RESEARCH ARTICLE

Genome-wide identification of protein phosphatase 2C family members in Glycyrrhiza uralensis Fisch. and their response to abscisic acid and polyethylene glycol stress

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1. Introduction

Abscisic acid (ABA), one of the six major hormones in plants, acts as an endogenous messenger of plant responses to biotic and abiotic stresses [1], such as drought stress [2], cold stress [3], salt stress [4] and pathogen infection [5]. Therefore, revealing the genes involved in the ABA signalling pathway is essential for improving plant quality and yield. Numerous proteins, especially ABA receptors, that are associated with the ABA signalling pathway and ABA stress responses have been identified. Type 2C protein phosphatase (PP2C), which is known as an interaction protein of the ABA receptor, plays an important role in response to various stresses, especially drought [6]. When the ABA signal is missing, the PP2C gene inactivates SnRK2 kinase through phosphorylation; in response to ABA signalling, the receptor PYR/PYL/RCAR binds to ABA and inhibits the activity of PP2C. SnRK2 kinase is then activated so that it can phosphorylate downstream factors to achieve signal transmission and cell response to ABA stress. In this manner, the ABA signalling pathway can be transmitted downwards, and the drought resistance of plants can be improved [7,8].

A total of 80 (13 subfamilies) and 78 (11 subfamilies) PP2C family members have been annotated in the genomes of Arabidopsis thaliana and Oryza sativa, respectively [9]. Several PP2Cs in plants have been found to be negative regulators within the ABA-mediated signalling network [10–14]. The PP2CA subfamily of A. thaliana has been identified as a key negative regulator in ABA signalling [15]. BdPP2CA6, a PP2CA gene of Brachypodium distachyon, reportedly plays a positive regulatory role in the ABA and stress signalling pathways [16]. Other subfamilies of PP2C, such as subfamilies PP2CB, PP2CC, PP2CD and PP2CE, have also been reported [17–19]. As key regulators of ABA responses, PP2Cs are central to understanding the integrative network of ABA signalling.

Glycyrrhiza uralensis is the main source of liquorice, which has great medical and economic value. Its roots and rhizomes are widely used as herbal medicine and as the raw materials of traditional Chinese medicine. It is also extensively utilized as food and chemical...
additives [20]. Pharmacological studies have shown that G. uralensis has anti-inflammatory, antiviral, antimicrobial, anticancer, immunomodulatory, gastroprotective, hepatoprotective, neuroprotective and cardioprotective properties [21]. Glycyrrhizic acid and liquiritin are the main secondary metabolites in G. uralensis. Previous studies have demonstrated that moderate drought or ABA stress can promote the accumulation of these active components, thereby improving the quality of G. uralensis [22,23]. Therefore, the PP2C genes of G. uralensis must be identified to understand the mechanism through which the accumulation of secondary metabolites is regulated under stresses, as well as to improve the quality of G. uralensis.

The availability of the whole G. uralensis genome provides convenience to the research on the PP2C genes of G. uralensis [24]. In this study, we identified PP2C genes by using the genomic data of G. uralensis. We then investigated the gene families of G. uralensis via the phylogenetic analysis of the PP2C genes of A. thaliana. The transcriptome of G. uralensis under treatment with different concentrations of ABA and a specific concentration of polyethylene glycol (PEG) and at different time points was sequenced to study the expression patterns of PP2C genes. Nine GuPP2C genes responding to ABA stress were screened by using transcriptomic data and verified via quantitative real-time polymerase chain reaction (qRT-PCR). The results of this work lay a foundation for further studies on the molecular and regulatory mechanisms of ABA signalling to secondary metabolites and pave the way for improving the cultivation and quality of G. uralensis.

2. Materials and methods

2.1. Identification and analysis of the PP2C genes of G. uralensis.

The amino acid sequences of each subfamily (A to L) in A. thaliana were downloaded from TAIR (https://www.arabidopsis.org/) [25] in March, 2021. The amino acids of G. uralensis with E-values of $1 \times 10^{-5}$ were aligned by using the protein basic local alignment search tool (BLASTp) [26]. PP2C gene sequences were extracted from the genome of G. uralensis [24]. The coding sequence (CDS), DNA and amino acid information of PP2Cs was controlled by using APOLLO [27]. The PFMA website (http://pfam.xfam.org/search/batch) was applied to predict the domains of PP2Cs [28]. The ExPaSy website (https://web.expasy.org/protparam/) was utilized to determine the properties of PP2Cs, including the number of amino acids, molecular weight (MW), theoretical isoelectric point (pI), instability index (II), aliphatic index (AI) and grand average of hydropathicity (GRAV). Gene Structure Display Server 2.0 (GSDS, http://gsds.cbi.pku.edu.cn/) was used to show the structural features of the PP2C genes of G. uralensis [29]. MEME (https://meme-suite.org/) was used to analyse the motifs of PP2Cs [30].

2.2. Phylogenetic analysis of PP2C genes of G. uralensis

The full-length PP2C protein sequences of G. uralensis and A. thaliana [9] were aligned by using MAFFT [31], and a maximum likelihood (ML) tree was constructed in accordance with the LG + F + R5 model and 1000 bootstraps by using IQ-TREE [32] to confirm the evolutionary relationships among the members of the PP2C gene family.

2.3. Transcriptomic sequencing and expression analysis of GuPP2C genes in response to ABA stress

The genome of G. uralensis was downloaded from http://ngs-data-archive.psc.riken.jp/Gur-genome/download.pl [24]. A total of 48 2-year-old G. uralensis samples were obtained from Beijing Medical Botanical Garden in Beijing City and identified by Professor Yulin Lin from the Institute of Medicinal Plant Development. G. uralensis plants with good and consistent growth were chosen. The leaves of G. uralensis were subjected to different stress conditions, including 10, 25, 50, and 100 mg/L ABA and 20% PEG4000. The leaves were collected at 0, 3, 6 and 12 h [33] after ABA and 20% PEG4000 treatments. The samples were immediately frozen in liquid nitrogen and stored in a freezer at $-80^\circ$C for total RNA extraction and transcriptome sequencing. Three samples were taken from each treatment as biological repeats. Total RNA was isolated by using RNAprep Pure Plant Plus Kit (TIANGEN, DP441, China) by following the manufacturer’s instructions. Transcriptomic data were obtained with high-throughput sequencing technology. The heat maps of transcriptomic data were drawn by using TBtools software [34]. Complementary DNA was obtained with the M-MLV reverse transcriptase synthesis system (Promega, Madison, WI, USA) by following the manufacturer’s protocols. Nine GuPP2C genes with relatively large differences in expression were screened for reverse qRT-PCR verification. The actin gene from the genome of G. uralensis was selected as the internal reference gene. The specific primers of GuPP2C genes were designed by the Primer5 programme (PREMIER Biosoft International, CA, USA). The primers of the nine genes are listed in Table S1. The amplification conditions were 95 $^\circ$C for 3 min, 95 $^\circ$C for 3 s, 57 $^\circ$C for 30 s and 72 $^\circ$C for 15 s for 40 cycles; 65 $^\circ$C for 5 s and 95 $^\circ$C for 0.5 s.

2.4. Statistical analysis

The results were calculated on the basis of three biological replicates (three technical replicates per biological replicate). Statistical analyses and significant
Figure 1. Phylogenetic relationships of the PP2C genes of G. uralensis and A. thaliana. All of the 76 GuPP2Cs from G. uralensis were included in the construction of the ML tree, and 26 AtPP2Cs from A. thaliana were used as references. Node labels represent values for bootstrap support.

correlations were performed by using SPSS (IBM, USA).

3. Results

3.1. Genome-wide identification and phylogenetic analysis of the PP2C genes of G. uralensis

A total of 76 PP2C genes were detected from the genomic sequence database of G. uralensis. All of these 76 GuPP2Cs were included to construct a ML tree, and 26 AtPP2Cs from A. thaliana were used as references (Figure 1). In accordance with the AtPP2C subfamily, the GuPP2Cs were divided into 12 subfamilies, namely, PP2CA, B, C, D, E, F1, F2, G, H, I, J and L, which contained 11, 2, 3, 15, 11, 7, 4, 8, 5, 2, 1 and 3 GuPP2C genes, respectively. The PP2CA, PP2CD and PP2CE subfamilies had the largest gene numbers, whereas the PP2J subfamily had only one gene. In contrast to A. thaliana, G. uralensis did not contain PP2CK genes. Four genes (GuPP2CX01–04) clustered into an independent branch that did not belong to the 12 subfamilies. As shown in Figure 1, the PP2CC and PP2CD subfamilies and the PP2CE and PP2CL subfamilies constituted a monophyletic sister branch with 100% bootstrap support, suggesting that the PP2CC and PP2CD subfamilies and the PP2CE and PP2CL subfamilies had a close evolutionary relationship.

3.2. Analysis of the gene structures and protein motifs of the PP2C genes of G. uralensis

The lengths of the GuPP2Cs varied from 797 bp to 13 598 bp with the CDSs ranging from 633 bp to 3 264 bp in length (Table S2). The protein amino acid number of GuPP2Cs ranged from 210 aa to 1 086 aa. The MW of GuPP2C proteins varied from 22.91 kDa to 122.74 kDa. The theoretical pl values ranged from 4.45–9.12. The II values ranged from 29.10–68.81 with an average value of 44.25. A total of 26 proteins (accounting for
The gene structure and protein motifs of the PP2C genes of *G. uralensis* were analysed. The results of gene structure analysis showed remarkable differences, which were mainly reflected in the numbers and lengths of introns (Figure 2a), amongst these subfamilies. The majority of these subfamilies, except for the PP2CL and PP2CA subfamilies, had similar numbers of exons. The PP2CE, PP2CI, PP2CB, PP2CC and PP2CG subfamilies had high genetic similarity. In GuPP2Cs, the numbers of introns ranged from 1 to 18, and the numbers of exons ranged from 2 to 19. The numbers of introns and exons greatly varied amongst the 12 subfamilies. The GuPP2CJ01 gene had the largest number of introns (18) and exons (19). Consistent with their evolutionary relationship depicted by the phylogenetic tree, most members in the same subfamily shared a similar intron/exon structure and gene length.

Twenty-six protein motifs were identified in the GuPP2C proteins (Figure 2b). Their sequences are presented in Table 1. Certain differences were observed in motif composition amongst these subfamilies. Some protein motifs were widespread amongst the GuPP2C proteins, whereas some were specific to only one or two subfamilies. Motifs 1, 2, 3 and 4 widely existed in over 90% of the GuPP2C protein sequences and were present in all of the subfamilies. However, 12 motifs were specific to a certain subfamily. Four motifs (18, 22, 23 and 26) were distributed in the PP2CH subfamily only; three motifs (14, 15 and 17) existed in the PP2CE subfamily only; two motifs (6 and 17) were present in the PP2CD subfamily only; two motifs (19 and 20) were found in the PP2CF1 subfamily only and motif 21 existed in the PP2CA subfamily only. Motifs 5 and 8 were specific to the PP2CC and PP2CD subfamilies; motif 10 was specific to the PP2CD and PP2CH subfamilies and motif 25 was specific to the PP2CA and PP2CD subfamilies. Amongst the different gene members of the same subfamilies, the main motifs were similar to each other. The composition of some subfamilies, such as PP2CC and PP2CD, was very conservative as reflected by their identical protein motifs, except for individual members. The motifs of the PP2CA subfamily greatly varied. The different motif distributions in the protein sequences may serve as a reference for studying the divergence of gene functions in different
subfamilies. Moreover, the highly similar motif distribution of the proteins in the same subfamily supported the close evolutionary relationship of these proteins [9].

3.3. Expression pattern analysis of GuPP2C genes under different stress conditions

The gene expression patterns of GuPP2Cs under different ABA and PEG stress conditions were investigated. The transcriptome from the leaves of *G. uralensis* were sequenced. Their fragment per kilobase of exon model per million mapped read (FPKM) values were calculated (Table S3). The results of differential expression analysis showed that 27.6% (n = 21) of the genes had a low expression (FPKM < 1), and included six genes with silent expression (FPKM < 1). By contrast, 72.4% (n = 55) of the genes had high expression (FPKM ≥ 10) (Figure 3). The expression levels of 18 GuPP2C genes increased under ABA stress. The average expression levels of GuPP2CA08, GuPP2CA12, GuPP2CA13, GuPP2CB02, GuPP2CC01, GuPP2CE04, GuPP2CE10, GuPP2CF14 and GuPP2CG02 in the ABA stress group were at least two times higher than those in the control group, indicating that these genes were more sensitive to ABA stress than other genes. These genes may respond to ABA stress and might respond to ABA treatment were screened by calculating FPKM values. The expression patterns of the nine candidate genes after treatment with 10, 25, 50 and 100 mg/L ABA for 3, 6 and 12 h were validated by qRT-PCR. The qRT-PCR results showed that the expression levels of six genes, i.e. GuPP2CA08, GuPP2CA12, GuPP2CE04, GuPP2CE10, GuPP2CF14 and GuPP2CG02, were significantly changed after ABA treatment (Figure 4). The expression patterns of GuPP2CA08, GuPP2CE10 and GuPP2CG02 were similar. Overall, the expression of these three genes significantly increased after 3 and 6 h of ABA treatment and then sharply decreased after 12 h of treatment. The differences amongst the three genes were as follows: GuPP2CA08 expression showed no significant change after 12 h of treatment; GuPP2CE10 expression significantly increased after 12 h of treatment with 50 and 100 mg/L ABA and GuPP2CG02 expression significantly decreased after 12 h of treatment. The gene expression levels of GuPP2CE04 and GuPP2CF14 were significantly increased under ABA treatment at all concentrations and treatment times. However, the expression of GuPP2CF14 significantly decreased under treatment with 50 mg/L ABA for 6 h. Compared with that under the control treatment, the expression of *GuPP2CA12* was significantly reduced under ABA treatment at all concentrations and treatment times but not under treatment with 25 mg/L ABA for 6 h.

Significant correlations between the expression values obtained through qRT-PCR and transcriptome analysis were identified (Table 2). The expression values of GuPP2CA08, GuPP2CE10 and GuPP2CG02 in different datasets were significantly correlated, whereas the others were not. Poor correlations may be due to sample differences.

| Table 1. Protein motifs and composition of PP2C of *G. uralensis*. |
| Motif | Width | Sites | Multilevel consensus sequence |
|-------|-------|-------|-------------------------------|
| 1     | 29    | 71    | LTDPDEFLILSDGLDLVLSNZEAVDIVR  |
| 2     | 15    | 72    | LVPANVGSRAVGLR                |
| 3     | 15    | 71    | TFFGFGDGHQGGGA                |
| 4     | 21    | 77    | GGLAVSRAGDVVLKPYGVSE          |
| 5     | 50    | 15    | IAKRLVKAALDAEAKKREMYSLSKIKDLEGKVRHRHDDITIVWYLYLHNL |
| 6     | 40    | 15    | AEQLSTEHANIEEERQRLSRLHPDPQIVLVKCHGWRSVK |
| 7     | 27    | 36    | AIALSSCDHPDDEPDERIEAAAGRRVI  |
| 8     | 50    | 17    | EQGSMEDVIRKASFAEEFGLSLVKKQLKEQQHQPJASVSCCLGVCNG |
| 9     | 21    | 45    | LVEALARGSDKDBTVWVDL           |
| 10    | 29    | 16    | PHASGSMSAQQNNLSDOSQVESGPL     |
| 11    | 11    | 53    | TSGSTAVTAIV                  |
| 12    | 41    | 17    | YLKKHFLSNIKLEKDPFDTPKPAKITKALATDDSFLESSSD |
| 13    | 27    | 27    | PTLLWGSSYSGRESRMEDAVAPVDL    |
| 14    | 41    | 11    | PSRSSAARALVESAVRAWRRKPTYPSKDVDAVCLFLOPPPP |
| 15    | 40    | 11    | PGRIFNNGSNSFASFSQKRKRGINODAMIVWEDFGSIZED |
| 16    | 15    | 26    | WKKAFLFCKFCMIDKE             |
| 17    | 21    | 15    | FRLPFPKRPLSAEPSISVR          |
| 18    | 41    | 5     | TVDHRLEENEEERERTASRGVEGRLNIFGAVISAEVGLRCPWP |
| 19    | 32    | 7     | VYKGSLSUKGHRANHMPEDYWHAKFOQLQGHEL |
| 20    | 29    | 7     | AVOQTVDKHEPNTERGIIHENGPGYFVSVMMP |
| 21    | 21    | 11    | NYCRERLHALMAEEFVEKG         |
| 22    | 21    | 5     | PWEGFPLCSNQCEQKIDVEGK       |
| 23    | 29    | 5     | EKPPFKYQQGQGAKKGDHYFKLTDQVRPV |
| 24    | 15    | 10    | ALQDPEFYRVRWLPID          |
| 25    | 11    | 14    | KDGgLWYKVDLG               |
| 26    | 38    | 5     | LFRKKESSNKTAKKLSAVGVVELFEEGSAMSLER |
Figure 3. Expression profile analysis of GuPP2Cs under different stresses at different times. CK indicates that the plants were treated with water and were used as the control. The 3, 6 and 12 h labels indicate the time after treatment. The 10, 25, 50 and 100 mg/L labels indicate different ABA concentrations. The bar on the lower right corner represents FPKM values, and different colours denote various expression levels.

Table 2. Significant correlations between FPKM values and qRT-PCR results.

| Genename   | Correlation coefficient | Sig. (2-tailed) |
|------------|-------------------------|-----------------|
| GuPP2CA08  | 0.846 **                 | 0.000266        |
| GuPP2CA12  | -0.401                  | 0.174357        |
| GuPP2CE04  | 0.275                   | 0.363675        |
| GuPP2CE10  | 0.736 **                | 0.000104        |
| GuPP2CF14  | 0.126                   | 0.680875        |
| GuPP2CG02  | 0.729 **                | 0.000005        |

** The correlation is significant at the 0.01 level.

4. Discussion and conclusions

The PP2C genes code for the interaction proteins of the ABA receptor, which can release SnRK2 kinase activity when its own activity is inhibited [6]. ABA plays an essential role in plant response to abiotic stress. Hence, in plants, ABA content increases to improve resistance to drought, salt stress and other abiotic stresses [35]. Therefore, identifying PP2C genes is vital for improving the abiotic stress resistance of plants. The functions of PP2C genes have been characterized in other plants, such as Salvia miltiorrhiza [36], Gossypium hirsutum [37], Brassica rapa [33] and Fragaria vesca [38], aside from A. thaliana.

To date, several studies have highlighted the importance of PP2C, which may play pivotal roles in various processes, including biotic–abiotic stresses and plant development [39]. In plants, PP2C members have been described as regulators of desiccation tolerance and as negative regulators in the ABA signalling pathway [12,13] Previous studies have reported that AtPP2CG1 regulates positively against salt tolerance in A. thaliana and is induced by drought, salt or exogenous ABA treatment [40]. In A. thaliana, two PP2C gene members show different responses. Specifically, AP2C1 expression is powerfully induced by drought, wounding and
Figure 4. Expression patterns of six GuPP2C genes in response to ABA stress. The relative expression of GuPP2Cs was examined after treatments with various concentrations of exogenous ABA for the indicated periods. The actin gene in *G. uralensis* was used as the internal control. Values are expressed as means ± SD (*n* = 3). *P* < 0.05, **P** < 0.01 vs the expression value at 0 h under 10 mg/L ABA treatments; *▲P* < 0.05, **▲P** < 0.01 vs the expression value at 0 h under 25 mg/L ABA treatments; *#P* < 0.05, **##P** < 0.01 vs the expression value at 0 h under 50 mg/L ABA treatments; *P* < 0.05, **P** < 0.01 vs the expression value at 0 h under 100 mg/L ABA treatments.

cold, whereas the same stresses only slightly induce AP2C2 expression [41]. Moreover, the expression profiles of PP2C in various species after exposure to abiotic and hormone stresses have been studied [42,43]. Shazadee et al. predicted the cis-regulatory elements in upland cotton and suggested that the *GhPP2C* genes in *G. hirsutum* may be involved in the response to heat, cold, drought and NaCl stresses. They subjected cotton seedlings to four various abiotic stress treatments and selected 30 genes for qRT-PCR. Their results showed that some *GhPP2C* genes exhibited high transcript levels after exposure to multiple treatments and that a few were induced by one or more treatments. For example, heat stress accounted for the dominant portion of down-regulated genes. However, cold stress up-regulated 70% of the genes and decreased the transcript level of 30% of the genes. Drought stress accounted for the up-regulation and down-regulation of 53% and 47% of *GhPP2C* genes, respectively, and salt stress resulted in the up-regulation and down-regulation of 56% and 44% of the genes, respectively. Taken together, all of the 30 genes were induced by different abiotic stresses; the diverse expression profiles of *GhPP2C* genes may suggest that these genes may be critical for abiotic stress responses [37]. Similarly, the diverse expression patterns of *BraPP2C* genes in *B. rapa* are suggestive of various roles after exposure to abiotic and hormone stress conditions [33]. In addition, studies have confirmed that some PP2C genes can regulate plant tolerance to drought. Yu et al. [44] reported that the wheat protein phosphatase PP2C-A10 can promote seed germination and decrease the drought tolerance of transgenic *A. thaliana* by interacting with the *TaDOG1L1* and *TaDOG1L4* genes. Miao et al. [45] showed that in rice, OsPP2C09 acts as a negative regulator of drought tolerance through ABA signalling. Under ABA treatment, the faster and higher accumulation of OsPP2C09 transcripts in roots than in shoots may increase the root-to-shoot ratio of plants under drought stress. Xiang et al. [46] found that *ZmPP2C-A10* was tightly associated with drought tolerance in *Zea mays*. Moreover, they found that allele-338 containing a deletion of the endoplasmic reticulum stress response element in the 5’-UTR region of *ZmPP2C-A10* enhanced plant drought tolerance.

Liquorice root is one of the most popular traditional Chinese medicines, and its main active components are flavonoids. *G. uralensis* is an important source of liquorice. Previous studies have confirmed that in plants, moderate drought or ABA stress can promote the accumulation of secondary metabolites [23], which are important components used in quality evaluation [22]. However, the characterization and functional analyses of PP2C genes in *G. uralensis* that respond to ABA treatment have not been reported yet. Here, we report the PP2C genes of *G. uralensis*. A total of 76 PP2C genes were identified in *G. uralensis*. The number of the PP2C genes of *G. uralensis* was similar to that of other higher plants, such as *A. thaliana* (80) [9], *O. sativa* (78) [9], *B. distachyon* (86) [42] and *Setaria italica* (80) [47]. The PP2C genes were divided into 12 subfamilies, namely, subfamilies GuPP2CA–L. Phylogenetic analysis revealed that most members in the same subfamily shared similar intron/exon structures. Moreover, the results suggested that the same subfamily
may perform similar biological functions. The analysis of the transcriptomic data of the GuPP2C genes indicated that GuPP2CA08, GuPP2CA12, GuPP2CA13, GuPP2CB02, GuPP2CC01, GuPP2CE04, GuPP2CE10, GuPP2CF14 and GuPP2CG02 were highly sensitive to ABA stress and might respond to ABA treatment. Gene expression pattern analysis via qRT-PCR showed that the expression levels of six genes were significantly changed after ABA treatment and were correlated with ABA concentration and treatment time. Overall, the expression patterns of GuPP2CA08, GuPP2CE10 and GuPP2CG02 changed dynamically with the treatment time. Moreover, the expression levels of GuPP2CE04 and GuPP2CF14 significantly increased, and the expression of GuPP2CA12 significantly reduced.

The present study conducted the first genome-wide identification and characterization of the phylogenetic relationships, gene structures and motifs of the PP2C gene family of G. uralensis. The expression patterns of the GuPP2C genes that responded to ABA treatment were then analysed. The results of this work provide a reference for the genome-wide identification of the PP2C gene family of other species and lay a foundation for future functional research on the PP2C genes of G. uralensis.

Data availability statements

The raw data of RNA-seq is stored in NCBI (https://www.ncbi.nlm.nih.gov/). The associated BioProject and BioSample numbers are PRJNA784170 and SAMN23475019, respectively.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Raghavendra AS, Gonugunta VK, Christmann A, et al. ABA perception and signalling. Trends Plant Sci. 2010;15(7): 395–401.
[2] Lim CW, Baek W, Jung J, et al. Function of ABA in Stomatal Defense against Biotic and Drought Stresses. Int J Mol Sci. 2015;16(7):15251–15270.
[3] Agurla S, Gahir S, Munemasa S, et al. Mechanism of Stomatal Closure in Plants Exposed to Drought and Cold Stress. Adv Exp Med Biol. 2018;1081:215–232.
[4] Julkowska MM, Testerink C. Tuning plant signaling and growth to survive salt. Trends Plant Sci. 2015;20(9): 586–594.
[5] Cao FY, Yoshioka K, Desveaux D. The roles of ABA in plant-pathogen interactions. J Plant Res. 2011;124(4):489–499.
[6] Ma Y, Sztostkiewicz J, Korte A, et al. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science. 2009;324(5930):1064–1068.
[7] Shi Y. Serine/threonine phosphatases: mechanism through structure. Cell. 2009;139(3):468–484.
[8] Zhu JK. Abiotic Stress Signaling and Responses in Plants. Cell. 2016;167(2):313–324.
[9] Xue T, Wang D, Zhang S, et al. Genome-wide and expression analysis of protein phosphatase 2C in rice and Arabidopsis. BMC Genomics. 2008;9:550.
[10] Merlot S, Gosti F, Guerrier D, et al. The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. Plant J. 2001;25(3):295–303.
[11] Tāhtihārju S, Palva T. Antisense inhibition of protein phosphatase 2C accelerates cold acclimation in Arabidopsis thaliana. Plant J. 2001;26(4):461–470.
[12] González-García MP, Rodríguez D, Nicolás C, et al. Negative regulation of abscisic acid signaling by the Fagus sylvatica FsPP2C1 plays a role in seed dormancy regulation and promotion of seed germination. Plant Physiol. 2003;133(1):135–144.
[13] Saez A, Apostolova N, Gonzalez-Guzman M, et al. Gain-of-function and loss-of-function phenotypes of the protein phosphatase 2C HAB1 reveal its role as a negative regulator of abscisic acid signalling. Plant J. 2004;37(3):354–369.
[14] Yoshida T, Nishimura N, Kitahata N, et al. ABA-hypersensitive germination3 encodes a protein phosphatase 2C (AtPP2CA) that strongly regulates abscisic acid signaling during germination among Arabidopsis protein phosphatase 2Cs. Plant Physiol. 2006;140(1): 115–126.
[15] Sheen J. Mutational analysis of protein phosphatase 2C involved in abscisic acid signal transduction in higher plants. Proc Natl Acad Sci U S A. 1998;95(3):975–980.
[16] Zhang F, Wei Q, Shi J, et al. Brachypodium distachyon BdPP2CA6 Interacts with BdPYLs and BdSnRK2 and Positively Regulates Salt Tolerance in Transgenic Arabidopsis. Front Plant Sci. 2017;8:264.
[17] Schweighofer A, Kazanaviciute V, Scheikl E, et al. The PP2C-type phosphatase AP2C1, which negatively regulates MPK4 and MPK6, modulates innate immunity, jasmonic acid, and ethylene levels in Arabidopsis. Plant Cell. 2007;19(7):2213–2224.
[18] Gagne JM, Clark SE. The Arabidopsis stem cell factor POLTERGEIST is membrane localized and phosphoplitid stimulated. Plant Cell. 2010;22(3):729–743.
[19] Chen C, Yu Y, Ding X, et al. Genome-wide analysis and expression profiling of PP2C clade D under saline and alkali stresses in wild soybean and Arabidopsis. Proteomics. 2018;18(25):643–654.
[20] Hong DY. Flora reipublicae Popularis Sinicae. Beijing: Science Press; 1983.
[21] Hosseinzadeh H, Nassiri-Asl M. Pharmacological Effects of Glycyrrhiza spp. and Its Bioactive Constituents: Update and Review. Phytother Res. 2015;29(12):1868–1886.
[22] Zhang CR, Sang XY, Qu M, et al. De novo sequencing and assembly of root transcriptome to reveal regulation of gene expression by moderate drought stress in Glycyrrhiza uralensis. China J Chin Mater Med. 2015;40(24): 4817–4823.
[23] Qiao J, Luo Z, Li Y, et al. Effect of Abscisic Acid on Accumulation of Five Active Components in Root of Glycyrrhiza uralensis. Molecules. 2017;22(11):1982.

[24] Mochida K, Sakurai T, Seki H, et al. Draft genome assembly and annotation of Glycyrrhiza uralensis, a medicinal legume. Plant J. 2017;89(2):181–194.

[25] Reiser L, Subramaniam S, Li D, et al. Using the Arabidopsis Information Resource (TAIR) to Find Information About Arabidopsis Genes. Curr Protoc Bioinformatics. 2017;60:1.11.1–1.11.45.

[26] Altschul SF, Madden TL, Schäffer AA, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 1997;25(17):3389–3402.

[27] Finn RD, Mistry J, Schuster-Böckler B, et al. Pfam: clans, web tools and services. Nucleic Acids Res. 2006;34:D247–D251.

[28] Hu B, Jin J, Guo AY, et al. GSDS 2.0: an upgraded gene feature visualization server. Bioinformatics. 2015;31(8):1296–1297.

[29] Bailey TL, Johnson J, Grant CE, et al. The MEME Suite. Nucleic Acids Res. 2015;43(W1):W39–W49.

[30] Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013;30(4):772–780.

[31] Nguyen LT, Schmidt HA, Von Haeseler A, et al. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol. 2013;32(1):1296–1297.

[32] Chen C, Chen H, Zhang Y, et al. Genome-Wide Identification, Evolution, and Transcriptional Profiling of PP2C Gene Family in Brassica rapa. Biomed Res Int. 2019;2019:Article ID 2965035.

[33] Yoshida T, Mogami J, Yamaguchi-Shinozaki K. ABA-dependent and ABA-independent signaling in response to osmotic stress in plants. Curr Opin Plant Biol. 2014;21:133–139.

[34] Xu ZC, Pu XD, Song JY. Genome-wide Characterization of PP2C Gene Family in Salvia miltiorrhiza. Modern Chinese Medicine. 2018;20(6):652–657.

[35] Shazadee H, Khan N, Wang J, et al. Identification and Expression Profiling of Protein Phosphatases (PP2C) Gene Family in Gossypium hirsutum L. Int J Mol Sci. 2019;20(6):Article ID 1395.

[36] Haider MS, Khan N, Pervaiz T, et al. Genome-wide identification, evolution, and molecular characterization of the PP2C gene family in woodland strawberry. Gene. 2019;702:27–35.

[37] Singh A, Pandey A, Srivastava AK, et al. Plant protein phosphatases 2C: from genomic diversity to functional multiplicity and importance in stress management. Crit Rev Biotechnol. 2016;36(6):1023–1035.

[38] Liu X, Zhu Y, Zhai H, et al. AtPP2CG1, a protein phosphatase 2C, positively regulates salt tolerance of Arabidopsis in abscisic acid-dependent manner. Biochem Biophys Res Commun. 2012;422(4):710–715.

[39] Chonnarit C. Characterization and functional analysis of a novel PP2C phosphatase AP2C2 from Arabidopsis. Vienna: University of Vienna; 2008.

[40] Cao J, Jiang M, Li P, et al. Genome-wide identification and evolutionary analyses of the PP2C gene family with their expression profiling in response to multiple stresses in Brachypodium distachyon. BMC Genomics. 2016;17:Article ID 175.

[41] Yang Q, Liu K, Niu X, et al. Genome-wide Identification of PP2C Genes and Their Expression Profiling in Response to Drought and Cold Stresses in Medicago truncatula. Sci Rep. 2018;8(1):Article ID 12841.

[42] Yu X, Han J, Li L, et al. Wheat PP2C-a10 regulates seed germination and drought tolerance in transgenic Arabidopsis. Plant Cell Rep. 2020;39(5):635–651.

[43] Miao J, Li X, Li X, et al. OsPP2C09, a negative regulatory factor in abscisic acid signalling, plays an essential role in balancing plant growth and drought tolerance in rice. New Phytol. 2020;227(5):1417–1433.

[44] Xiang Y, Sun X, Gao S, et al. Deletion of an Endoplasmic Reticulum Stress Response Element in a ZmPP2C-A Gene Facilitates Drought Tolerance of Maize Seedlings. Mol Plant. 2017;10(3):456–469.

[45] Min DH, Xue FY, Ma YN, et al. Characteristics of PP2C Gene Family in Foxtail Millet (Setaria italica). Acta Agronomica Sinica. 2013;39(12):2135.