56 °C, and 20 s at 72 °C. The quantification results were then calculated according to the method of 2⁻ΔΔCT (Livak and Schmittgen, 2001).

### Table 2. Primers used for sequence cloning and RT-PCR verification.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Product length (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IbPP2A-F1</td>
<td>5’-CGAATTCTGATGCCCCTTCTTCTTCTTTC-3’</td>
<td>1748</td>
</tr>
<tr>
<td>IbPP2A-R1</td>
<td>5’-ATAGTGACGGACACGGGCTTTGTTATCCT-3’</td>
<td>1748</td>
</tr>
<tr>
<td>IbPP2A-F2</td>
<td>5’-TGTGATAATGGCCATTGTTGATGTC-3’</td>
<td>143</td>
</tr>
<tr>
<td>IbPP2A-R2</td>
<td>5’-GGTTGTGTTGAGGGGACACACAC-3’</td>
<td>143</td>
</tr>
<tr>
<td>IbACT-F1</td>
<td>5’-CTGGTGTTATGGTGAGGATGGGAC-3’</td>
<td>195</td>
</tr>
<tr>
<td>IbACT-R1</td>
<td>5’-GAAGGACAGGGTGCTCTTCAAG-3’</td>
<td>195</td>
</tr>
</tbody>
</table>

The underlined sequences are the recognition and cleavage site of restriction enzymes.
2.6. Functional analysis of IbPP2A1 expressed in *Saccharomyces cerevisiae*

Plasmids were extracted from the verified JM109 recombinants and transformed into *S. cerevisiae* strain FGY217 (MATa, pep4Δ, ura3-52, lys2Δ201; laboratory stock) by a LiAc/SS-DNA/PEG procedure (Gietz and Schiestl, 2007). After the transformation, cells were spread on an Ura-selection plate (2% agar, 0.2% yeast synthetic drop-out medium without uracil, 0.67% yeast nitrogen base without amino acids, and 2% glucose). The transformants were cultured at 30 °C for 48 h. For functional assays, a single yeast colony hosting the *IbPP2A1* gene was cultivated in liquid YPD medium. The culture was subsequently centrifuged to collect the yeast cells. Resistance to environmental stresses were assayed by exposing the recombinant yeast culture and the control yeast culture to cold, heat, and drought stresses, which were simulated by incubating the strain at –20 °C, at 45 °C, or in sorbitol solution (4 mol/L and 6 mol/L), respectively, for 0, 24, 72, 120, and 168 h. The treated strains were then cultured on solid YPD medium and incubated at 30 °C for 48 h before observation.

3. Results and discussion

3.1. Identification and clustering of sweet potato PP2A-encoding genes

The most comprehensive transcriptome of sweet potato cultivar Xushu 18 (Tao et al., 2013) was used for the sequence similarity search using the PP2A sequences downloaded from GenBank (Benson et al., 2013) as a query. Results showed that a total of 15 PP2A-encoding transcripts were identified, including nine regulatory subunit-encoding transcripts and six catalytic subunit-encoding transcripts (Table 1). Among the nine regulatory subunits, seven were annotated as 57-kDa regulatory subunits, one was a 59-kDa regulatory subunit, and one was a TAP46 regulatory subunit. A neighbor-joining tree was constructed by MEGA6 (Tamura et al., 2013). Results showed that the 15 PP2A transcripts could be clustered into three groups (Figure 1). All of the catalytic subunit-encoding transcripts were clustered into one group, Contig_2901 in a separate group, and the others in another group. Comparatively, the subunit-encoding genes identified in this study were

![Figure 1. Clustering analyses of PP2As in sweet potato. The neighbor-joining tree was constructed by the MEGA6 program (Tamura et al., 2013) using the deduced protein sequence of the 15 PP2A transcripts.](image-url)
fewer than those reported in Arabidopsis (Farkas et al., 2007). This result could be due to the incompleteness of the referenced transcriptome (Tao et al., 2013).

The 21-bp DGE sequencing tags of seven different tissues, including young leaves (YL), mature leaves (ML), stems (ST), fibrous roots (FR), initial tuberous roots (ITR), expanding tuberous roots (ETR), and harvest tuberous roots (HTR) (Tao et al., 2012), were retrieved from the NCBI's SRA database (http://www.ncbi.nlm.nih.gov/Traces/sra) and aligned to the assembled transcriptome to quantify each transcript. Results showed that different transcripts had varying expression patterns. Contig_1240, 1513, 6229, and 9483 encoded the primary expressed regulatory subunit, while Contig_4602, 4816, and 3207 encoded the primary expressed catalytic subunits. These results indicated that different subunits play specialized roles in diverse tissues and at different developmental stages. The predicted open reading frame (ORF) of the PP2A transcripts ranged from 735 to 1590 bp. However, only Contig_1240 encoded a complete regulatory subunit.

3.2. Sequence cloning and characterization
In this study, one full-length PP2A-encoding transcript, Contig_1240, was cloned and characterized. Primers IbPP2A-F1 and IbPP2A-R1 were used for cDNA cloning of Contig_1240. PCR was performed by using the KOD-Plus-Neo DNA polymerase (TOYOBO, Japan). The resulting 1748-bp product was extracted and purified from the gel and cloned into a pEX-tag vector. The recombinant plasmids were transformed into the E. coli strain JM109 and then sequenced using the Sanger DNA sequencing method. Results showed that this PP2A gene had an ORF of 1590 bp, which encoded a deduced protein containing 529 amino acid residues (termed IbPP2A1). The molecular weight of this protein was 59.66 kDa. Three conserved domains were detected in this protein, including a B56 domain and two PLN00122 domains (Figure 2). The two PLN00122 domains were located at 56–122 and 352–518 of this protein, while the B56 domain was located at 106–513. A sequence similarity search showed that this protein shared the highest identity (79%) with the PP2A domain and two PLN00122 domains. To verify the polymorphic site and PLN00122 domain. To verify the polymorphic site, as presented in Figure 3.

3.3. Nucleotide polymorphisms of IbPP2A1
To analyze the sequence diversity of IbPP2A1, a nucleotide sequence fragment (30–40 bp) was used as bait to search the RNA-Seq reads of sweet potato cultivar Xushu 18 (Tao et al. 2012, 2013). Ten polymorphic sites were found to be distributed in the coding region, including nucleotide polymorphisms located at sites 492, 1002, 1009, 1029, 1050, 1143, 1161, 1223, 1233, and 1481 (Figure 3). Five of the polymorphisms were transitions and five were transversions. The most frequent transition was an A-G transition. The RNA-Seq method also provides the opportunity to detect the nucleotide frequency of each polymorphic site, as presented in Figure 3.

Most of the polymorphic sites did not result in amino acid change, but the nucleotide polymorphisms at 1009, 1223, and 1481 resulted in amino acid changes of I→V, Y→S, and L→S, respectively, which are located in the B56 domain and PLN00122 domain. To verify the polymorphic sites detected by RNA-Seq, RT-PCR was performed using the same RNA samples that were used in the RNA-Seq study. The PCR product was submitted to the ABI 3730 DNA sequencer (Life Technologies, USA) for Sanger DNA sequencing. The sequencing chromatogram was analyzed and results showed that all ten polymorphic sites described above were also identified by Sanger DNA sequencing (Figure 3). These observations indicated that identification of polymorphic sites using RNA-Seq data for specific genes is a reliable method.

3.4. Spatial expression patterns analyses
All DGE tags of the seven tissue samples were used for tag mapping analyses using Bowtie (Langmead et al., 2009). The tag number of each contig was counted and normalized by the TPM algorithm (Morrissy et al., 2009). Results showed that the cloned IbPP2A1 was ubiquitously expressed in all of the seven tissues, although expression levels varied (Figure 4). During root development, the expression level of IbPP2A1 showed a decreasing trend. The expression abundances were 32.7, 28.9, 11.5, and 11.9 TPM in FR, ITR, ETR, and HTR, respectively. Compared with YL (14.0 FPKM), ML had a higher expression level (21.0 TPM), while ST (11.0 TPM) showed a similar expression abundance as ETR and HTR. Indeed, PP2As are usually ubiquitously expressed, but they are differentially regulated in a tissue-specific and stage-specific manner (Pérez-Callejón et al., 1993; McCright et al., 1996; Yu et al., 2003). The spatial expression pattern suggested that the IbPP2A1 gene probably plays specialized roles in diverse tissues during plant development.
Figure 3. Sequence diversity and alignment of IbPP2A1 with PP2As of some other species. A) Sequence diversity identified by Sanger DNA sequencing. Underlined numbers represent the position of polymorphic sites. The fractional numbers represent the nucleotide frequency. B) Alignment of IbPP2A1 with PP2As of other species. Arrows indicate the amino acid diversity sites.
3.5. Stress response of IbPP2A1 in sweet potato

Sweet potato is highly tolerant to adverse environmental conditions. However, very little is known about the expression of PP2As during plant stress responses. In this study, sweet potato cultivar Xushu 18 plants were grown under natural growth conditions for 3 months and subsequently exposed to drought, salinity, or alkaline stress treatment. Young leaves, stems, and expanding tuberous roots were collected at 0, 2, 11, and 20 days after stress treatment and used for a qRT-PCR study. The primers used for qRT-PCR analyses were IbPP2A-F2 and IbPP2A-R2 (Table 2). Following drought stress treatment, the IbPP2A1 expression level in stems was upregulated 26.9 times more than that of the control at 2 days, but afterwards the expression abundance declined (Figure 5A). IbPP2A1 expression was upregulated in young leaves at 2 days after treatment, reaching the highest abundance at 11 days, and then it was downregulated at 20 days. As a result of exposure to salinity and alkaline stress treatment, IbPP2A1 expression clearly increased in stems and expanding tuberous roots (Figures 5B–5D). Generally, environmental stresses result in the accumulation of reactive oxygen species (ROS) and immediate ROS-induced damage of plant proteins, DNA, and lipids (Foyer and Noctor, 2000; Dat et al., 2001; Pastori and Foyer, 2002). Higher plants have evolved two important antioxidant systems to decrease the oxidative damage. At the beginning of the stress response, plants activate the enzymatic antioxidant system (glutathione, glutathione peroxidase, catalase, or other oxidant enzymes) (Hu et al., 2005; Janssens et al., 2005; DeLong, 2006; Yang et al., 2006), but as time goes on, the nonenzymatic antioxidant system is activated to decrease oxidative damages (Silva et al., 2016) and reduce the expression of antioxidant genes (Janssens et al., 2005). As regulators of ROS signaling in plants, PP2A subunits contribute to transcriptional and posttranslational regulation of some antioxidant enzymes (Uhrig et al., 2013; Rahikainen et al., 2016). In Arabidopsis, PP2A-B1 has been shown to be a negative regulator of organellar ROS accumulation and consequent salicylic acid signaling elicited by these ROS (Li et al., 2014). Previous literature has also shown that OsPP2A-1-5, StPP2Ac1, StPP2Ac2a, StPP2Ac2b, and StPP2Ac3 become transcriptionally upregulated under salinity stress (Yu et al. 2003, 2005; País et al., 2009). However, other reports have shown that PP2A subunits mediate negative regulation of abiotic stress responses. For instance, PP2A-A3 of Arabidopsis inhibits the cold adaption response (Luo et al., 2006). Mutants of regulatory B-subunit B”a developed a longer main root than wild-type plants when exposed to salt stress (Leivar et al., 2011). Therefore, when plants were exposed to 10% PEG 6000 and 200 mmol/L NaCl, IbPP2A1 was initially upregulated and then gradually decreased after 11 days. This indicated that the cloned IbPP2A1 may play a positive role in abiotic stress responses. However, it was found that the expression of IbPP2A1 was suppressed at 2 and 11 days in young leaves following
salinity and alkaline stress treatment, but upregulated by a combination of these stresses at 11 and 20 days after treatment. Since there are different PP2A holoenzymes constructed by different combinations of A, B, and C subunits, the quantification results described here indicated that the different subunits constructed different heterotrimers that played specialized roles in diverse tissues and developmental stages, and are even localized in different subcellular structures to determine what substrates are dephosphorylated (McCright et al., 1996). However, it can still be concluded that *IbPP2A1* should be an important gene involved in stress responses.

3.6. Resistance assay of *IbPP2A1* in *Saccharomyces cerevisiae*

To investigate whether the cloned PP2A regulatory subunit could improve stress tolerance in vitro, *IbPP2A1* was cloned and expressed in *S. cerevisiae*. First, BlastP was employed to search for the homologous gene in the *S. cerevisiae* genome. It was found that *IbPP2A1* showed the highest identity with a PP2A regulatory subunit RTS1 in *S. cerevisiae* S288c with an identity of 46%. However, this PP2A of *S. cerevisiae* has a length of 757 amino acids, which is relatively longer than the *IbPP2A1* cloned in this study. This result suggested that no high identity homologous gene is encoded by the genome of *S. cerevisiae*. Stress resistances were assayed by exposing the recombinant yeast to cold, heat, and drought, which were simulated by incubating the strain at −20 °C, at 45 °C, or in sorbitol solution (4 mol/L and 6 mol/L) for 24, 72, 120, and 168 h, respectively. Results showed that the *IbPP2A1* gene cloned in this study could not improve cold, heat, and drought stress tolerance in *S. cerevisiae* (data not shown). This could be due to the fact that the 57-kDa regulatory subunit could not work alone, but needed to play regulatory roles together with additional scaffold and catalytic subunits to construct heterotrimers.

In conclusion, a total of 15 PP2A-encoding transcripts were identified from the transcriptome of sweet potato.
The one full-length PP2A was cloned and characterized (IpPP2A1). Ten nucleotide polymorphisms sites were detected in the coding sequence. Drought, salinity, and alkaline stresses lead to the upregulation of IpPP2A1 expression, but this gene could not enhance stress tolerance in *S. cerevisiae* alone.

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References


