Genome-Wide Identification and Expression Analyses of AnSnRK2 Gene Family under Osmotic Stress in Ammopiptanthus nanus

Yueming Tang, Fengzhong Lu, Wenqi Feng, Yuan Liu, Yang Cao, Wanchen Li, Fengling Fu * and Haoqiang Yu *

Key Laboratory of Biology and Genetic Improvement of Maize in Southwest Region, Maize Research Institute, Sichuan Agricultural University, Chengdu 611130, China; tangyueming@stu.sicau.edu.cn (Y.T.); luwf@stu.sicau.edu.cn (F.L.); fwq@stu.sicau.edu.cn (W.F.); 18394159617@163.com (Y.L.); caoy@stu.sicau.edu.cn (Y.C.); aumdyms@sicau.edu.cn (W.L.)

* Correspondence: ffl@sicau.edu.cn (F.F.); yhq1801@sicau.edu.cn (H.Y.)

Abstract: Sucrose non-fermenting-1 (SNF1)-related protein kinase 2’s (SnRK2s) are plant-specific serine/threonine protein kinases and play crucial roles in the abscisic acid signaling pathway and abiotic stress response. Ammopiptanthus nanus is a relict xerophyte shrub and extremely tolerant of abiotic stresses. Therefore, we performed genome-wide identification of the AnSnRK2 genes and analyzed their expression profiles under osmotic stresses including drought and salinity. A total of 11 AnSnRK2 genes (AnSnRK2.1-AnSnRK2.11) were identified in the A. nanus genome and were divided into three groups according to the phylogenetic tree. The AnSnRK2.6 has seven introns and others have eight introns. All of the AnSnRK2 proteins are highly conserved at the N-terminus and contain similar motif composition. The result of cis-acting element analysis showed that there were abundant hormone- and stress-related cis-elements in the promoter regions of AnSnRK2s. Moreover, the results of quantitative real-time PCR exhibited that the expression of most AnSnRK2s was induced by NaCl and PEG-6000 treatments, but the expression of AnSnRK2.3 and AnSnRK2.6 was inhibited, suggesting that the AnSnRK2s might play key roles in stress tolerance. The study provides insights into understanding the function of AnSnRK2s.

Keywords: protein kinase; SnRK2; osmotic stress; expression profiles; Ammopiptanthus nanus

1. Introduction

Plants frequently encounter surrounding environment changes and are vulnerable to these stimuli, including drought, extreme temperatures, and salinity. After long-term evolution, plants have evolved a variety of mechanisms to adapt to abiotic stresses. Protein phosphorylation or dephosphorylation is one of the most important cures for plant response to environmental stresses [1–3]. Protein phosphorylation catalyzed by protein kinases interconnects various signal pathways and plays a vital role in plant response to abiotic stresses [4]. For instance, the plant mitogen-activated protein kinase (MAPK) mediates MAPKKK–MAPKK–MAPK cascade reaction through phosphorylation to respond to a variety of biotic and abiotic stresses [5,6]. Phosphorylated AtMKKK1 interacts with AtM KK1 and AtMKK2 to activate MPK4 in drought response [7]. Calcium-dependent protein kinase (CDPK) can phosphorylate and activate transcription factors, thereby regulating gene expression in response to environmental stimuli [8,9]. AtCPK4 and AtCPK11 phosphorylate two ABA-responsive transcription factors (ABF1 and ABF4) in response to abscisic acid (ABA) and drought stress [10]. The sucrose non-fermenting-1 (SNF1)-related protein kinases (SnRKs) are plant-specific serine/threonine protein kinases. They widely exist in higher plants, where they regulate plant growth and development and response to adversity stresses [11–15]. According to the sequence similarity and conservation of the C-terminal domain, the SnRK proteins are classified into three subfamilies, namely...
SnRK1, SnRK2, and SnRK3 [16,17]. The SnRK1 subfamily has been reported to participate in sugar and ABA signaling pathways and metabolic regulation [18–20]. SnRK2 and SnRK3 subfamilies mediate plant response to drought, salinity, and osmotic stress [21,22].

Notably, SnRK2s control plant growth, development, and stress response via the ABA signaling pathway [23]. In plants, SnRK2s were first identified by the description of the PKABA1 gene in wheat, which is involved in ABA signal transduction. The expression of PKABA1 is induced by dehydration, salinity, low-temperature, and osmotic stress [24]. Likewise, PKABA1 phosphorylates the ABA response element-binding factor (TaABF) to regulate grain maturation and seed dormancy [25,26]. Furthermore, the expression of the SnRK2 genes was induced by osmotic stress of mannitol and NaCl, or ABA, in *Arabidopsis thaliana* [27]. The SnRK2.2/2.3/2.6 phosphorylate ABA-responsive element-binding protein (AREB) and thus positively regulate ABA signaling in response to drought stress in *Arabidopsis* [28]. In addition, SnRK2s regulate root morphogenesis, flowering, fruit maturation, yield formation, and plant height [15,29–32]. Under saline conditions, the *AtSnRK2.4/2.10* knockout mutant shows reduced root length and fewer lateral roots than the wild type [33]. Overexpression of the *TaSnRK2.9-5A* gene increases the grain yield in transgenic rice [31].

*A. nanus* is a relict broadleaf shrub. It has survived in the desert and arid regions of Central Asia since the disappearance of the ancient Mediterranean in the tertiary period [34]. It is extremely tolerant to abiotic stresses such as drought, salinity, barrenness, and extreme temperatures [35,36]. Considering the crucial roles of SnRK2s in stress response, we analyzed and identified the *SnRK2* genes in the genome of *A. nanus* through bioinformatics. Subsequently, quantitative real-time PCR was performed to analyze the expression pattern of the *AnSnRK2* genes under osmotic stresses including drought and salinity. The study will provide useful information for further functional studies of the *AnSnRK2* genes.

### 2. Results

#### 2.1. The SnRK2 Genes in *A. nanus*

The amino acid sequences of SnRK2s from *Arabidopsis* and rice were used to perform local blast in *A. nanus*. In total, 224 candidate sequences were obtained. After removing the redundancy, 215 candidate sequences were aligned with the SnRK2 sequences of *Arabidopsis* and rice to construct a phylogenetic tree. The results showed that 11 SnRK2s of *A. nanus* and all SnRK2s of *Arabidopsis* and rice were clustered into the same branch with a bootstrap value of 100 (Figures S1 and S2). The 11 *AnSnRK2* candidate genes were named *AnSnRK2.1–AnSnRK2.11*. Their coding sequences were 1017 to 1152 bp in length, encoding 338 to 383 amino acids, with a molecular weight of 38.42 to 43.07 KDa, respectively. The theoretical isoelectric point of *AnSnRK2* proteins ranged from 4.71 to 6.21, indicating that they were acidic proteins. Ten *AnSnRK2* were predicted to be hydrophilic proteins with grand average of hydropathicity (GRAVY) < 0. No signal peptides were detected, and only *AnSnRK2.1* showed a transmembrane domain (Figure S3). Prediction of subcellular location indicated that *AnSnRK2* localized in cytoskeleton and cytosol, which was consistent with their hydrophilic nature. Among them, only *AnSnRK2.1* localized in chloroplast, which might be involved in the photosynthetic metabolism of cells (Table 1).
### Table 1. Characteristics of putative *AnSnRK2* genes in *A. nanus.*

| Gene Name | Gene ID     | CDs(bp) | Amino Acid | Isoelectric Point | Molecular Weight (KDa) | Introns | Grand Average Hydrophy | Subcellular Localization (Probability) |
|-----------|-------------|---------|------------|-------------------|------------------------|---------|-----------------------|-----------------------------------|
| *AnSnRK2.1* | EVM0003959.1 | 1152    | 383        | 5.92              | 43.07                  | 8       | −0.25                 | chloroplast                        |
| *AnSnRK2.2* | EVM0011565.1 | 1062    | 353        | 5.81              | 40.52                  | 8       | −0.53                 | cytoskeleton                       |
| *AnSnRK2.3* | EVM0013530.1 | 1071    | 356        | 6.21              | 40.71                  | 8       | −0.47                 | cytoskeleton                       |
| *AnSnRK2.4* | EVM0016282.1 | 1098    | 365        | 4.89              | 41.55                  | 8       | −0.34                 | cytoskeleton                       |
| *AnSnRK2.5* | EVM0017722.1 | 1059    | 352        | 5.95              | 40.60                  | 8       | −0.50                 | cytoskeleton                       |
| *AnSnRK2.6* | EVM0020312.1 | 1074    | 357        | 5.69              | 40.43                  | 7       | −0.19                 | cytoplasm                          |
| *AnSnRK2.7* | EVM0025729.1 | 1062    | 353        | 5.00              | 40.22                  | 8       | −0.35                 | cytoskeleton                       |
| *AnSnRK2.8* | EVM0028012.1 | 1029    | 342        | 5.24              | 38.76                  | 8       | −0.31                 | cytoplasm                          |
| *AnSnRK2.9* | EVM0029135.1 | 1035    | 344        | 5.23              | 38.96                  | 8       | −0.3                 | cytoskeleton                       |
| *AnSnRK2.10* | EVM0032503.1 | 1017    | 338        | 5.3               | 38.42                  | 8       | −0.2                  | cytoplasm                          |
| *AnSnRK2.11* | EVM0034033.1 | 1089    | 362        | 4.71              | 41.16                  | 8       | −0.27                 | cytoskeleton                       |

#### 2.2. Multiple Alignment and Phylogenetic Analysis of *AnSnRK2s*

The results of multiple alignment indicated that 11 *AnSnRK2s* were highly conserved with an average of 66.7% sequence identity. Specifically, all *AnSnRK2s* possessed a highly conserved protein kinase domain in the N-terminal, including an ATP-binding region, the serine/threonine protein kinase active-site, and an abiotic stress activation region in C-terminal. *AnSnRK2.4/7/8/9/10/11* contained abundant aspartic acids (D) and glutamic acids (E) at the C-terminus, which were proved to regulate ABA signaling activity (Figure 1) [37,38].

Furthermore, the SnRK2 amino acid sequences of *A. nanus, Arabidopsis, rice, maize,* and soybean were used to construct a phylogenetic tree. As shown in Figure 2, these SnRK2s were divided into three subgroups (I, II, and III), which was consistent with previous classifications [27,38]. The AnSnRK2s showed a closer phylogenetic relationship with GmSnRK2s of soybean than the SnRK2s of *Arabidopsis, rice,* and *maize.* The *AnSnRK2.2, AnSnRK2.3,* and *AnSnRK2.5* were classed into one group (Subgroup I) and showed a diversity at C-terminal compared to other AnSnRK2s (Figure 1). The *AnSnRK2.1, AnSnRK2.6,* *AnSnRK2.8, AnSnRK2.9,* and *AnSnRK2.10* were divided into one subgroup (Subgroup II). The *AnSnRK2.4, AnSnRK2.7,* and *AnSnRK2.11* were clustered into the same subgroup (Subgroup III) and exhibited high conservation at C-terminal. The result indicates that the SnRK2 genes are relatively conservative in evolution.
Figure 1. Multiple alignment of amino acid sequences of AnSnRK2s. The ATP-binding domain and the serine/threonine protein kinase active-site are marked by red and green boxes, respectively. The C-terminal domain contains two subdomains. The brown box represents the structural domain response to abiotic stress; the blue box represents ABA inducible regulatory domain.
2.3. Gene Structure and Motifs of AnSnRK2s

As shown in Figure 3, gene structure analysis showed that all AnSnRK2s had untranslated regions (UTRs) at both 5 and 3 terminals. Only the AnSnRK2.6 gene had eight exons; other AnSnRK2s possessed nine exons with different lengths. The lengths of the first and last exon of every AnSnRK2 were different. The lengths of the second to the seventh exons of AnSnRK2.6 were 75, 102, 231, 93, 105, and 99 bp, respectively. However, the length of the second to the eighth exons of the other AnSnRK2s were 75, 102, 54, 93, 93, 105, and 99 bp, respectively. All introns of the AnSnRK2 genes were 0-phase, which interrupted the
exons between two triplet codons, showing high conservation of exon–intron structure and similar splicing patterns of AnSnRK2s [39].

To further understand the systematic relationship among AnSnRK2 proteins, 15 conserved motifs of AnSnRK2s were predicted using MEME (Figure 4). All AnSnRK2s possessed nine conserved motifs and exhibited similar motif composition within subgroups. The motif 13 was only shared by Subgroup I AnSnRK2s, which was different from other subgroups. The motifs 12, 14, and 15 were only shared by subgroup III AnSnRK2s. Among them, the AnSnRK2s of subgroups II and III showed motif diversity at the C-terminal.

![Figure 3. The exon–intron organizations of AnSnRK2s.](image)

2.4. The cis-Elements in the Promoter of AnSnRK2s

Through PlantCARE analysis, a total of 75 cis-elements were found in the promoter region of the AnSnRK2 genes, including 9 types of hormone-related elements (responding to ABA, auxin, gibberellin, ethylene, salicylic acid, and methyl jasmonate), 9 types of stress-related response elements (response to drought, salt, low-temperature, wound, anaerobic, defense, and stress responses), and 13 types of light-related response elements (Figure 5). Notably, ABA-responsive elements (ABREs) were found in the promoters of most AnSnRK2s except AnSnRK2.3 and AnSnRK2.11, indicating their potential roles in ABA signaling. Meanwhile, ethylene-responsive elements (EREs) were found in the promoters of
AnSnRK2s except for AnSnRK2.7 and AnSnRK2.9. MYB binding sites (MBSs), MYC-binding sites, and low-temperature inducing elements (LTRs) were also found in the promoters of most AnSnRK2s. The results suggest that AnSnRK2s not only respond to ABA but also are involved in stress response.

Figure 5. The *cis*-elements in AnSnRK2 promoters. The number represents the number of *cis*-elements.

### 2.5. Expression of AnSnRK2 Genes under Drought and Salinity Stress

To investigate the expression of the AnSnRK2 genes in abiotic stress response, their expression patterns under the high osmotic pressure (PEG-6000 or NaCl) were analyzed by qRT-PCR. The results of qRT-PCR showed that the expression of AnSnRK2.1, AnSnRK2.5, AnSnRK2.7, AnSnRK2.8, AnSnRK2.9, AnSnRK2.10, and AnSnRK2.11 was significantly upregulated by salt treatment (250 mM NaCl) and peaked at 1, 12, 6, 1, 1, 1, and 1 h of treatment, respectively. The expression of AnSnRK2.8, AnSnRK2.9, and AnSnRK2.11 was upregulated more than 17-, 18-, and 8-fold, respectively. However, the expression of AnSnRK2.2 AnSnRK2.3 AnSnRK2.4, and AnSnRK2.6 was inhibited by salt and reached a minimum at 24, 6, 1, and 6 h of treatment, respectively (Figure 6A). After 20% PEG-6000 treatment, the expression of AnSnRK2.3 and AnSnRK2.6 was significantly downregulated and decreased to 55% and 13% at 24 h, respectively, compared to control (0 h). The expression of AnSnRK2.6 was significantly inhibited by both treatments and 33% lower than control. The AnSnRK2.8 exhibited no differential expression. Only AnSnRK2.7 was continuously induced during stress treatment. The results indicate that the AnSnRK2s may play crucial roles in osmotic stress response.
3. Discussion

SnRK2s are plant-specific Ser/Thr protein kinases and play crucial roles in plant growth and stress response [40]. To date, the SnRK2 genes have been identified from different species, such as rice (10), maize (10), sorghum (10), pak choi (13), Chinese white pear (10), and pepper (9) [17,41–45]. It has been shown that the number of SnRK2 genes ranges from 8 (Solanum tuberosum) to 22 (Glycine max) [46,47]. However, most of them have 9–11 SnRK2s. In the case of soybeans, it may be due to two whole-genome duplication events [48]. In this study, 11 AnSnRK2s were identified from the A. nanus (Table 1).
The phylogenetic tree showed that AnSnRK2s were divided into three groups (Figure 2). The AnSnRK2s from the same subgroup showed similar conserved domains, motif composition, and gene architecture and slight diversity among different subgroups (Figures 1, 3 and 4). Most of SnRK2 genes identified from different species have eight introns, but few of them have one (ZmSnRK2.5), two (SbSnRK2.8), three (SAPK5), five (AtSnRK2.8), six (SAPK10/SlSnRK2.6), seven (GmSnRK2.6/SbSnRK2.2/ZmSnRK2.9/ZmSnRK2.10), or nine (AtSnRK2.6/SbSnRK2.7) introns [17,27,41,42,46,47]. It is speculated that the number of introns of the plant SnRK2 gene is highly conservative at eight. In this study, the AnSnRK2 genes all had eight introns except AnSnRK2.6 (Figure 3). The lengths of the second to eighth exons of all SnRK2s from maize and Arabidopsis are 75, 102, 54, 93, 105, and 99 bp, respectively [41], which is likewise found in A. nanus. The AnSnRK2.6 gene only possessed seven introns because of an extended fourth exon. These results indicate SnRK2s evolve structure conservation among the same subclades and diversity between different subgroups.

When plants are exposed to stress, the stress-related transcription factors will be activated through a series of signal transmissions. These activated factors will combine with the cis-acting elements of downstream target gene promoters and regulate their expression to respond to stress [49]. The study of cis-acting elements of genes is particularly important to reveal their potential roles. In this study, the AnSnRK2s promoters possessed abundant hormone- and stress-responsive cis-elements. The MYC, ABRE, or ERE cis-elements were enriched in most of the AnSnRK2s promoters, which are also found in pepper and cotton [45,50]. Meanwhile, a huge number of cold-inducing elements (LTRs) were identified in AnSnRK2s promoters (Figure 5). The result suggests that AnSnRK2s play an important role in the ABA signaling pathway and stress response.

It has been proved that SnRK2 is involved in abiotic stresses and used to improve plants’ stress resistance via the expression of SnRK2 genes. The expression of TaSnRK2.4 in wheat, SAPK4 in rice, NtSnRK2.2 in tobacco, and MpSnRK2.10 in apple can be activated by salt, water deficit, low temperature, or oxidative stresses. Overexpression of TaSnRK2.4, SAPK4, NtSnRK2.2, or MpSnRK2.10 increases the tolerance to salt, drought, cold, or oxidative stress of transgenic plants, respectively [12,14,51,52]. In this study, all AnSnRK2s were found to respond to osmotic stress except for SnRK2.8. Only AnAnRK2.7 can be upregulated by both NaCl and PEG, while AnSnRK2.6 is inhibited by these stresses (Figure 6). It is speculated that these two genes may play an extraordinary role under osmotic stress.

In summary, we identified 11 AnSnRK2 members from A. nanus and analyzed their gene structures, conservative motifs, phylogenetic relationships, cis-acting elements, and expression profiles under osmotic stress. The results provide valuable information for further elucidating the function of AnSnRK2s.

4. Materials and Methods

4.1. Identification of AnSnRK2 Genes in A. nanus

In order to identify the AnSnRK2 gene of A. nanus, the protein and genome sequences were obtained from A. nanus genome project [53]. The 10 SnRK2 protein sequences from Arabidopsis and rice were retrieved from the Arabidopsis Information Resource (http://www.arabidopsis.org/, accessed on 15 October 2020) and Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/index.shtml, accessed on 15 October 2020), respectively [17,27], and used as queries to perform local BLASTP (blast-2.9.0) with an E-value 1 × 10⁻¹⁰ and homology minimum 50% to obtain the SnRK2 of A. nanus. The amino acid sequences of candidates were analyzed by using the Hidden Markov Model (HMM) seed profiles of the protein kinase domain (PF00069) and protein serine/threonine kinase (PF07714) from the Pfam database (http://pfam.xfam.org/, accessed on 15 October 2020). After removing the redundancy sequences, the candidate genes were obtained and used to construct a phylogenetic tree with SnRK2s of the Arabidopsis and rice. The candidate genes clustered with Arabidopsis and rice SnRK2s were identified as putative AnSnRK2s. The motif composition of AnSnRK2s was analyzed using the
online tool MEME V4.12.0 (http://meme.sdsc.edu/meme/meme.html, accessed on 20 October 2020) with the motif length set at 10–100 and motif maximum number set at 15. The protein molecular weight, isoelectric point, and protein hydrophobicity of AnSnRK2s were predicted using online ProtParam software provided by ExPaSy (available online: http://expasy.org/tools/protparam.html, accessed on 20 October 2020). The subcellular location and transmembrane structure of the AnSnRK2s were predicted by the WoLF PSORT tools (https://wolfpsort.hgc.jp/, accessed on 20 October 2020) and the TMHMM v.2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/, accessed on 20 October 2020), respectively.

4.2. Multiple Alignment and Phylogenetic Analysis

DNAMAN (version 8) software was used for the multiple alignment of the amino acid sequences of AnSnRK2s. The conserved domains were analyzed using the Conserved Domain Search Database (CDD, http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml, accessed on 15 October 2020). Subsequently, the phylogenetic tree of SnRK2s between A. nanus, Arabidopsis, rice, maize, and soybean was constructed using MEGA X (version X, Hachioji, Tokyo, Japan) with neighbor-joining method and 1000 bootstrap replicates. The SnRK2 gene IDs of Arabidopsis, rice, maize, and soybean are listed in Table S1.

4.3. Gene Structure and Analysis of cis-Acting Elements

The encoding and genomic sequences of AnSnRK2s were used to analyze exon–intron organizations and intron type by using Gene Structure Display Server (GSDS) (http://gsds.cbi.pku.edu.cn/index.php, accessed on 20 October 2020).

In order to analyze the cis-acting elements of AnSnRK2 family, the 2000 bp region upstream of the start codon was obtained and used to analyze the cis-acting elements using PlantCARE online software (available online: http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 25 October 2020).

4.4. Plant Materials and Treatments

As described by Ding [54] with minor modification, the seeds of A. nanus collected from Tarim Basin in China were surface-sterilized with 75% (v/v) ethanol, immersed in sterilized water for swelling 24 h at 25 °C, sown in pots filled with nutrient soil (nutrient soil/vermiculite = 3:1), and cultured in a growth chamber under a photoperiod of 14 h light at 30 °C/10 h dark at 22 °C with 60–70% relative humidity. The five-leaf-old seedlings with the same size were treated with 20% PEG-6000 or 250 mM NaCl, with three replicates, as described by Yu et al. [55,56]. At 0 (control), 3, 6, 12 and 24 h of the treatments, the shoots from six seedlings were collected, ground in liquid nitrogen, and used for RNA extraction. Total RNA was extracted using RNAiso Plus kit and reversely transcribed into cDNA by using PrimeScript RT reagent Kit with gDNA Eraser (TAKARA, Dalian, China). The cDNA samples were stored at −20 °C.

4.5. qRT-PCR Analysis

A set of specific primers of AnSnRK2s and a pair of specific primers of AnActin (GenBank accession number: KJ873129) for the internal control were designed by Primer5.0 and synthesized at Sangon (China) (Table S2). The qRT-PCR was performed using SYBR Green I kit (TAKARA, Dalian) in CFX-96 system (Bio-Rad, Hercules, CA, USA) as described by Yu et al. [57]. The 2−ΔΔCT method of the CFX Manager software version 2.0 (Bio-Rad, USA) was used to normalize the expression differentiation between the internal control and the AnSnRK2s [58]. The data are presented as the mean values ± standard deviation (SD). The statistical significance among three biological replicates was tested by Microsoft Excel 2017 and SPSSS 17.0 software based on Student’s t-tests.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants10050882/s1, Figure S1: The phylogenetic tree constructed using 215 candidate sequences from A. nanus and SnRK2s of Arabidopsis and rice to identify AnSnRK2s. Figure S2: CDD results of 11
candidate members and EVM0005794 and EVM0001332. Figure S3: The trans-membrane structure prediction of AnSnRK2.1. Table S1: SnRK2 genes in Arabidopsis, rice, maize, and soybean. Table S2: The primers for qRT-PCR.

Author Contributions: Conceptualization, H.Y. and F.E.; software, Y.T. and F.L.; formal analysis, W.F.; investigation, Y.T., F.L., Y.L., and Y.C.; data curation, Y.T.; writing—original draft preparation, Y.T.; writing—review and editing, W.L. and H.Y.; funding acquisition, W.L. and H.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Sichuan Science and Technology Program (2018Y0470, 2020YJ0353) and the National Natural Science Foundation of China (32001552).

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Liu, L.; Hu, X.; Song, J.; Zong, X.; Li, D.; Li, D. Over-expression of a Zea mays L. protein phosphatase 2c gene (ZmPP2C2) in arabidopsis thaliana decreases tolerance to salt and drought. J. Plant Physiol. 2009, 166, 531–542. [CrossRef] [PubMed]
2. Tougane, K.; Komatsu, K.; Bhyan, S.B.; Sakata, Y.; Ishizaki, K.; Yamato, K.T.; Kohchi, T.; Takezawa, D. Evolutionarily conserved regulatory mechanisms of abscisic acid signaling in land plants: Characterization of abscisic acid insensitive1-like type 2C protein phosphatase in the liverwort marchantia polymorpha. Plant Physiol. 2010, 152, 1529–1543. [CrossRef]
3. Nie, L.L.; Peng, J.J.; Fan, P.X.; Chen, X.Y.; Guo, J.; Lv, S.L.; Bao, H.; Jia, W.T.; Tai, F.; Jiang, P.; et al. Comparative proteomics of root plasma membrane proteins reveals the involvement of calcium signalling in nacl-facilitated nitrate uptake in salicornia europaea. J. Exp. Bot. 2015, 66, 4497–4510. [CrossRef]
4. Umezawa, T.; Sugiyama, N.; Takahashi, F.; Anderson, J.C.; Ishihama, Y.; Peck, S.C.; Shinozaki, K. Genetics and phosphoproteomics reveal a protein phosphorylation network in the abscisic acid signaling pathway in arabidopsis thaliana. Sci. Signal. 2013, 6, rs8. [CrossRef]
5. Teige, M.; Scheikl, E.; Eulgem, T.; Döczi, R.; Ichimura, K.; Shinozaki, K.; Dangel, J.L.; Hirt, H. The MKK2 pathway mediates cold and salt stress signaling in Arabidopsis. Mol. Cell 2004, 15, 141–152. [CrossRef]
6. Kong, X.; Pan, J.; Zhang, D.; Jiang, S.; Cai, G.; Wang, L.; Li, D. Identification of mitogen-activated protein kinase gene
7. Hadiarto, T.; Nanmori, T.; Matsuoka, D.; Iwasaki, T.; Sato, K.; Fukami, Y.; Azuma, T.; Yasuda, T. Activation of Arabidopsis MAPK plasma membrane proteins reveals the involvement of calcium signalling in nacl-facilitated nitrate uptake in salicornia europaea. J. Exp. Bot. 2015, 66, 4497–4510. [CrossRef] [PubMed]
8. Boudsocq, M.; Sheen, J. CDPKs in immune and stress signaling. Trends Plant Sci. 2013, 18, 30–40. [CrossRef] [PubMed]
9. Zhao, R.; Li, Y.; Fan, R.C.; Shang, Y.; Du, S.Y.; Wang, X.F.; Wu, F.Q.; Xu, Y.H.; Zhang, X.Y.; Zhang, D.P. The Arabidopsis Ca2+-dependent protein kinase CPK12 negatively regulates abscisic acid signaling in seed germination and post-germination growth. New Phytol. 2011, 192, 61–73. [CrossRef]
10. Zhu, S.Y.; Yu, X.C.; Wang, X.J.; Rui, Z.; Zhang, D.P. Two calcium-dependent protein kinases, CPK4 and CPK11, regulate abscisic acid signal transduction in Arabidopsis. Plant Cell 2007, 19, 3019–3036. [CrossRef]
11. Boudsocq, M.; Laurière, C. Osmotic signaling in plants: Multiple pathways mediated by emerging kinase families. Plant Physiol. 2005, 138, 1185–1194. [CrossRef]
12. Mao, X.; Zhang, H.; Tian, S.; Chang, X.; Jing, R. TaSnRK2.4, an SNF1-type serine/threonine protein kinase of wheat (Triticum aestivum L.), confers enhanced multistress tolerance in Arabidopsis. J. Exp. Bot. 2010, 61, 683–696. [CrossRef]
13. Nakashima, K.; Fujita, Y.; Kanamori, N.; Katagiri, T.; Umezawa, T.; Kidokoro, S.; Maruyama, K.; Yoshida, T.; Ishiyama, K.; Kobayashi, M.; et al. Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, are involved in ABI3 and ABI5 regulation and contribute to multiple stress responses. J. Exp. Bot. 2009, 60, 316–328. [CrossRef]
14. Shao, Y.; Zhang, X.; Nocker, S.V.; Gong, X.Q.; Ma, F.W. Overexpression of a protein kinase gene MpSnRK2.10 from Malus pumila confers tolerance to drought stress in transgenic Arabidopsis thaliana and tomato. Planta 2009, 230, 882–892. [CrossRef]
15. Wang, P.; Xue, L.; Batelli, G.; Lee, S.; Hou, Y.J.; Van Oosten, M.J.; Zhang, H.; Tao, W.A.; Zhu, J.K. Quantitative phosphoproteomics reveals SnRK-mediated regulation of SnRK2 protein kinases. Proc. Natl. Acad. Sci. USA 2013, 110, 11205–11210. [CrossRef] [PubMed]
16. Hrabak, E.M.; Chan, C.W.M.; Gribskov, M.; Harper, J.F.; Choi, J.H.; Halford, N.; Kudla, J.; Luan, S.; Nimmo, H.G.; Sussman, M.R.; et al. The Arabidopsis CDPK-SnRK superfamily of protein kinases. Plant Physiol. 2003, 132, 666–680. [CrossRef] [PubMed]
17. Shao, Y.; Zhang, X.; Nocker, S.V.; Gong, X.Q.; Ma, F.W. Overexpression of a Protein Kinase gene MpSnRK2.10 from Malus prunifolia confers tolerance to drought stress in transgenic Arabidopsis thaliana and tomato. Genes 2019, 692, 26–34. [CrossRef] [PubMed]
18. Wang, P.; Xue, L.; Lee, S.; Hou, Y.J.; Van Oosten, M.J.; Zhang, H.; Tao, W.A.; Zhu, J.K. Quantitative phosphoproteomics identifies SnRK2 protein kinase substrates and reveals the effectors of abscisic acid action. Proc. Natl. Acad. Sci. USA 2013, 110, 11205–11210. [CrossRef] [PubMed]
19. Kobayashi, Y.; Yamamoto, M.; Minami, H.Y.; Hattori, T. Differential activation of the rice sucrose nonfermenting1–related protein kinase2 family by hyperosmotic stress and abscisic acid. Plant Cell 2004, 16, 1163–1177. [CrossRef] [PubMed]
20. Jossier, M.; Bouly, J.P.; Meimoun, P.; Arjmand, A.; Lessard, P.; Hawley, S.; Hardie, D.G.; Thomas, M. SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in Arabidopsis thaliana. Plant J. 2009, 59, 316–328. [CrossRef] [PubMed] [PubMed] [PubMed]
21. Halford, N.G.; Hey, S.; Jhurreea, D.; Laurie, S.; McKibbin, R.S.; Paul, M.; Zhang, Y.H. Metabolic signalling and carbon partitioning: Role of Snf1-related (SnRK1) protein kinase. J. Exp. Bot. 2003, 58, 467–475. [CrossRef] [PubMed]
Plants 2021, 10, 882

20. Haldorf, N.G.; Hey, S. Snf1-related protein kinases (SnRKs) act within an intricate network that links metabolic and stress signaling in plants. *Biochem. J.* 2009, 419, 247–259. [CrossRef] [PubMed]

21. Coello, P.; Hey, S.J.; Haldorf, N.G. The sucrose non-fermenting-1-related (SnRK) family of protein kinases: Potential for manipulation to improve stress tolerance and increase yield. *J. Exp. Bot.* 2011, 62, 883–893. [CrossRef]

22. 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. [CrossRef]

23. Fujita, Y.; Yoshida, T.; Yamaguchi-Shinozaki, K. Pivotal role of the AREB/ABF-SnRK2 pathway in ABRE-mediated transcription in response to osmotic stress in plants. *Physiol. Plant.* 2013, 147, 15–27. [CrossRef]

24. Anderberg, R.J.; Walker-Simmons, M.K. Identification of a wheat cDNA clone for an abscisic acid-inducible transcript with homology to protein kinases. *Proc. Natl. Acad. Sci. USA* 1992, 89, 1767–1772. [CrossRef] [PubMed]

25. Johnson, R.R. The Abscisic Acid-Responsive Kinase PKABA1 Interacts with a Seed-Specific Abscisic Acid Response Element-Binding Factor, TaAEB, and Phosphorylates TaABF Peptide Sequences. *Plant Physiol.* 2002, 130, 837–846. [CrossRef] [PubMed]

26. Boudsocq, M.; Barbier-Brygoo, H.; Lauriere, C. Identification of nine sucrose nonfermenting 1-related protein kinases 2 activated by hyperosmotic and saline stresses in *Arabidopsis thaliana*. *J. Biol. Chem.* 2004, 279, 41758–41766. [CrossRef] [PubMed]

27. Fujita, Y.; Nakashima, K.; Katagiri, T.; Kidokoro, S.; Kanamori, N.; Umezawa, T.; Fujita, M.; Maruyama, K.; Ishiyama, K.; et al. Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in Arabidopsis. *Plant Cell Physiol.* 2009, 50, 2123–2132. [CrossRef]

28. Julkowska, M.M.; McLoughlin, F.; Galvan-Ampudia, C.S.; Rankenberg, J.M.; Kawa, D.; Klimiecka, M.; Haring, M.A.; Munnik, T.; Kooijman, E.E.; Testerink, C. Identification and functional characterization of the Arabidopsis Snf1-related protein kinase SnRK2.4 phosphatidic acid-binding domain. *Plant Cell Environ.* 2015, 38, 614–624. [CrossRef] [PubMed]

29. Shen, X.J.; Guo, X.; Zhao, D.; Zhang, Q.; Jiang, Y.Z.; Wang, Y.T.; Peng, X.; Wei, Y.; Zhai, Z.F.; Zhao, W.; et al. Cloning and expression profiling of the PacSnRK2 and PacPP2C gene families during fruit development, ABA treatment, and dehydration stress in sweet cherry. *Plant Physiol. Bioch.* 2017, 119, 275–285. [CrossRef]

30. Rehman, S.U.; Wang, J.Y.; Chang, X.P.; Zhang, X.Y.; Mao, X.G.; Jin, R.L. A wheat protein kinase gene TaSnRK2.9-5A associated with yield contributing traits. *Theor. Appl. Genet.* 2019, 132, 907–919. [CrossRef]

31. Tian, S.J.; Mao, X.G.; Zhang, H.Y.; Chen, S.S.; Zhai, C.C.; Yang, S.M.; Jin, R.L. Cloning and characterization of TaSnRK2.3, a novel SnRK2 gene in common wheat. *J. Exp. Bot.* 2013, 64, 2063–2080. [CrossRef]

32. McLoughlin, F.; Galvan-Ampudia, C.S.; Julkowska, M.M.; Caarls, L.; Does, D.V.D.; Lauriere, C.; Munnik, T.; Haring, M.A.; Testerink, C. The Snf1-related protein kinases SnRK2. 4 and SnRK2. 10 are involved in maintenance of root system architecture during salt stress. *Plant J.* 2012, 72, 436–449. [CrossRef] [PubMed]

33. Cheng, S.H. Ammopiptanthus Cheng, F. a new genus of leguminosae from central asia. *J. Bot. USSR* 1959, 44, 1381–1386.

34. Jia, X.H.; Li, X.R.; Zhang, J.G.; Zhang, Z.S. Analysis of spatial variability of the fractal dimension of soil particle size in Ammopiptanthus mongolicus’ desert habitat. *Environ. Geol.* 2009, 58, 953–962. [CrossRef]

35. Fei, Y.B.; Cao, P.; Gao, S.Q.; Wang, B.; Wei, L.B.; Zhao, J.; Chen, G.; Wang, B.H. Purification and structure analysis of antifreeze proteins from Ammopiptanthus mongolicus. *Prep. Biochem. Biotech.* 2013, 43, 614–624. [CrossRef] [PubMed]

36. Shao, Y.; Qin, Y.; Zou, Y.J.; Ma, F.W. Genome-wide identification and expression profiling of the SnRK2 gene family in Malus prunifolia. *Gene* 2014, 552, 87–97. [CrossRef]

37. Umezawa, T.; Nakashima, K.; Miyakawa, T.; Kurokami, T.; Tanokura, M.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Molecular basis of the core regulatory network in ABA responses: Sensing, signaling and transport. *Plant Cell Physiol.* 2010, 51, 1821–1839. [CrossRef] [PubMed]

38. Ruvinsky, A.; Eskesen, S.T.; Eskesen, F.N.; Hurst, L.D. Can codon usage bias explain intron phase distributions and exon symmetry? *J. Mol. Evol.* 2005, 60, 99–104. [CrossRef] [PubMed]

39. Boudsocq, M.; Driollard, M.; Barbier-Brygoo, H.; Laurière, C. Different phosphorylation mechanisms are involved in the activation of sucrose non-fermenting 1-related protein kinases 2 by osmotic stresses and abscisic acid. *Plant Mol. Biol.* 2007, 63, 491–503. [CrossRef]

40. Huai, J.L.; Wang, M.; He, J.G.; Zheng, J.; Dong, Z.G.; Lv, H.K.; Zhao, J.F.; Wang, G.Y. Cloning and characterization of the SnRK2 gene family from *Zea mays*. *Plant Cell Rep.* 2008, 27, 1861–1868. [CrossRef]

41. Li, L.B.; Zhang, Y.R.; Liu, K.C.; Ni, Z.F.; FANG, Z.J.; Sun, Q.X.; GAO, J.W. Identification and Bioinformatics Analysis of SnRK2 and CIPK Family Genes in Sorghum. *Agric. Sci. China* 2010, 9, 19–30. [CrossRef]

42. Huang, Z.; Tang, J.; Duan, W.; Wang, Z.; Song, Z.; Hou, X. Molecular evolution, characterization, and expression analysis of SnRK2 gene family in Pak-choi (*Brassica rapa* ssp. *chinensis*). *Front. Plant Sci.* 2015, 6, 879–892. [CrossRef]

43. Chen, G.; Wang, J.; Qiao, X.; Jin, C.; Wu, J. Genome-wide survey of sucrose non-fermenting 1-related protein kinase 2 in *Rosaceae* and expression analysis of PbrSnRK2 in response to ABA stress. *BMC Genom.* 2020, 21, 781. [CrossRef] [PubMed]

44. Wu, Z.M.; Cheng, J.W.; Hu, F.; Qin, C.; Xu, X.W.; Hu, K.L. The SnRK2 family in pepper (*Capsicum annuum* L.): Genome-wide identification and expression analyses during fruit development and under abiotic stress. *Genes Genom.* 2020, 42, 1117–1130. [CrossRef]
46. Bai, J.P.; Mao, J.; Yang, H.Y.; Khan, A.; Fan, A.; Liu, S.; Zhang, J.L.; Wang, D.; Gao, H.J.; Zhang, J.L. Sucrose non-ferment 1 related protein kinase 2 (SnRK2) genes could mediate the stress responses in potato (*Solanum tuberosum* L.). *BMC Genet.* 2017, 18, 41. [CrossRef]

47. Zhao, W.; Cheng, Y.H.; Zhang, C.; Shen, X.J.; You, Q.B.; Guo, W.; Li, X.; Song, X.J.; Zhou, X.A.; Jiao, Y.Q. Genome-wide identification and characterization of the GmSnRK2 family in soybean. *Int. J. Mol. Sci.* 2017, 18, 1834–1855. [CrossRef]

48. Schmutz, J.; Cannon, S.B.; Schlueter, J.; Ma, J.X.; Mitros, T.; Nelson, W.; Hyten, D.L.; Song, Q.J.; Thelen, J.J.; Cheng, J.L.; et al. Genome sequence of the palaeopolyploid soybean. *Nature* 2010, 463, 178–183. [CrossRef]

49. Ali, G.M.; Komastu, S. Proteomic analysis of rice leaf sheath during drought stress. *J. Proteome Res.* 2006, 5, 396–403. [CrossRef] [PubMed]

50. Liu, Z.; Ge, X.; Yang, Z.; Liu, Z.; Ge, X.; Yang, Z.; Zhang, C.; Zhao, G.; Chen, E.; Liu, J.; et al. Genome-wide identification and characterization of SnRK2 gene family in cotton (*Gossypium hirsutum* L.). *BMC Genet.* 2017, 18, 54. [CrossRef] [PubMed]

51. Diédhiou, C.J.; Popova, O.V.; Dietz, K.J.; Golldack, D. The SNF1-type serine-threonine protein kinase *SAPK4* regulates stress-responsive gene expression in rice. *BMC Plant Biol.* 2008, 8, 49. [CrossRef]

52. Liu, M.; Wang, J.; Gou, J.; Wang, X.; Sun, S. Overexpression of NtSnRK2.2 enhances salt tolerance in Nicotiana tabacum by regulating carbohydrate metabolism and lateral root development. *Funct. Plant Biol.* 2020, 47, 537–543. [CrossRef]

53. Gao, F.; Wang, X.; Li, X.; Xu, M.; Li, H.; Abla, M.; Sun, H.; Wei, S.; Feng, J.; Zhou, Y. Long-read sequencing and de novo genome assembly of *Ammopiptanthus nanus*, a desert shrub. *GigaScience* 2018, 7, giy074. [CrossRef]

54. Ding, L.; Guo, X.; Wang, K.X.; Pang, H.W.; Liu, Y.; Yang, Q.Q.; Fu, F.L.; Li, W.C.; Yu, H.Q. Genome-wide analysis of *BES1/BZR1* transcription factors and their responses to osmotic stress in *Ammopiptanthus nanus*. *J. Forest Res-JPN.* 2020, 1, 1–9. [CrossRef]

55. Yu, H.Q.; Yong, T.M.; Li, H.J.; Liu, Y.P.; Zhou, S.F.; Fu, F.L.; Li, W.C. Overexpression of a phospholipase Dα gene from *Ammopiptanthus nanus* enhances salt tolerance of phospholipase Dα1-deficient Arabidopsis mutant. *Planta* 2015, 242, 1495–1509. [CrossRef] [PubMed]

56. Yu, H.Q.; Zhang, Y.Y.; Yong, T.M.; Liu, Y.P.; Zhou, S.F.; Fu, F.L.; Li, W.C. Cloning and functional validation of molybdenum cofactor sulfurase gene from *Ammopiptanthus nanus*. *Plant Cell Rep.* 2015, 34, 1165–1176. [CrossRef]

57. Yu, H.Q.; Feng, W.Q.; Sun, F.A.; Zhang, Y.Y.; Qu, J.T.; Liu, B.L.; Lu, F.Z.; Yang, L.; Fu, F.L.; Li, W.C. Cloning and characterization of *BES1/BZR1* transcription factor genes in maize. *Plant Growth Regul.* 2018, 86, 235–249. [CrossRef]

58. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2−∆∆CT method. *Methods* 2001, 25, 402–408. [CrossRef] [PubMed]