Plant Science 237 (2015) 33–45

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Genome-wide analysis of *SnRK* gene family in *Brachypodium distachyon* and functional characterization of *BdSnRK2.9*

Lianzhe Wang[1], Wei Hu[1], Jiutong Sun, Xiaoyu Liang, Xiaoyue Yang, Shuya Wei, Xiatian Wang, Yi Zhou, Qiang Xiao, Guangxiao Yang*, Guangyuan He*

The Genetic Engineering International Cooperation Base of Chinese Ministry of Science and Technology, The Key Laboratory of Molecular Biophysics of Chinese Ministry of Education, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China

ARTICLE INFO

Article history:
Received 16 March 2015
Received in revised form 11 May 2015
Accepted 12 May 2015
Available online 18 May 2015

Keywords:
SnRK
Expression
Protein interaction
Evolution
Abiotic stress
B. distachyon

ABSTRACT

The sucrose non-fermenting 1 (SNF1)-related protein kinases (SnRKs) play key roles in plant signaling pathways including responses to biotic and abiotic stresses. Although SnRKs have been systematically studied in Arabidopsis and rice, there is no information concerning SnRKs in the new Poaceae model plant *Brachypodium distachyon*. In the present study, a total of 44 *BdSnRKs* were identified and classified into three subfamilies, including three members of *BdSnRK1*, 10 of *BdSnRK2* and 31 of *BdSnRK3* (*CIPK*) subfamilies. Phylogenetic reconstruction, chromosome distribution and synteny analyses suggested that *BdSnRK* family had been established before the dicot-monocot lineage parted, and had experienced rapid expansion during the process of plant evolution since then. Expression analysis of the *BdSnRK2* subfamily showed that the majority of them could respond to abiotic stress and related signal molecules treatments. Protein–protein interaction and co-expression analyses of BdSnRK2s network showed that SnRK2s might be involved in biological pathway different from that of dicot model plant Arabidopsis. Expression of *BdSnRK2.9* in tobacco resulted in increased tolerance to drought and salt stresses through activation of NtABF2. Taken together, comprehensive analyses of *BdSnRKs* would provide a basis for understanding of evolution and function of *BdSnRK* family.

1. Introduction

During the processes of growth and development, plants are constantly confronted with multiple adverse stresses, such as high salinity, drought, extreme temperatures and pathogens, which seriously affect their growth, development and productivity. To survive and complete life cycle, plants have developed various complicated mechanisms to deal with these biotic and abiotic stresses [1]. Protein kinases and phosphatases, the major components of intracellular signal transduction, play important roles in stress responses [2]. Of them, sucrose non-fermenting 1 (SNF1)-related protein kinases (SnRKs) are highly conserved in organisms and involved in various physiological processes. Plant SnRKs that belong to Ser/Thr protein kinase are grouped into three subfamilies (SnRK1, SnRK2 and SnRK3) based on the sequence similarity and gene structures [3]. The SnRK1 subfamily, which contains three domains: N-terminal protein kinase (Pkinase) domain, ubiquitin-associated (UBA) domain and kinase-associated 1 (KA1) domain, is highly conserved with SNF1 in yeast and AMP-activated protein kinases (AMPKs) in animals, and is involved in carbon metabolism regulation [4]. *SnRK1s* have also been reported to be induced by ABA, implying their roles in cross-talk with metabolic signaling and stress pathways [5,6].

Unlike SnRK1, the other two subfamilies are unique in plants and participate in stress signaling pathways. The SnRK2s harbor

Abbreviations: ABA, Abscisic acid; ABI, ABA-insensitive-clade protein phosphatases PP2Cs; ABRE, ABA-responsive element; AREB/ABF, ABA-response-element-binding proteins; BAP, Benzylaminopurine; CIPK, CBL-interacting protein kinases; DRE, Dehydration-responsive element; HAB, Homology to ABI; IAA, Indole-3-acetic acid; IL, Ion leakage; LTRE, Low temperature-responsive element; MDA, Malonaldehyde; PP2C, 2C-type protein phosphatase; qRT-PCR, Quantitative real-time polymerase chain reaction; SAPK, Osmotic stress/abscisic acid-activated protein kinases, SnRK2; SCS, SnRK2-interacting calcium sensor; SnRK, Sucrose non-fermenting 1 (SNF1)-related protein kinases; VC, Vector control; WT, Wild-type.

* Corresponding authors. Tel.: +86 27 87792271; fax: +86 27 87792272.
E-mail addresses: jijy99@126.com (L. Wang), huwei2010916@126.com (W. Hu), sunjiutong@126.com (J. Sun), hnnhlxy@163.com (X. Liang), yangxiaoyuex@126.com (X. Yang), weishuya1011@126.com (S. Wei), wangxiatiancwz@163.com (X. Wang), phyllisyy@126.com (Y. Zhou), xqiangiexk@gmail.com (Q. Xiao), ygx@hust.edu.cn (G. Yang), hegy@hust.edu.cn (G. He).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.plantsci.2015.05.008
0168-9452/

two domains including the N-terminal Pkinase domain and the C-terminal regulatory domain. Further, C-terminal domain consists of two subdomains, i.e. Domain I and Domain II [7]. Domain I with about 30 amino acid residues is characteristic for all SnRK2 subfamily members while Domain II with about 40 amino acid residues is specific to the 2c group SnRK2s (AtSnRK2.2, AtSnRK2.3 and AtSnRK2.6) and mediates interaction with the clade A type 2C protein phosphatases (PP2Cs) [8,9]. To date, a large number of studies have focused on the involvement of SnRK2s in stress signaling pathways. All 10 *SnRK2s* in *Arabidopsis thaliana* (*AtSnRK2.1-2.10*) except *AtSnRK2.9* as well as all 10 *SnRK2s* in *Oryza sativa* (*OsSAPK1-10*) could be activated by hyperosmotic stress. Five *AtSnRK2s* (*SnRK2.2*, *SnRK2.3*, *SnRK2.6*, *SnRK2.7* and *SnRK2.8*) and three *OsSAPKs* (*OsSAPK8*, *OsSAPK9* and *OsSAPK10*) were activated by abscisic acid (ABA). Some members of *AtSnRKs* and *OsSAPKs* have been confirmed to play crucial roles in response to salinity and water stresses [8,10–12]. For example, overexpression of *AtSnRK2.8* and *OsSAPK4* significantly enhanced tolerance to drought and salt respectively in transgenic plants [13,14]. In other species such as maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.), most *SnRK2s* could also respond to one or more abiotic stresses and overexpression of some *SnRKs* could increase plant tolerance to abiotic stresses [15–17]. These studies suggest that *SnRK2s* are activated by abiotic stresses and play important roles in enhancing tolerance to multi-environmental stresses in plants.

SnRK3 kinases, designated as CIPKs (CBL-interacting protein kinases), interact with calcium sensor calcineurin B-like proteins (CBLs) to mediate calcium signaling pathway. Generally, CIPKs consist of a conserved Pkinase domain in N-terminal region, a NAF domain and a PPI domain in C-terminal regulatory region [18]. The NAF domain has been identified to mediate the CBL interaction and the PPI domain has been shown to mediate the interaction with PP2C [19]. To date, CIPKs have been demonstrated to participate in regulating the Na^+, K^+ and NO_3^- transportation, abiotic stress responses and some developmental processes in Arabidopsis [20–22]. The first identified CIPK in Arabidopsis is CIPK24 (SOS2) which interacts with CBL4 (SOS3) to function on Na^+/H^+ antiporter (SOS1) and H^+-ATPase, improving plant tolerance to salt stress [23]. The expression patterns and functions of the *CIPKs* have been widely studied in other species besides the Arabidopsis, including rice [24–26]; maize [27,28] and wheat [29]. These researches emphasize the importance of SnRKs function in the stress response and nutritional efficiency, and ultimately improve crop tolerance to stress by genetically manipulating these proteins.

Bioinformatic analysis of SnRK family has identified a total of 39 SnRKs in Arabidopsis [3,30–32], and 48 SnRKs in rice [8,31,33,34]. However, genome-wide identification of SnRKs has not been reported in *Brachypodium distachyon*, which is the first sequenced Poaceae grass and has close relationships with important crops such as wheat (*T. aestivum* L.), barley (*Hordeum vulgare* L.) and sorghum (*Sorghum bicolor* L.) [35]. In this study, we identified 44 *BdSnRK* genes from *B. distachyon*, and analyzed their genomic structures, chromosomal locations, phylogenetic expansion and evolutionary mechanism. Additionally, expression patterns and interaction analyses of *BdSnRK2* subfamily were preformed to detect their responses to abiotic stress and their interaction network responding to abiotic stress in *B. distachyon*. Further, functional analysis of *BdSnRK2.9* revealed its positive role in response to drought and salt stresses. These systematical analyses will be helpful for understanding the roles of SnRK family in *B. distachyon* under abiotic stress and provides valuable information for further functional characterization of SnRKs in other monocot crops.

2. Materials and methods

2.1. Identification and phylogenetic analysis of SnRK gene family in B. distachyon

The non-redundant amino acid sequences of the Arabidopsis and rice SnRKs were collected from TAIR v10 (http://www.arabidopsis.org/) and RGAP v7 databases (http://rice.plantbiology.msu.edu/) respectively. More than 100 SnRKs sequences belonging to some other plant species were downloaded from Uniprot (http://www.uniprot.org/) (Table S1). The whole protein sequences of *B. distachyon* were downloaded from the Brachypodium Genome Database v1.2 (http://www.brachypodium.org/). To identify the predicted SnRKs in *B. distachyon*, the local Hidden Markov Model-based searches in the protein sequence dataset were performed separately with HMM profiles built from each subfamily of known SnRKs as queries using HMMER software [36]. In addition, BLAST searches with all SnRK sequences of rice as queries were performed to identify the predicted SnRKs in the Brachypodium Genome Database. All the potential BdSnRK proteins identified from HMM search and BLAST search were validated for the presence of conserved domain with the PFAM (http://pfam.sanger.ac.uk/) and CDD databases (http://www.ncbi.nlm.nih.gov/cdd/). Further, the sequences were reciprocally searched against the Arabidopsis and rice database to identify the best hit among all the SnRK genes. The proteins that did not contain the known conserved domains and motifs or the best hits of reciprocally searches were not the SnRKs, were removed manually.

Due to high variation in the C-terminal sequences of the SnRK proteins, the conserved Pkinase domain regions of SnRK family from Arabidopsis, rice and *B. distachyon* were selected to perform multiple alignment using Clustal X 2.0 [37]. The amino acid substitution model was calculated by the ModelGenerator v0.85 and the optimal model of "JTT + I + G + F" was selected [38]. The maximum-likelihood (ML) tree was constructed using MEGA5 program with bootstrap values for 1000 replicates [39].

2.2. Protein properties and sequence analyses

The relative molecular mass and isoelectric points of putative proteins were obtained by the ExPASy proteomics server (http://expasy.org/). The motifs were identified using the MEME program (http://meme.sdsc.edu/meme/intro.html), with optimum motif width $\geq$6 and $\leq$200 bp, maximum number of motifs 23. The motifs were annotated by InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan/). The gene information of *BdSnRKs* was retrieved from the Brachypodium database and the gene structures were drawn with the GSDS (http://gsds.cbi.pku.edu.cn/). To analyze the *cis*-element in promoter regions, the 1.5 kb upstream regions of the coding sequence region were selected from the Brachypodium Genome Database and analyzed with the PLACE (http://www.dna.affrc.go.jp/PLACE/) and PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) databases. Among them, the ABA-responsive element (ABRE; ACGTGG/TC) [40], dehydration-responsive element (DRE)/C-repeat (DRE; TACCGACAT) [41], and low temperature-responsive element (LTRE) [42] were selected for analyses.

2.3. Chromosomal location, genome synteny and gene duplication analyses

The chromosomal location information of *BdSnRK* family was downloaded from Brachypodium database. The syntenic blocks information of Arabidopsis, rice and *B. distachyon* was downloaded from the Plant Genome Duplication Database (http://chibba.agtec.