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Genome-Wide Identification and Expression Analysis of the *BvSnRK2* Genes Family in Sugar Beet (*Beta vulgaris* L.) Under Salt Conditions

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Abstract

The sucrose non-fermenting-1-related protein kinase 2s (SnRK2s) have been shown to play critical roles in the response to environmental stresses in higher plants. Although the *SnRK2* genes family has been identified in various plants, little is reported regarding *SnRK2s* in sugar beet (*Beta vulgaris* L.), which is one of the most important crops for both food and sugar production. In the current study, the *SnRK2s* genes are identified in the sugar beet genome by bioinformatics, and their expression patterns under salinity conditions are tested by the qRT-PCR method. Results showed that a total of six *BvSnRK2* genes are identified and characterized from the genome of sugar beet and are further classified into three distinct groups (Group 1, 2, and 3). All BvSnRK2s contained a highly conserved N-terminal kinase region and a greatly divergent C-terminal region. Except for *BvSnRK2.4*, most of the *BvSnRK2* genes were disrupted by eight introns with size ranging from 82 to 2164 bp. Moreover, the expression levels of the *BvSnRK2s* genes were strongly enhanced by salt treatments, which may be an indicator of potential roles in the response to salinity. The present work is the first systematic analysis of the *SnRK2* family genes in sugar beet. The results from this study provide a novel insight for the functional exploration and application of the *SnRK2s* genes for crop improvement, especially in sugar crops.

Keywords Sugar beet · *SnRK2s* · Salt tolerance · Protein kinase domain · Expression pattern

Introduction

Plants are often subjected to various abiotic stresses, including high salinity, drought, and extreme temperatures, which severely limit the growth and development of plants and the production of crops (Hussain et al. 2016). To adapt with these environmental stresses, plants have developed a variety of sophisticated protective strategies at morphological, physiological, and molecular levels during the long-term evolution process (Choudhary et al. 2018). Protein kinases and phosphatases, the major components of the intracellular signal transduction, play prominent roles in growth, development, and stress response of plants (Hunter 1995). Particularly, the sucrose non-fermenting-1-related protein kinases (*SnRKs*) family has been found widely in plant species and is involved in the abiotic stress response of plants (Fujii et al. 2011; Kulik et al. 2011; Mao et al. 2020).

Plant *SnRKs* family can be split into three major subgroups, *SnRK1*, *SnRK2* and *SnRK3*, according to sequences similarities, domain structures, and cellular functions (Hrabak et al. 2003). Among these subgroups, *SnRK2s* have been studied well in various plant species due to their crucial functions in ABA signal transduction pathways and abiotic stress responses of plants (Yoshida et al. 2014; Zhu 2016; Mao et al. 2020). To date, the *SnRK2s* family genes have been identified and characterized in different plants, including 6 in sweet cherry (*Prunus avium*) (Shen et al. 2017), 8 in grapevine (*Vitis vinifera*) (Liu et al. 2016), 8 in tea plant (*Camellia sinensis*) (Zhang et al. 2018), 10 in *Brachypodium distachyon* (Wang et al. 2015), 10 in wheat (*Triticum aestivum*) (Zhang et al. 2016), 11 in maize (*Zea mays*) (Huai et al. 2008), 12 in apple (*Malus prunifolia*) (Shao et al. 2014), 12 in poplar (*Populus trichocarpa*) (Song et al. 2015), 14 in *Brassica napus* (Yoo et al. 2016), 20 in cotton (*Gossypium hirsutum*) (Liu et al. 2017), and 22 in soybean (*Glycine max*)

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(Zhao et al. 2017). SnRK2s contain the highly conserved kinase region at the N-terminus and the divergent regulatory region at the C-terminus (Mao et al. 2020). The C-terminal region is classified functionally into two parts: Domain I and Domain II (Yoshida et al. 2006; Mao et al. 2020). Domain I was required for the ABA-independent activation, while Domain II was responsible only for the ABA-dependent activation (Yoshida et al. 2006; Mao et al. 2020). In general, the members of *SnRK2s* family can be classed into three major groups as Groups 1, 2, and 3 (Kulik et al. 2011). The *SnRK2s* of Group 1 were ABA-independent kinases, the kinases of Group 2 were not or very weakly dependent ABA, and the *SnRK2s* of Group 3 were strongly dependent on ABA (Kobayashi et al. 2004; Kulik et al. 2011).

There are evidences that the *SnRK2s* genes play crucial roles in the plant growth, development, and the response to abiotic stresses (Song et al. 2016; Feng et al. 2019; Kawa et al. 2020; Zhonget al. 2020). In *Arabidopsis thaliana*, over expression of *PtSnRK2.5* and *PtSnRK2.7* conferred salt tolerance in transgenic plants through maintaining chlorophyll content and root elongation (Song et al. 2016). Furthermore, in apple, transcript levels of *MpSnRK2.10* were up-regulated by ABA, drought, and salt stresses (Shao et al. 2014), and the conferred drought tolerance was found in both transgenic apple and *Arabidopsis* (Shao et al. 2019). Moreover, in wheat, the expression of *TaSnRK2s* was induced by salt, water deficit, and low temperature (Zhang et al. 2016). Additionally, the overexpression of *TaSnRK2.9* in tobacco significantly enhanced plant tolerance to both salt and drought stresses through improving ROS scavenging ability, specific SnRK–ABF(ABRE-binding factor) interaction and ABA-dependent signal transduction (Feng et al. 2019). Similarly, in tea plant, *CsSnRK2.5* was induced by PEG 6000 and ABA treatments (Zhang et al. 2018), and the improved drought tolerance was found in transgenic *Arabidopsis* plants (Zhang et al.2020). In rice, the expression of *OsSAPK8*, one gene of *SnRK2s* family, was promoted under stresses of cold, salt, and drought (Zhonget al. 2020). Further studies showed that the *OsSAPK8*-defective lines displayed lower tolerance to drought, high salinity and low temperature, verifying that *OsSAPK8* played an important role in response to abiotic stresses (Zhong et al. 2020). Recently, in *Arabidopsis*,*AtSnRK2s* were found to control root development and growth under salinity stress by changing the expression levels of two aquaporin genes *AtPIP2, 3* and *AtPIP2, 5*, as well as *AtCYP79B2*, an enzyme involved in the biosynthesis of auxin (Kawa et al. 2020). All of these results implied that SnRK2s act as the omnipotent players in the environmental stimuli response as well as in the growth and development of plants.

Sugar beet (*Beta vulgaris* L. ssp. *vulgaris*) is one of greatly important crops for both food and sugar industry, accounting for approximately 20% of the world's annual sugar production (Ribeiro et al. 2016; Monteiro et al. 2018). Sugar beet is able to survive well to high salt concentrations (Wakeel et al. 2011) and is planted widely in the arid and semi-arid areas of northern China, including Gansu province (Wu et al. 2013). Although the *SnRK2s* genes have been reported widely in many plants, the comprehensive analysis of the *SnRK2s* genes family has not still been conducted in sugar beet. Recently, the genome sequences of sugar beet have been completed (Dohm et al. 2014) and provide a good opportunity to conduct a genome-wide identification of the *SnRK2s* genes family.

In the current work, a genome-wide analysis of the *SnRK2s* genes family in sugar beet was performed, and their phylogeny, chromosome distribution, gene structures, conserved motifs, *cis*-acting regulatory elements, and expression patterns in response to salt were further investigated. The systematic study would provide a novel insight for the further functional exploration and application of the *SnRK2* genes for crop improvement, especially in sugar crops.

Materials and Methods

Identification and Characterization of SnRK2s in the Sugar Beet Genome

To identify all members of *SnRK2s* in the genome of sugar beet, the protein sequences of 10 *AtSnRK2s* are downloaded from The *Arabidopsis* Information Resource (TAIR, https://www.arabidopsis.org/) (Supplementary Table S1). The sequences of *AtSnRK2s* were used as queries to search sugar beet *SnRK2s* family genes from The *Beta vulgaris* Resource (TBR, https://bvseq.boku.ac.at/) (Rodríguez Del Río et al. 2019), by BLAST with the parameters of expected values $\leq 1e^{-10}$. All candidate sequences were analyzed using the online tools CDD v3.17 (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi/) and Pfamv32.0 (https://pfam.xfam.org/) to confirm the presence and competence of protein kinase-acting domain (Shao et al. 2014). The isoelectric point (*p*I), molecular weight (MW), and the grand average of hydropathicity (GRAVY) of BvSnRK2s proteins was computed using the online tool ProtParam (https://web.expasy.org/protparam/) (Gasteiger et al. 2005).

Chromosomal Location of SnRK2s in Sugar Beet

The chromosomal locations were retrieved from TBR, and the distribution graph of the *BvSnRK2s* genes on the chromosome were drawn by the MapInspect v1.0 software.

Gene Structure and Protein Motif Analysis of SnRK2s in Sugar Beet

The intron/exon structure of *BvSnRK2s* was predicted using online tool GSDS v2.0 (https://gsds.cbi.pku.edu.cn/) (Hu et al. 2015) according to the comparison of their coding sequences (CDS) and the corresponding genomic sequences. The conserved motifs of BvSnRK2s proteins were predicted using MEME v5.1.1 (https://meme-suite.org/tools/meme/), with the motif widths ranging from 6 to 50 residues and the maximum number of motifs set at 10.

Protein Sequences Alignment and Phylogenetic Analysis of SnRK2s

DNAMAN v10.0 software was used to align the protein sequences of the *BvSnRK2s* genes. ScanProsite tool (https://prosite.expasy.org/scanprosite/) was used to detect the protein kinase conserved domains (Sigrist et al. 2013). To build a phylogenetic tree, the protein sequences of the *SnRK2s* genes for *Arabidopsis thaliana*, apple (*Malus prunifolia*), maize (*Zea mays*), wheat (*Triticum aestivum*), cotton (*Gossypium hirsutum*), sweet cherry (*Prunus avium*), tea plant (*Camellia sinensis*), rubber tree (*Hevea brasiliensis*), and potato (*Solanum tuberosum*) were downloaded from the NCBI protein database (https://www.ncbi.nlm.nih.gov/protein/). The accession number and protein sequences of *SnRK2s* from various plant species are shown in Supplementary Table S1. MEGA v10.0 software (https://www.megasoftware.net/) was used to construct an unrooted phylogenetic tree based on the protein kinase conserved domains with 1000 bootstrap replicates (Kumar et al. 2018).

Three-Dimensional Structure Prediction of SnRK2s in Sugar Beet

The online sever I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) was used to predict three-dimensional (3-D) structures of BvSnRK2s (Yang et al. 2015).

Cis-Acting Regulatory Element Prediction of Promoter Regions in BvSnRK2s

The promoter sequences were identified in 1.5 kb upstream of the translation initiation site (TIS) of *BvSnRK2s*. The *cis*-acting regulatory elements of *BvSnRK2s* were analyzed using the database PlantCARE (Lescot et al. 2002).

Protein–Protein Interaction (PPI) Prediction of BvSnRK2s

The functional interacting networks of BvSnRK2s were predicted using the online STRING v11.0 program (https://string-db.org/cgi/input.pl) with the confidence limits set at 0.400 (Franceschini et al. 2013).

Plant Materials, Growth Conditions, and Salinity Treatments

All the sugar beet (*B. vulgaris* L. cv. "Gantang No. 7") seedlings were grown in the same growth room where the temperature was set at 25 °C/20 °C (day/night), the relative humidity was regulated between 65 and 75%, and the daily photoperiod was 16 h/8 h (day/night). The seedlings were irrigated with the modified Hoagland nutrient solution containing 2.5 mM KNO_3, 1 mM $NH_4H_2PO_4$, 0.5 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 60 μM Fe-Citrate, 92 μM H_3BO_3, 0.7 μM $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 18 μM $MnCl_2{\cdot}4H_2O$, 1.6 μM $ZnSO_4{\cdot}7H_2O$, and 0.6 μM $CuSO_4{\cdot}5H_2O$, once every 3 days. After 4 weeks, they were exposed to 0 (control), 50, 100, and 150 mM NaCl solutions, respectively. Shoot and root of plants were collected separately at 48 h after salinity treatment. All the tissue samples were frozen immediately in liquid nitrogen and kept at − 80 °C refrigerator until total RNA isolation.

Expression Analysis of BvSnRK2s in Sugar Beet

To evaluate the expression levels of six *BvSnRK2s* genes in response to salinity treatments, the qRT-PCR analysis was performed. The primers of *BvSnRK2s* and *BvACTIN* for genes expression analysis were designed to avoid the conserved region and are listed in Table 1. Total RNAs were isolated from shoots and roots of seedlings under salt treatments based on the previous report (Wu et al. 2019). First strand cDNAs were synthesized from total RNAs using the PrimeScript™ RT Master reagents (Takara, Dalian, China) according to the manufacturer's instructions. The qRT-PCR analysis was performed using the RT-PCR System MA-6000 (Molarray, Suzhou, China) and the TB Green™ Master reagents (Takara, Dalian, China). The relative quantification values of *BvSnRK2s* were calculated by $2^{-\Delta\Delta Ct}$ methods (Livak and Schmittgen 2001) with *BvACTIN* as the reference gene.

Results

Identification of the SnRK2s Genes in the Genome of Sugar Beet

A total of six *SnRK2s* genes were identified from the genome of sugar beet through homologous search and domain confirmation by InterProScan, and named from *BvSnRK2.1* to *BvSnRK2.6* (Supplementary Data S1 and Table 2). The CDS lengths of *BvSnRK2s* varied from

1008 bp (*BvSnRK2.1*) to 1095 bp (*BvSnRK2.6*). The BvSnRK2s proteins lengths ranged from 335 to 364 aa, whereas their molecular weights (MWs) varied from 37.80 to 41.15 kDa. The isoelectric point (p*I*) varied from 4.85 to 5.73, which revealed that BvSnRK2s were acidic. The grand average of hydropathy (GRAVY) scores of all BvSnRK2s was negative, indicating that BvSnRK2s were hydrophilic proteins and possible located in the cytosol.

To investigate the putative genome location of each *BvSnRK2*, MapInspect v1.0 was used to map on the sugar beet chromosomes. Six *BvSnRK2* genes could be mapped onto five of total 9 sugar beet chromosomes (Fig. 1). Two genes (*BvSnRK2.4* and *-2.5*) were putatively located on

Table 1 The sequences of primers used for qRT-PCR in this study

Genes	Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)
BvSnRK2.1	ACCTCTTCAACCGCCTAATACCA	TCATACAAATTCGCCACTGCTTTCT
BvSnRK2.2	GAAAATTGTGGGCGAGGCAAGA	CTCTCCGCTTGCATGAACCTG
BvSnRK2.3	GATTGATGAGAATGTGCAGAGGGAG	CCAGCAGCATACTCCATGACAA
BvSnRK2.4	AAAAAATGGACGGCGGTGGTAG	AGCAACACCGAAGTTTCCAGA
BvSnRK2.5	AAGGAAGGTGGGCTAGAAGAGA	AATCCAAGCTGCCGCCTAAGA
BvSnRK2.6	AAAGATATGGATCGTACGGCGG	TCTCTCATCAACCTAGCAACTCCA
BvACTIN	ACTGGTATTGTGCTTGACTC	ATGAGATAATCAGTGAGATC

Table 2 Identification and characterization of *BvSnRK2s* in sugar beet

Gene name	Gene ID	Locus	CDS (bp)	Protein length (aa)	MW (kDa)	p*I*	GRAVY
BvSnRK2.1	Bv1_008630_pooe	Bvchr1.sca002:2741270..2746454 (+)	1008	335	37.8	5.55	− 0.266
BvSnRK2.2	Bv4_084120_kqpu	Bvchr4.sca009:591979..598669 (−)	1068	355	40.61	5.73	− 0.511
BvSnRK2.3	Bv5_105400_zmyx	Bvchr5.sca004:1857715..1865037 (−)	1026	341	38.48	5.45	− 0.383
BvSnRK2.4	Bv6_138530_ywks	Bvchr6.sca005:1761879..1766076 (−)	1047	348	39.38	5.39	− 0.332
BvSnRK2.5	Bv6_134600_gdoe	Bvchr6.sca003:3266521..3271221 (+)	1086	361	41.04	4.90	− 0.313
BvSnRK2.6	Bv9_218570_fxdy	Bvchr9.sca026:2267026..2271288 (−)	1095	364	41.15	4.85	− 0.338

CDS coding sequence, *MW* molecular weight, *pI* isoelectric point, *GRAVY* grand average of hydropathicity

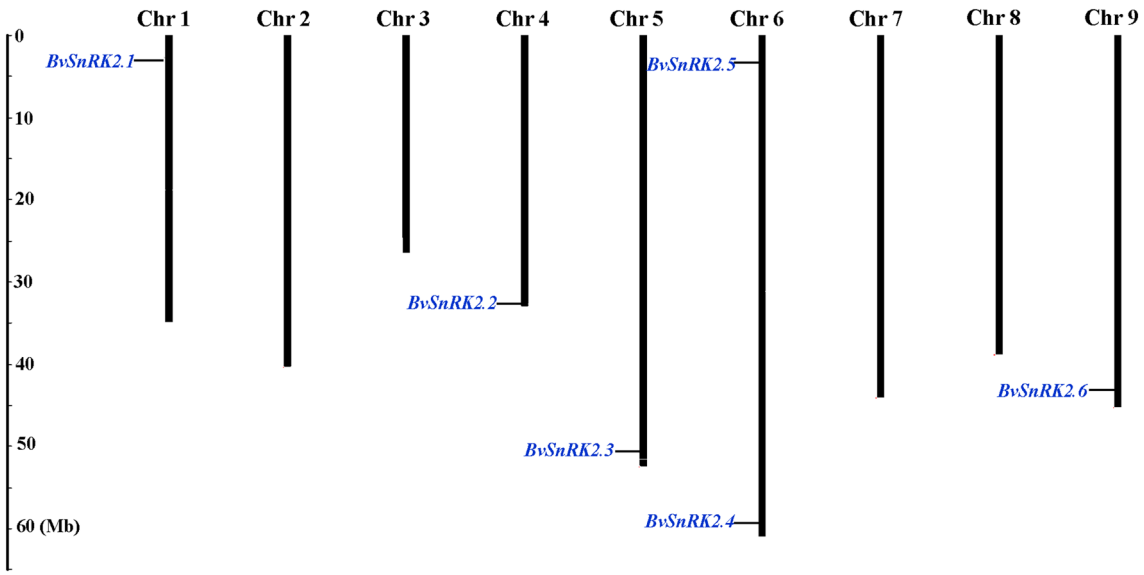

Fig. 1 The location of *BvSnRK2s* on nine chromosomes of sugar beet. The number of chromosomes is represented at the top of each chromosome (Chr). The scale of genome size is indicated on the left

Chromosomal 6, while *BvSnRK2.1*, *-2.2*, *-2.3*, and *-2.6* were distributed on Chromosomal 1, 4, 5, and 9, respectively (Fig. 1).

Multiple Sequences Alignments and Phylogenetic Analysis of BvSnRK2s

To investigate the phylogenetic relationship among *SnRK2s* family members from different plant species, an unrooted phylogenetic tree was constructed with MAGA 10.0 according to multiple alignments of the predicated protein sequences of 6 *BvSnRK2s* from sugar beet, 6 *PaSnRK2s* from sweet cheery, 8 *CsSnRK2s* from tea plant, 8 *StSnRK2s* from potato, 10 *AtSnRK2s* from *Arabidopsis*, 10 *HbSnRK2s* from rubber tree, 11 *ZmSnRK2s* from maize, 12 *MpSnRK2s* from apple, 12 *TaSnRK2s* from wheat, and 20 *GhSnRK2s* from cotton. All of these *SnRK2s* family genes can be clustered into three distinct groups (Groups 1, 2, and 3) (Fig. 2). Consistent with previous classification in *Arabidopsis*, six *BvSnRK2s* in this study were distributed within the three

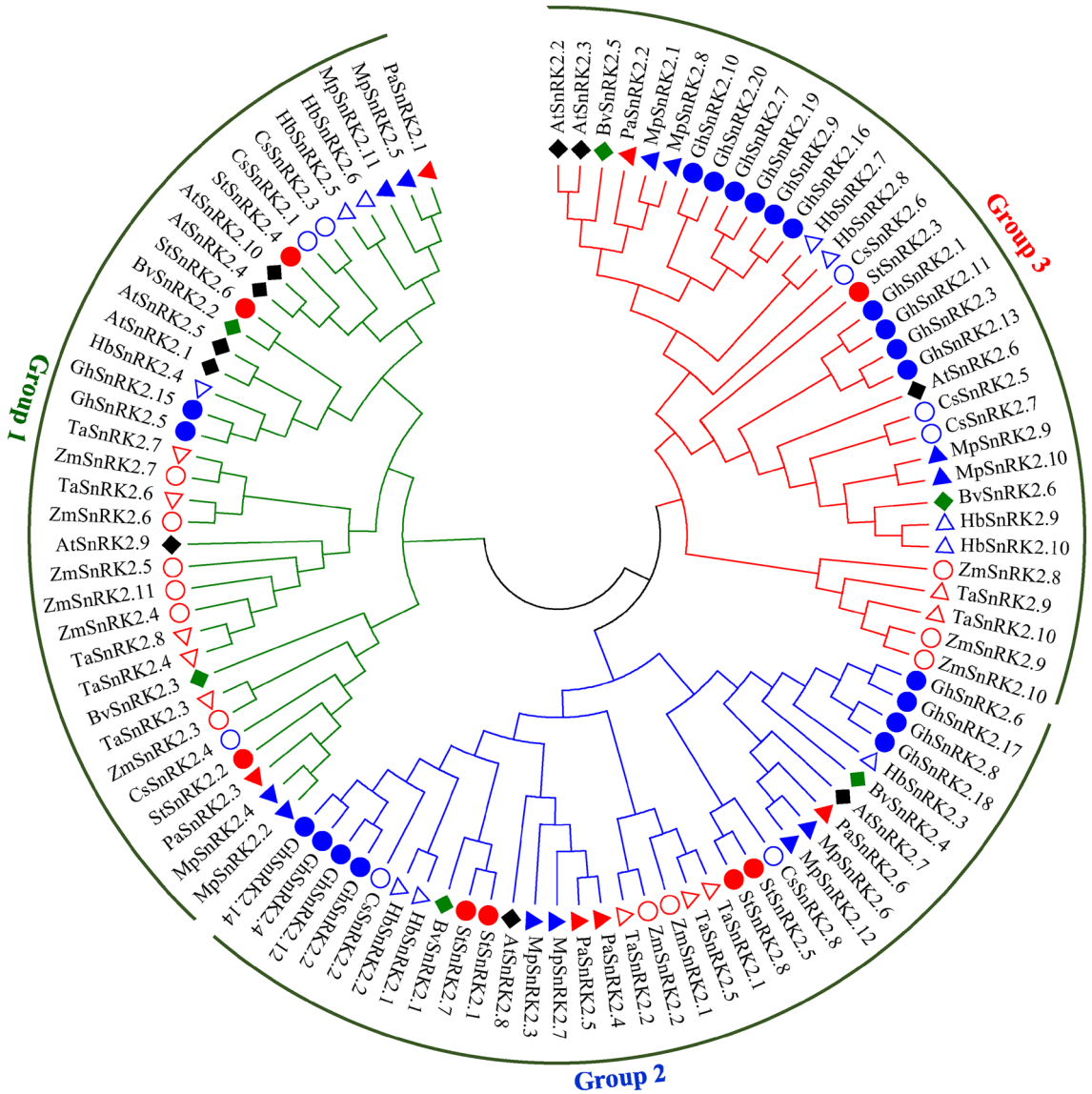

Fig. 2 Phylogenetic analysis of the*SnRK2s* family genes from *Arabidopsis thaliana* (black solid rhombus, *AtSnRK2s*), sugar beet (*Beta vulgaris*) (green solid rhombus, *BvSnRK2s*), cotton (*Gossypium hirsutum*) (blue solid round, *GhSnRK2s*), tea plant (*Camellia sinensis*) (blue hollow round, *CsSnRK2s*), apple (*Malus prunifolia*) (blue solid triangle, *MpSnRK2s*), rubber tree(*Hevea brasiliensis*) (blue hollow triangle, *HbSnRK2s*), sweet cherry (*Prunus avium*) (red solid triangle, *PaSnRK2s*), wheat (*Triticum aestivum*) (red hollow triangle, *TaSnRK2s*), potato (*Solanum tuberosum*) (red solid round, *StSnRK2s*), and maize (*Zea mays*) (red hollow round, *ZmSnRK2s*). The unrooted phylogenetic tree was built using software MEGA v10.0 by the neighbor-joining method. The accession number and protein sequences of all *SnRK2s* genes used in the present study are listed in Supplementary Table S1

groups. Group 1 comprised two members (*BvSnRK2.2* and *BvSnRK2.3*). *BvSnRK2.1* and *BvSnRK2.4* belonged to Group 2, while *BvSnRK2.5* and *BvSnRK2.6* were included in Group 3. This phylogenetic analysis showed that *BvSnRK2s* are more closely clustered with *SnRK2s* from dicots potato, *Arabidopsis*, apple, and rubber tree than those with genes from monocots maize and wheat (Fig. 2).

To explore the structural domains of *BvSnRK2s*, the sequences of 6 proteins were aligned using DNAMAN10.0. The results indicated that six *BvSnRK2s* genes showed at least 70.1% amino acid similarity (data not shown), with the very highly conserved N-terminus region and the greatly divergent C-terminus region (Fig. 3). An ATP-binding region (D/Q/NI/LGS/AGNFGVA) and a protein kinase-acting domain (CHRDLKLENTLLD) were found in the N-terminal domain of six BvSnRK2s (Fig. 3); these two parts constituted a conserved Ser/Thr protein kinases domain, which was necessary for the activity of BvSnRK2s. Two distinct domains are also observed in the C-terminus region of BvSnRK2s. Domain II may be critical to ABA response and is found only in members of Group 3, whereas Domain I is represented in all SnRK2s and was a key component needed for the activation by osmotic stresses (Fig. 3).

Gene Structures and Protein Motifs of BvSnRK2s

Most of the *SnRK2s* genes from high plants have been documented to display a highly conserved distribution

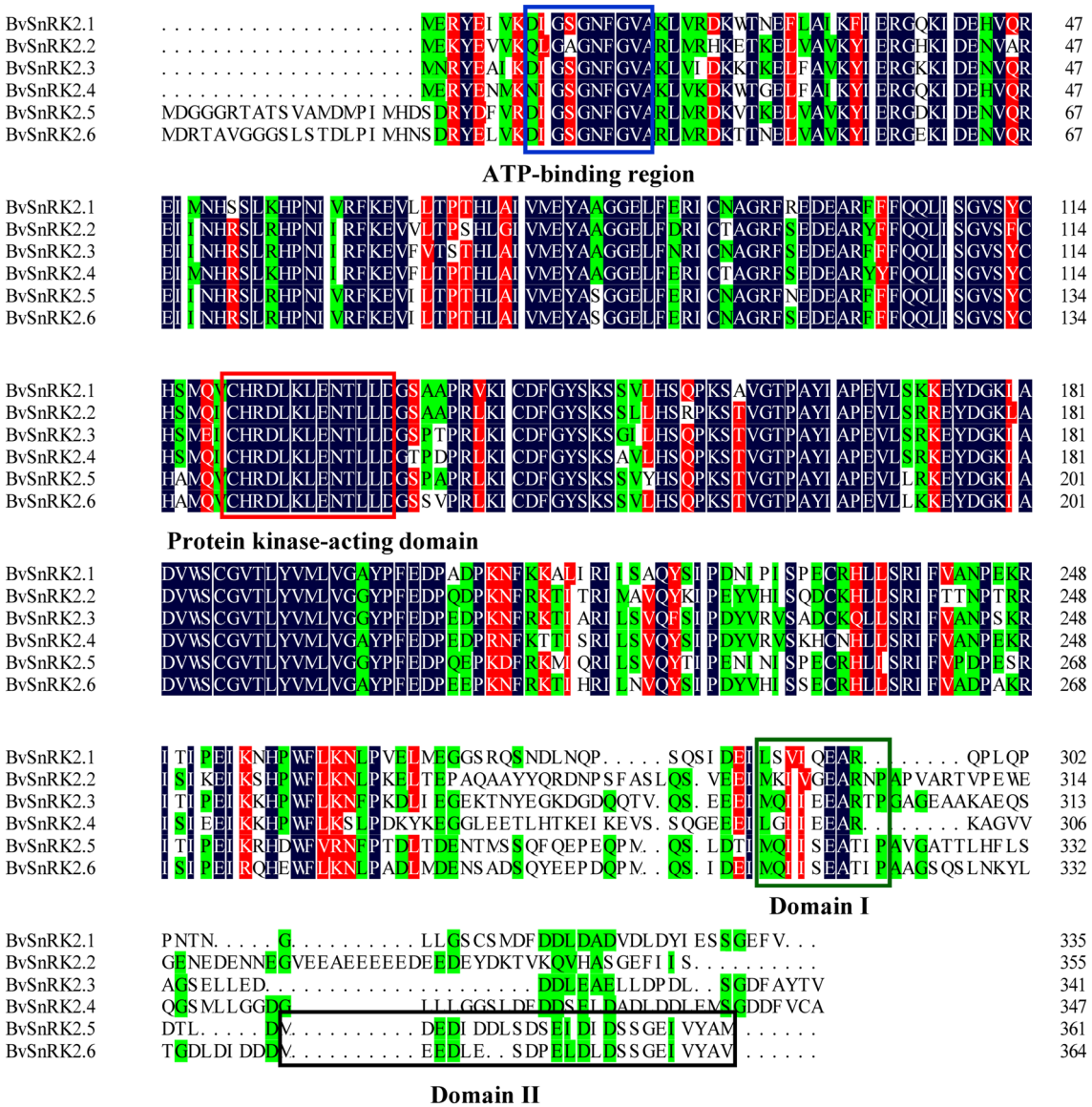

Fig. 3 Multiple sequences alignments of six BvSnRK2s. The ATP-binding region and the protein kinase-acting domain are marked by the blue and red boxes, respectively. Domain I and II in the C-terminal region are represented by the green and black boxes, respectively

of introns and exons and have eight introns (Huai et al. 2008). According to the analysis of gene structures in online tool GSDS, *BvSnRK2s* exhibited the similar intron–exon organizations (Fig. 4a). The CDS of *BvSnRK2s* were disrupted by eight introns except for *BvSnRK2.4* (seven introns), and the size of intron varied greatly, ranging from 82 to 2164 bp.

The 10 motifs are predicted with online program MEME to search all BvSnRK2s (Fig. 4b), and the multilevel consensus sequences and E-value of all motifs are shown in Table 3. It was found that nine motifs (Motif 1 to 9) constituted the highly conserved N-terminal and were shown in six BvSnRK2s. Motif 10 is located in the N-terminus extension peptide specific to members (BvSnRK2.5 and BvSnRK2.6) of Group 3.

Cis-Acting Regulatory Elements in the Promoter Region of BvSnRK2s

To explore the putative *cis*-acting regulatory element of *BvSnRK2s*, 1.5 kb upstream sequences from TIS using online tool PlantCARE was analyzed. As shown in Table 4, there are five hormone-related elements, including abscisic acid-responsive element (ABRE), salicylic acid-responsive element (TCA-element), ethylene-responsive element (ERE), auxin-responsive element (TGA-element), and MeJA-responsive element (TGACG-motif). Among these elements, ABREs were found in the promoter regions of *BvSnRK2.1*, *BvSnRK2.2*, and *BvSnRK2.6*; TCA-elements were found in both *BvSnRK2.2* and *BvSnRK2.3*; TGACG-motifs were found in both *BvSnRK2.1* and *BvSnRK2.6*, while ERE was only present in promoter region of *BvSnRK2.6*. Furthermore, six light-responsive elements, such as I-box, Sp1,

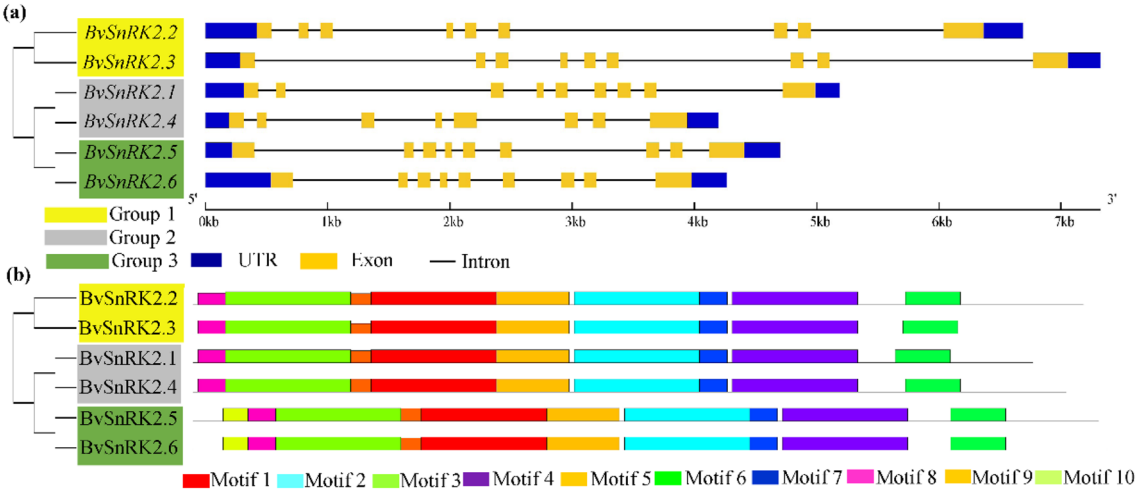

Fig. 4 Phylogenetic evolutionary relationship, intron–exon organization and conserved motifs analysis of *BvSnRK2s*. **a** Phylogenetic evolutionary relationship and gene structure of *BvSnRK2s*. Exon and intron are represented by the orange box and the single line, respectively. 5′- and 3′-untranslated regions (UTRs) are represented by blue boxes. **b** Conserved motif analysis of BvSnRK2s. The ten conserved motifs are represented by the colored boxes at the bottom. The details of multilevel consensus motifs are shown in Table 3

Table 3 Multilevel consensus sequences of motifs in BvSnRK2s

Motif	E-value	Site	Width	Multilevel consensus sequence
1	2.90E−188	6	50	HLAIVMEYAAGGELFERICNAGRFSEDEARFFFQQLISGVSYCHSMQICH
2	3.10E−188	6	50	HSQPKSTVGTPAYIAPEVLSRKEYDGKIADVWSCGVTLYVMLVGGYPFED
3	1.90E−151	6	50	NFGVAKLVRDKWTKELVAVKYIERGZKIDENVQREIINHRSLRHPNIIRF
4	4.60E−124	6	50	ILSVQYSIPDYVHISPECRHLLSRIFVANPEKRISIPEIKKHPWFLKNLP
5	1.80E−87	6	29	RDLKLENTLLDGSPAPRLKICDFGYSKSS
6	1.80E−21	6	22	QPMQSIDEIMQIIEEARIPAVG
7	1.70E−17	6	11	PZDPKNFRKTI
8	5.40E−11	6	11	RYEIVKDIGSG
9	9.00E−10	2	8	KEVILTPT
10	1.10E+01	2	10	MDLPIMHDSD

Table 4 The *cis*-acting regulatory element analysis in promoter regions of *BvSnRK2s*

Cis-acting element	Function	Sequence	*BvSnRK2s* genes					
			2.1	*2.2*	*2.3*	*2.4*	*2.5*	*2.6*
ABRE	Abscisic acid-responsive element	CACGTG	1	1	0	0	0	2
TCA-element	Salicylic acid-responsive element	CCATCTTTTT	0	1	1	0	0	0
TGA-element	Auxin-responsive element	AACGAC	1	0	0	0	0	0
ERE	Ethylene-responsive element	ATTTCATA	0	0	0	0	0	1
TGACG-motif	MeJA-responsive element	TGACG	1	0	0	0	0	1
I-box	Light-responsive element	TAGATAACC	0	1	0	0	1	0
Sp1	Light-responsive element	GGGCGG	0	1	0	0	0	0
TCCC-motif	Light-responsive element	TCTCCCT	0	1	0	0	0	0
G-box	Light responsiveness	CACGTT	1	0	0	0	0	1
Box 4	Light responsiveness	ATTAAT	0	3	0	0	1	0
MRE	Light responsiveness	AACCTAA	0	0	0	0	0	1
LTR	Low-temperature responsiveness	CCGAAA	0	0	0	0	0	1
TC-rich repeats	Defense and stress responsiveness	ATTCTCTAAC	0	0	0	0	0	1
TCT-motif	Responsive element	TCTTAC	0	0	0	0	0	1
STRE	Stress-responsive element	AGGGG	1	0	0	0	0	1
MBS	MYB binding site involved in drought	CAACCA	0	0	0	0	1	1
W-box	WRKY binding site	TTGACC	0	0	1	1	0	0

MeJA methyl jasmonic acid

TGACG-motif, Box 4, G-box, and MRE, were found in promoter regions of the *BvSnRK2s* genes. Moreover, there are also four stress-related elements, including low-temperature responsiveness (LTR), defense and stress responsiveness element (TC-rich repeats), stress responsive element (STRE), and MYB binding site involved in drought (MBS). Among these elements, MBS was found in the promoter regions of both *BvSnRK2.5* and *BvSnRK2.6*, while LTR was only found in *BvSnRK2.6*. Additionally, the promoter regions of both *BvSnRK2.3* and *BvSnRK2.4* contained a W-box element, which is a specific binding site of transcription factor WRKY (Table 4).

Three-Dimensional Structures Analysis of BvSnRK2s

To further explore the structures of BvSnRK2s proteins, the 3-D structure models were constructed using online program I-TASSER. The results showed that the number of α-helices in BvSnRK2s ranged from 13 to 16, such as 13 in BvSnRK2.1 and -2.6, 15 in BvSnRK2.2 and -2.5, and 16 in BvSnRK2.3 and -2.4. Both BvSnRK2.1 and -2.5 had 8 β-strands, while the other four proteins contained 9 β-strands (Fig. 5 and Supplementary Fig. S1). Seven β-strands were present in the N-terminal regions of all BvSnRK2s (Fig. 5), which are necessary for maintaining the stability of tertiary structures in BvSnRK2s. The ATP binding loop was found between the first β-strand and the second β-strand in the N-terminus of BvSnRK2s (Figs. 3, 5, and Supplementary Fig. S1). Similar structures were present in the VvSnRK2s proteins from grapevine as reported by Liu et al. (2016). Additionally, to quantify the accuracy of constructed model, the C-score is used to assess the predicted protein models. In general, the C-score ranges from − 5 to 2, where the higher value represents the model with higher accuracy. In the present study, all the C-scores of the predicted BvSnRK2s models ranged from − 1.89 (BvSnRK2.5) to − 0.31 (BvSnRK2.1) (Table 5), indicating the constructed protein structures were highly accurate.

Protein–Protein Interaction Prediction of BvSnRK2s

To investigate roles of BvSnRK2s during the possible interacted with other proteins, STRING database was used to construct the PPI network (Fig. 6). No interacted relationship was predicted among BvSnRK2s proteins. However, four proteins, BvSnRK2.1, -2.3, -2.4, and -2.5, shared the same interaction protein, protein phosphatase 2C50 (PP2C50, XP_010696551.1). BvSnRK2.2 was hypothesized to interact with four proteins, including AMPK1 (XP_010674700.1), ABI5-2 (XP_010691227.1), EBF1 (XP_010687976.1), and EBF2 (XP_010696236.1). BvSnRK2.6 was putatively interacted with three PP2C proteins, PP2Cc (XP_010688278.1), PP2C24 (XP_010685683.1), and PP2C37 (XP_010691227.1), and two ABI5 proteins, ABI5-1(XP_010683741.1) and ABI5-5 (XP_010685236.1) (Fig. 6).

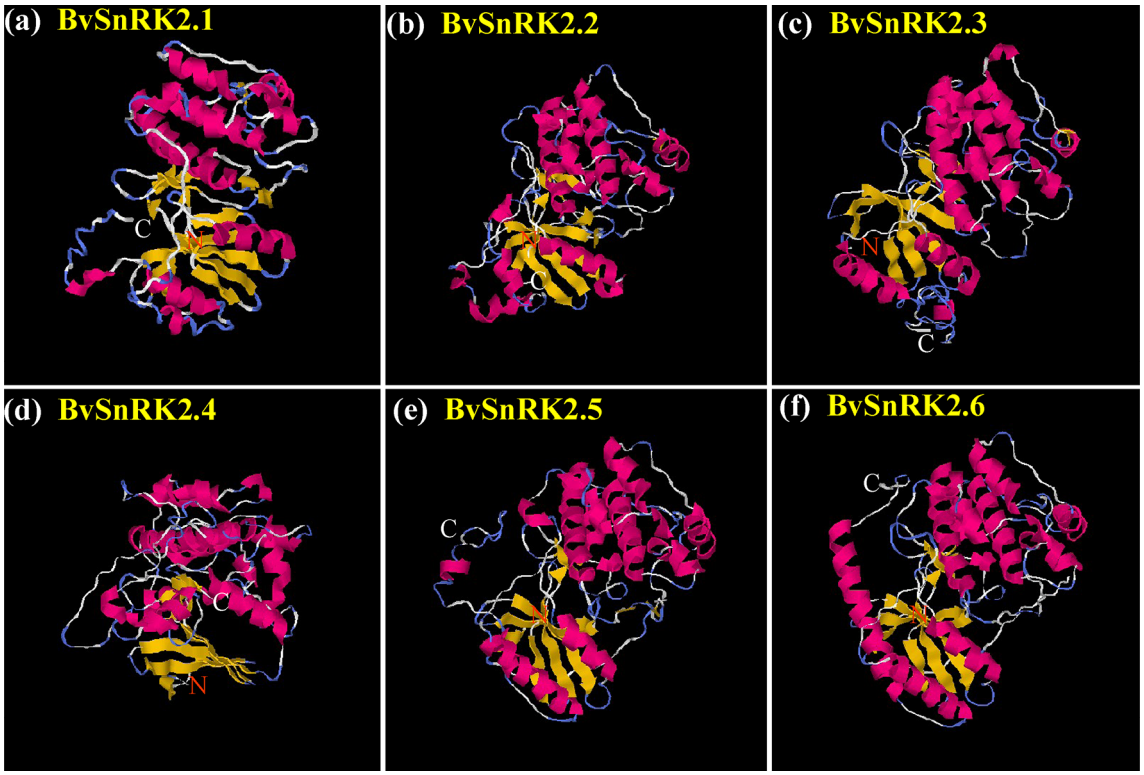

Fig. 5 Three-dimensional structures of six BvSnRK2s. **a** BvSnRK2.1; **b** BvSnRK2.2; **c** BvSnRK2.3; **d** BvSnRK2.4; **e** BvSnRK2.5; **f** BvSnRK2.6. The models of proteins were obtained by the online server I-TASSER. The α-helix, β-strand, and random coil are marked by red, orange, and blue, respectively. The parameters of the best PDB structure for BvSnRK2s are listed in Table 4. The detailed information of secondary structures of BvSnRK2s is shown in Supplementary Fig. S1

Table 5 Structural dependent modeling parameters for BvSnRK2s

Protein	C-score	TM-score	RMSD (Å)	Best identified structural analogs in PDB				
				PDB Hit	TM-score[a]	RMSD[a]	IDEN[a]	Cov
BvSnRK2.1	− 0.31	0.65 ± 0.13	7.5 ± 4.3	6c9dA	0.881	2.61	0.342	0.955
BvSnRK2.2	− 0.45	0.66 ± 0.13	7.5 ± 4.3	6c9gA	0.891	2.46	0.317	0.955
BvSnRK2.3	− 1.87	0.59 ± 0.14	8.7 ± 4.6	3q5iA	0.766	3.03	0.267	0.868
BvSnRK2.4	− 0.32	0.67 ± 0.13	7.6 ± 4.2	6c9dA	0.903	2.09	0.349	0.951
BvSnRK2.5	− 1.89	0.68 ± 0.12	7.1 ± 4.1	6c9dA	0.895	1.38	0.328	0.920
BvSnRK2.6	− 1.04	0.65 ± 0.13	7.8 ± 4.4	6c9dA	0.875	1.84	0.343	0.923

Expression Analysis of BvSnRK2s in Sugar Beet Under Salt Conditions

To determine the potential functions of *SnRK2s* in the response to salinity conditions, the transcript abundances of *BvSnRK2s* were assayed by the qRT-PCR method. As shown in Fig. 7, *BvSnRK2.1* in shoot was significantly up-regulated by NaCl concentrations of 50, 100, and 150 mM compared with control (0 mM). NaCl concentrations of 100 and 150 mM obviously increased transcript levels of four genes (*BvSnRK2.2*, *-2.3*, *-2.5*, and-*2.6*) in shoots. However, *BvSnRK2.4* in shoot was significantly down-regulated by 50 mM and up-regulated by 100 mM NaCl. All three concentrations of NaCl significantly up-regulated the expression levels of four genes (*BvSnRK2.2*, *-2.4*, *-2.5*, and-*2.6*) in roots. Moreover, 50 and 100 mM NaCl significantly increased transcript abundances of *BvSnRK2.3* in root by 63.0% and 57.8% compared to control, respectively. These results implied that the *BvSnRK2s* genes were involved in the salinity response in sugar beet.

Discussion

It is well known that SnRK2 is a plant-specific Ser/Thr kinase family involved in ABA signaling transduction and plays a vital role in the response to abiotic stress (Kawa

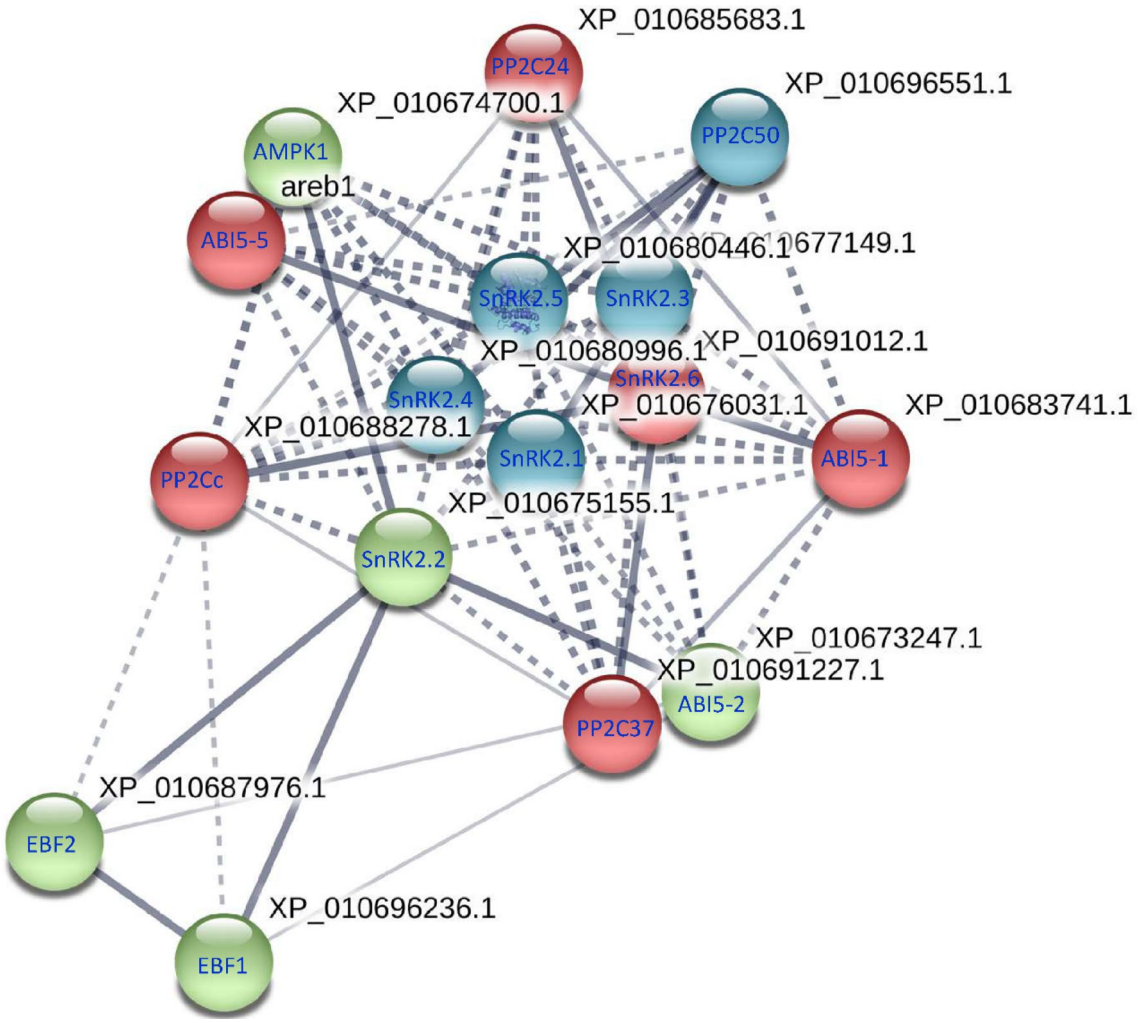

Fig. 6 Protein–protein interaction network of BvSnRK2s with other proteins. Line thickness showed the strength of data support. Network is clustered into three major clusters, which are marked by red, green, and blue nodes, respectively. Details of string analysis for individual BvSnRK2s are shown in Supplementary Fig. S2. The detailed information of all proteins was indexed in Supplementary Table S2

et al. 2020; Mao et al. 2020; Zhong et al. 2020). In the current study, a comprehensive identification and characterization of sugar beet *SnRK2s* genes (*BvSnRK2s*) were conducted. According to the previously released sugar beet genome (Dohm et al. 2014), a total of six genes encoding the SnRK2 kinases were identified and mapped onto five of 9 chromosomes (Table 2; Fig. 1). Additional numbers of *SnRK2s* would likely be found out as improvement of the sugar beet genome annotation. The current number of *BvSnRK2s* is the same as the 6 in sweet cherry (Shen et al. 2017). However, there are much more *SnRK2s* genes identified in other plant species, such as eight *SnRK2s* in grapevine (Liu et al. 2016), 10 in wheat (Zhang et al. 2016), 11 in maize (Huai et al. 2008), 12 in poplar (Song et al. 2015), 20 in cotton (Liu et al. 2017), and 22 in soybean (Zhao et al. 2017). These results suggested that the number of the*SnRK2s* genes differs remarkably among different plant species, reflecting the occurrence of gene duplication and deletion during the evolutional process of *SnRK2s* family (Mao et al. 2020).

According to ABA activation, the ten *Arabidopsis SnRK2s* were divided into two subgroups: one group corresponded to kinases activated by both ABA and hyperosmolarity and the other one composed of proteins only activated by hyperosmolarity in the previous report (Boudsocq et al. 2004). However, Kobayashi et al. (2004) further classed the *SnRK2s* genes from *Arabidopsis* and rice into three major groups based on the sequence similarity of protein kinase domain. To further determine the evolutionary relationships of *BvSnRK2s* with related genes from other species, in the present study, an unrooted phylogenetic tree was constructed according to the amino acid sequences of *SnRK2s* (Fig. 2). The *BvSnRK2s* genes can be split into three groups as Group 1, Group 2, and Group 3 (Fig. 2), which is in agreement with the grouping of *AtSnRK2s* and *OsSnRK2s* as described by

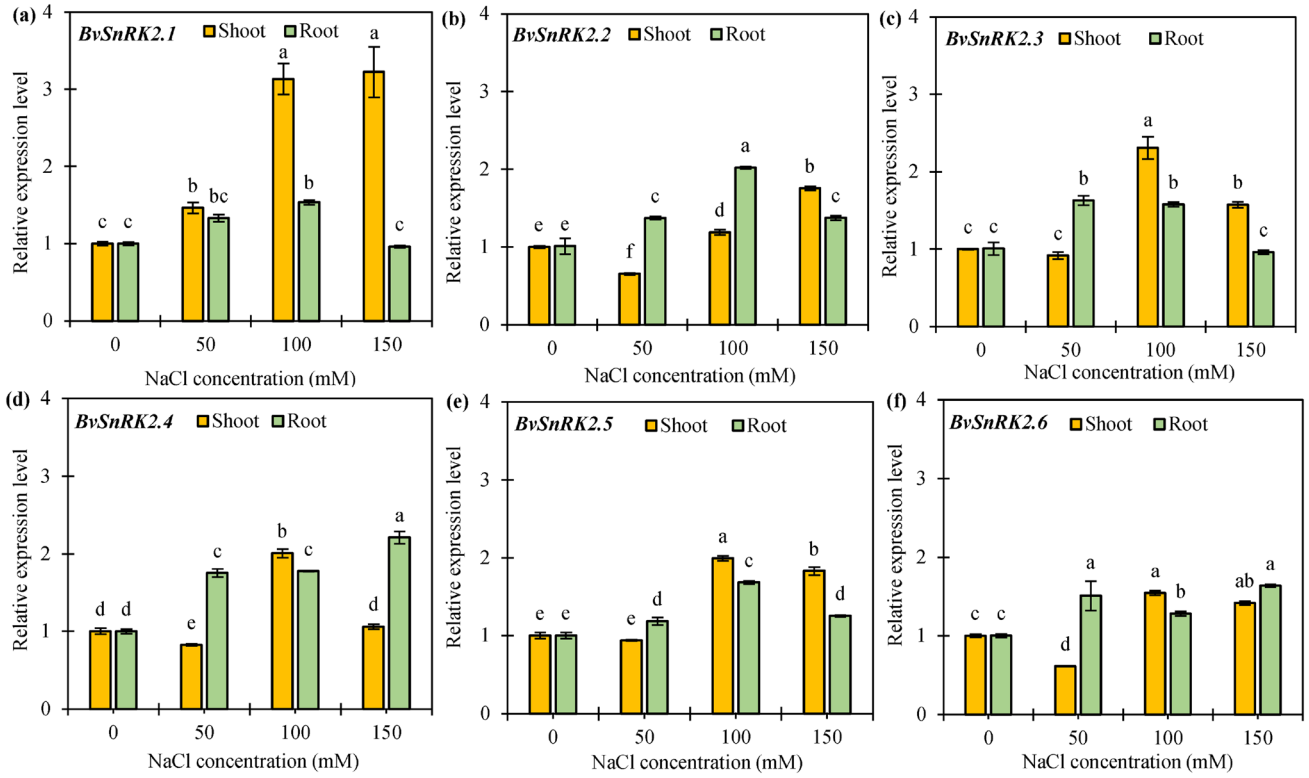

Fig. 7 The relative expression level of *BvSnRK2s* in shoots and roots of sugar beet plants treated with 0, 50, 100, and 200 mM NaCl for 48 h. **a** *BvSnRK2.1*; **b** *BvSnRK2.2*; **c** *BvSnRK2.3*; **d** *BvSnRK2.4*; **e** *BvSnRK2.5*; **f** *BvSnRK2.6*. Data are normalized to *BvACTIN* expression level. Vertical bars indicate standard error (SE) ($n=3$). Lowercase letters on top of each bar represent significant difference at $P<0.05$ level

Kobayashi et al. (2004). It is interesting that *BvSnRK2s* are more closely grouped with kinases from dicots *Arabidopsis*, potato, apple, and rubber tree than those with kinases from monocots maize and wheat. The similar evolutional relationships were also reported in the previous studies (Liu et al. 2017; Zhang et al. 2018). Moreover, the protein sequence of all *SnRK2s* could be split into two parts, the highly conserved N-terminal kinase region and the regulatory C-terminal region with "acidic patch" domain, and the functional diversities of the SnRK2s proteins were shown to be closely related to their divergent C-terminus (Kulik et al. 2011). The acidic patch was Asp rich in *BvSnRK2s* of Group 2 and Group 3 while Glu rich in the members of Group 1 (Fig. 3). Similar acidic patch region was found in *SnRK2s* from *Arabidopsis* and apple (Umezawa et al. 2010; Shao et al. 2014). Furthermore, the C-terminus region of BvSnRK2s is also split into two domains, Domain I and Domain II (Fig. 3). Domain I is relatively similar in all BvSnRK2s, whereas Domain II is present in the members of Group 3 (Figs. 2, 3). It has been shown that Domain I is involved in ABA-independent activation in the osmotic stress response, whereas Domain II is required for ABA response in BvSnRK2s of Group 3 (Kuliket al. 2011).

The exon–intron structures of *BvSnRK2s* of sugar beet displayed high similarity with soybean (Zhao et al. 2017). The size of introns of *BvSnRK2s* varied from 82 to 2164 bp, whereas the number of introns is highly conserved (Fig. 4a). Except for *BvSnRK2.4*, the CDS of *BvSnRK2s* were disrupted by eight introns, and most of *BvSnRK2s* had nine exons (Fig. 4a). Similar distribution of nine exons and eight introns was also showed in other plant species, such as apple (Shao et al. 2014), soybean (Zhao et al. 2017), and tea plant (Zhang et al. 2018). These results implied that the gene structures of *SnRK2s* were evolutionarily conserved in higher plants.

The expression pattern of genes can provide key a clue to the functions of gene, which is considered to relate to divergence in the promoter region (Liu et al. 2017).The *cis*-acting regulatory elements of the promoter regions act as important players in the regulating expression of genes (Liu et al. 2017). According to the previous reports, the *SnRK2s* genes of Group 3 were significantly induced by ABA treatments (Kulik et al. 2011) and in this group classification, two genes (*BvSnRK2.5* and *BvSnRK2.6*) belonged to Group 3 (Fig. 2). Two ABREs were found in the promoter region of *BvSnRK2.6*, while no ABRE was

present in *BvSnRK2.5* (Table 4). In cotton, all of the members in Group 3 *SnRK2s* contained ABRE-elements (Liu et al. 2017). Additionally, ABREs are also observed in the promoter regions of *BvSnRK2.1* and *BvSnRK2.2*, which belonged to Group 1 and Group 2, respectively (Table 4). In wheat, four members (*TaSnRK2.4*, *TaSnRK2.5*, *TaSnRK2.6*, and *TaSnRK2.7*) of Group 1 *SnRK2s* had several ABRE-elements in their promoter regions, although they were not activated by ABA treatments (Zhang et al. 2016). Besides ABRE, some other hormone-related elements, such as TCA-element, TGA-element, ERE, and TGACG-motif, were also found in the promoter regions of *BvSnRK2s*, which suggested that the expression of *BvSnRK2s* might be induced by salicylic acid, auxin, ethylene, and methyl jasmonic acid (Table 4). The similar *cis*-acting elements were also present in the promoters of the *SnRK2s* genes from other plants species, such as cotton (Liu et al. 2017), soybean (Zhao et al. 2017), and tea plant (Zhang et al. 2018). The regulation of adapting to certain abiotic stress, such as low temperature and drought, through *SnRK2s* has been well studied (Kawa et al. 2020; Zhong et al. 2020). In the present study, LTR was found in the promoter region of *BvSnRK2.5*. Similarly, in maize, *ZmSnRK2.3*, *ZmSnRK2.8*, and *ZmSnRK2.1*, which were significantly induced by low temperature, contained 5, 1, and 3 LTR-elements in their promoter regions, respectively (Huai et al. 2008). Additionally, both *BvSnRK2.5* and *BvSnRK2.6* had one MBS element, which is a specific binding site of MYB transcription factor (Table 4). These results implied that *BvSnRK2s* might be induced by low temperature and drought stress.

There are evidences that *SnRK2s* were involved in the multiple abiotic stress response (Shao et al. 2014; Wang et al. 2015; Zhanget al. 2016; Liu et al. 2017; Zhang et al. 2018). In the current study, the changes of transcript level of *BvSnRK2s* in root and shoot of sugar beet were analyzed under salinity conditions. Most *BvSnRK2s* were observably up-regulated by different concentrations of NaCl compared to control (0 mM) (Fig. 7). Particularly, the expression levels of *BvSnRK2* in shoots under 100 and 150 mM NaCl were 2.1- and 2.2-fold higher than those under control condition, respectively (Fig. 7a). In maize, two genes, *ZmSnRK2.3* and *ZmSnRK2.6*, were induced strongly by salinity treatments (Huai et al. 2008). In cotton, salinity also significantly up-regulated the expression levels of *GhSnRK2.8*, *GhSnRK2.9*, and *GhSnRK2.10* in both roots and stems of seedlings (Liu et al. 2017). The expression level of *BvSnRK2s* was variously induced by salinity treatment, which might be indicative of potential functions in the salinity response. However, the precise functions of *BvSnRK2s* in the response to abiotic stress need to be further explored.

Conclusions

In the current study, six *BvSnRK2s* genes were identified in the genome of sugar beet and were further split into three major groups as Group 1, 2, and 3. All BvSnRK2s contained two distinct parts, the highly conserved N-terminal protein kinase region and the greatly divergent C-terminal region. Most of the *BvSnRK2s* genes were disrupted by eight introns, except for *BvSnRK2.4*. Furthermore, the expression levels of *BvSnRK2s* were significantly raised by salinity treatments, which might be indicative of potential functions in the salinity response. The present work was the first systematic analysis of the *SnRK2s* genes family in sugar beet. These results could provide a solid foundation for further exploring functions of the *BvSnRK2s* genes in plant abiotic stress responses.

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Author Contributions G-QW designed the research and wrote the article. Z-XL, L-LX, and J-LW conducted the research and analyzed the data. All the authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

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