Systematic analysis of the OSCA family members in Vigna radiata and their involvement in osmotic resistance

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Abstract

Background

Mung bean (*Vigna radiata*) is a warm-season legume crop and belongs to papilionoid subfamily of the Fabaceae. China is the leading producer of mung bean in the world. It has significant economic and health benefits and is a promising species with broad adaptation and high tolerance to stress environments. The OSCA family members play an important role in the modulation of hypertonic stresses, such as drought and salinity. However, genome-wide analysis of the OSCA family in mung bean is lacking.

Results

We identified a total of 13 *OSCA* genes in the mung bean genome and named according to their homology with *AtOSCAs*. All the *OACAs* were phylogenetically splitted into four clades. Phylogenetic relationship and synteny analyses showed that the *VrOSCAs* in mung bean and soybean shared a relatively conserved evolutionary history. In addition, three duplicated *VrOSCA* gene pairs were identified and the duplicated *VrOSCA s*have mainly undergone purifying selection pressure during evolution. Protein domain, motif and transmembrane analysis indicated that most of the VrOSCAs shared similar structures with their homologs. The expression pattern showed that exception of *VrOSCA2.1*, the other 12*VrOSCA*s were up-regulated expression under treatment with ABA, PEG and NaCl, among which *VrOSCA1.4* showed the largest increased expression levels. The duplicated genes *VrOSCA2.1/ VrOSCA2.2* showed divergence expression, which might experience functionalization during subsequent evolution. The expression profiles under ABA, PEG and NaCl stress revealed a functional divergence of *VrOSCA* genes, which agreed with the cis-acting elements analysis in the promoter of *VrOSCA* genes.

Conclusions

Collectively, the study provided a systematic analysis of the VrOSCA family in mung bean. Our results would lay an important foundation for functional and evolutionary analysis of *VrOSCAs*, and provide promising genes for further investigation of abiotic stress tolerance in mung bean.

Background

In natural environmental conditions, plants are subjected to many stresses. The osmotic stress caused by drought and salinity is one of the key stress factors affecting plants growth and yield [1]. Osmotic stress usually broke the plants' osmotic balance and finally caused damage of cell membrane system[2]. Previous studies have found plant responses to stresses mainly include the perception, transmission of signals through various pathways and the regulation of stress-responsive gene expression, resulting physiological and morphological modifications to resist the stresses [3–5]. During the signal perception, calcium is an important second messenger in the signal transduction pathway when plants respond to stresses [6, 7]. Under Osmotic stress, plants induce a rapid increase of free calcium ion concentration in intracellular, thereby inducing the expression of many stress-related genes to regulate plant tolerance to osmotic stress [5, 8, 9]. The increase of calcium ion concentration in intracellular is mainly regulated by calcium transport systems such as calcium channels and calcium pumps [10]. Previous studies showed that stimuli-gated Ca^{2+}-permeable channels served as osmosensors in bacteria and animals [11, 12], which indicated that there might be specific calcium permeable channels that function as osmosensors in plants.

In plants, the hyperosmolality-gated calcium-permeable channels (OSCA) is the calcium non-selective cation channel protein as well as the receptor protein for hypertonic stress [13–15]. Studies of the functional domain have shown OSCA family contained the calcium-dependent channel domain (DUF221) that may participate in osmotic adjustment[16, 17]. In rice, the entire OSCA family is characterized by the presence of a conserved DUF221 domain, which functions as an osmotic-sensing calcium channel [18]. In *Arabidopsis*, OSCA1, a hyperosmolality-gated calcium-permeable channel, was characterized as an osmosensor and mediated osmotic-stress-evoked Ca^{2+} concentration increases [14]. Studies have shown maize gene *ZmOSCA2.4* could enhance drought tolerance in transgenic *Arabidopsis* [19]. It can be seen that OSCA family numbers play a crucial role in plants resistance to osmotic stress. Therefore, it is an important path to excavate and study potential genes for breeding osmotic-stress-resistant varieties. The predecessors have systematically identified and analyzed the OSCA family in dicotyledon such as *Arabidopsis thaliana* and soybean, and monocotyledon rice[14, 18, 20]. However, genome-wide analysis of the OSCA family in mung bean is lacking.

Mung bean (*Vigna radiata* (L.) R. Wilczek, 2n = 2×=22) belongs to the papilionoid subfamily of the Fabaceae and is always grown in poor-soil regions because its broad adaptation and high tolerance to stresses. Mung bean seeds are rich in proteins and contain higher levels of folic acid and iron than most other legumes [21]. With the completion of mung bean genome sequencing, an opportunity was opened to systematic research the OSCA family in mung bean [22]. In the present study, we identified putative OSCA family members in mung bean and analyzed their phylogeny, syntenic relationship, conserved motifs, transmembrane regions and cis-elements responsive to abiotic stresses in promoters. In addition, we studied the expression profiles of *OSCAs* under PEG, NaCl and ABA treatments. These findings will facilitate further research on the biological function of this gene family and provide promising genes for cultivation of genetically modified osmotic-stress-resistant plants.

Results

Genome-wide identification of *OSCA* gene family members in mung bean

The Hidden Markov Model of DUF221 domain (Pfam accession number: 02714) was used to search against the mung bean genome. Ultimately, a total of 13 *VrOSCA* genes were identified in mung bean and named according to the *Arabidopsis* orthologs (Table 1). Among the 13 genes, 12 *VrOSCA* genes were distributed randomly on all the 11 chromosomes except chr2, 8 and 10, while *VrOACA2.5* was located on scaffold_100. The amino acid numbers of all identified VrOACAs varied from 592 (VrOACA2.2) to 880 (VrOACA4.1). The molecular weight of the VrOSCA proteins varied from 67.16 (VrOACA2.2) to 99.16 kDa (VrOACA4.1) and the isoelectric points ranged from 6.28 (VrOACA4.1) to 9.44 (VrOACA2.5).

Table 1
Detailed information for 13 *VrOSCA* genes in the *V. radiata* genome.

Gene Name	Gene Identifier	Chromosome	Gene length(bp)	Protein length (aa)	ORF (bp)	Isoelectric Point	Molecular Weight (KDa)	Clade
VrOACA1.1	Vradi07g26860	7	6435	775	2328	8.91	88.17	1
VrOACA1.2	Vradi10g01410	10	7609	773	2322	9.22	88.68	1
VrOACA1.3	Vradi06g03460	6	5939	760	2283	9.08	87.48	1
VrOACA1.4	Vradi03g00620	3	7957	640	1923	6.68	72.55	1
VrOACA1.5	Vradi11g08350	11	6738	863	2592	9.06	98.71	1
VrOACA2.1	Vradi06g14350	6	8460	721	2166	8.53	81.83	2
VrOACA2.2	Vradi05g11970	5	16125	592	1779	8.37	67.16	2
VrOACA2.3	Vradi01g07680	1	11006	709	2130	8.52	80.50	2
VrOACA2.4	Vradi04g08970	4	6275	637	1914	6.71	72.90	2
VrOACA2.5	Vradi0100s00520	Scaffold_100	5251	671	2016	9.44	76.95	2
VrOACA2.6	Vradi05g17480	5	10518	600	1803	9.03	67.43	2
VrOACA3.1	Vradi10g09440	10	4444	728	2187	9.36	82.44	3
VrOACA4.1	Vradi07g01560	7	7051	880	2643	6.28	99.16	4

Phylogenetic analysis of the *OSCA* gene family in mung bean

To elucidate the phylogenetic relationships of OSCA proteins in mung bean, *Arabidopsis*, soybean and rice, the phylogenetic tree based on the alignment of 60 full-length OSCA protein sequences was build (Fig. 1, Additional file 1). The 60 OSCA proteins were classified into four major group, clade 1, 2, 3, and 4. The clade 1 and 2 contained more members than clade 3 and 4. Phylogenetic analysis result showed that the OSCA family experienced a similar evolutionary history between the mung bean, *Arabidopsis*, soybean and rice genomes. Moreover, OSCA proteins derived from mung bean had a higher similarity with those from soybean, demonstrating a closer phylogenetic relationship between mung bean and soybean since both of them belong to the leguminous family.

Collinearity analysis of *OSCA* genes in mung bean, *Arabidopsis*, soybean and rice

Comparative genomics analyses of gene collinearity reveal homologous gene functions and phylogenetic relationships between species. Thus, we analyzed the colinearity relationship of *OSCA* genes with three representative species, including one monocot (rice) and two dicots (*Arabidopsis*, soybean) (Fig. 2, Additional file 2). We found that the *OSCA* genes of mung bean had the most homologous gene pairs with the *OSCA* genes of *Glycine ma* (17), followed by *Arabidopsis* (7) and *Oryza sativa* (1), indicating that in comparison with *Arabidopsis* and rice, mung bean *OSCAs* genes show a closer phylogenetic relationship with soybean. The result was consistent with the phylogenetic analysis (Fig. 1), affirming the accuracy of our analysis. Some *VrOSCAs* (*VrOSCA1.1, -1.4, -1.5, -2.4, -2.5, -3.1* and − *4.1*) were found to be associated with two syntenic gene pairs in mung bean and soybean. These genes may play a crucial role during evolution. Meanwhile, no collinear segments of *VrOSCA1.2* and *VrOSCA2.2* were found in the genome of mung bean and soybean. The results indicated that large-scale expansion of *OSCAs* probably occurred before mung bean-soybean

division, and certain *VrOSCAs* might originate from duplication of the mung bean genome after phylogenetic divergence of mung bean.

Gene duplication of *VrOSCAs* in mung bean

To better understand the evolutionary relationship, gene duplication events were analyzed to elucidate the expansion patterns of the *OSCA* genes in mung bean. Three segmental duplication events with five *OSCAs* were identified, which are located on duplicated segments on chromosomes 1, 4, 5, 6 and scaffold_100 (Fig. 3). Moreover, the Ka/Ks ratio of the duplicated *VrOSCA* gene pairs was calculated to evaluated the molecular evolutionary (Table 2). All of the Ka/Ks ratios were less than 1.

Table 2
Ka/Ks analysis for duplicated gene pairs of *OSCAs* in mung bean.

Duplicated Gene 1	Duplicated Gene 2	Type of duplication	Ka	Ks	Ka/Ks	Purifying Selection
VrOACA2.1	*VrOACA2.2*	segmental	0.1478	0.7539	0.1960	Yes
VrOACA2.3	*VrOACA2.4*	segmental	0.1397	0.6934	0.2015	Yes
VrOACA2.4	*VrOACA2.5*	segmental	0.3050	2.2950	0.1329	Yes

The conserved domains, motifs and TMs analysis of VrOSCAs protein

Analysis of the protein conserved domains of VrOSCAs revealed that most VrOSCAs contained three domains: late exocytosis (pfam13967), cytosolic domain of 10TM putative phosphate transporter (pfam14703, DUF4463) and calcium-dependent channel (pfam02714, DUF221), while VrOSCA4.1 contained four domains including two protein domain DUF221, as shown in Fig. 4a. It is noteworthy that protein domain pfam13967 and DUF221 are located at the N-terminal and C- terminal of all VrOSCAs, respectively, and protein domain DUF4463 is located in the middle of pfam13967 and DUF221. These results indicated that the three domains are relatively conservative in the VrOSCA family. Meanwhile, it was found that protein domain pfam13967 and DUF221contained a different number of TMs, while no TMs were detected in the protein domain DUF4463 in all VrOSCAs. All the VrOSCAs contained at least eight TMs (Fig. 4a, Additional file 3).

To further explore potential function of VrOSCAs, we detected additional conserved motifs using the MEME tool and a total of 20 conserved motifs were detected (Fig. 4b, Additional file 4). It is noteworthy that all clades contained motif1, 2 and 4, indicating that all genes perform the three functions. Among them, motif1 and motif2 located in the calcium-dependent channel domain and motif4 located in the late exocytosis domain. Some conserved domains were restricted to specific clades. For example, motif 16 and motif 12 were only detected in clade1 and clade2 respectively, which indicated functional difference between clade1 and clade2. We also observed different motifs within the same clade, suggesting that there were different action mechanisms within each clade. For example, VrOSCA1.4 in clade1 lacked motifs 5, 14, 15,17 and19, whereas the other four VrOSCAs (VrOSCA1.1, -1.2, -1.3 and − 1.5) in clade1 contained these motifs. This phenomenon was also observed in other clades. VrOSCA4.1 in clade 4 contained the least motifs. The results of the conserved motif analysis were generally consistent with the phylogenetic analysis.

Cis-acting elements analysis in the promoter of *VrOSCA* genes

The cis-acting elements in promoter regions of genes participate in various pathways, for example, the ABA and abiotic stress response signal transduction pathway [23]. Therefore, we analyzed the cis-acting elements involved in ABA and abiotic stress response in -1.5 kb promoter region of *VrOSCA* genes, including ABRE, DRE, MBS, TC-rich and LTR elements (Fig. 5, Additional file 5). We found all the *VrOSCA* genes, except *VrOSCA1.5*, contained at least one of these cis-acting elements. Moreover, the cis-acting elements of the *VrOSCA*s among clades were different. For example, clade1 and clade2

contained DER and MBS elements associated with drought stress, but genes in clade3 and clade4 did not. Clade1, clade2 and clade3 contained LTR element associated with low temperature stress, while gene in clade4 did not. It indicated that *VrOSCA* genes in different clades might response to stress collectively. In the clade2, only *VrOSCA2.2* and *VrOSCA2.4* contained MBS element, and only *VrOSCA2.1* contained TC-rich element, and only *VrOSCA2.2* contained LTR element. This phenomenon was also observed in clade 1. These results suggested that *VrOSCAs* in the same clade may have different action mechanisms.

Expression of VrOSCAs under ABA and abiotic stresses

PEG and NaCl stresses may cause similar cellular damage and lead to osmotic stress [24]. Plants adapt and respond to drought and salt stress by inducing a range of gene expression. ABA is an important plant hormone that regulates the expression of stress-responsive genes in plants [25]. We studied the expression profiles of the 13 *VrOSCA* genes under ABA, PEG and NaCl treatments for 4h, 12h and 24h. Analysis of expression profiles showed that the exception of *VrOSCA2.1*, the other12 *VrOSCA* genes were up-regulated expression by ABA, PEG and NaCl treatments. *VrOSCA2.1* was significantly down-regulated under ABA, PEG and NaCl treatments (Fig. 6, Additional file 6). The expression patterns of all the up-regulated genes showed increased at 4 h or 12 h and then decreased at 24 h of stress. The relative expression values of *VrOSCA1.4, -2.2, -2.3, -2.4, -2.5, -2.6, -3.1* and − *4.1*were relatively higher than *VrOSCA1.1, -1.2* and − *1.3* under the three types of osmotic stress. Additionally, under ABA treatment, the genes increased expression level by a factor of more than 10-fold compared with 0 h were *VrOSCA1.4, -2.2, -2.3, -2.4, -2.5, -2.6* and − *3.1*. Under PEG treatment, the expression of *VrOSCA1.4, -2.2, -2.4, -2.5, -2.6* and − *3.1* increased by a factor of more than 10-fold compared with 0 h. Under NaCl treatment, the expression of *VrOSCA1.4, -1.5, -2.2, -2.4, -2.5* and − *3.1* increased by a factor of more than 10-fold compared with 0 h. Among these genes, *VrOSCA1.4*showed the largest folds change in relative expression levels under the three types of osmotic stress compared with normal growth conditions. These results indicated that mung bean *OSCA* genes responded to osmotic stress caused by ABA, PEG and NaCl treatment.

Discussion

With mung bean being a broadly adapted and highly stress-tolerant crop, the whole genome sequencing of mung bean is conducive to identification of resistance genes and genetic improvement of crops. In the present study, we performed a genome-wide analysis of the *OSCA* genes in mung bean and identified a total of 13 *VrOSCA* genes. The VrOSCA proteins varied substantially in the sequence and physicochemical properties (Table 1), which were comparable with *OSCA* genes from other plant species [14, 18, 20, 26]. Phylogenetic tree (Fig. 1) analysis showed that OSCAs can be divided into four clades, which was consistent with evolutionary analysis of *Arabidopsis*, soybean and rice[14, 18, 20]. Each clade included OSCA members from mung bean, *Arabidopsis*, soybean and rice, indicated that the OSCA family originated and diversified prior to divergence of mung bean, *Arabidopsis*, soybean and rice. The clade3 and clade4 contained fewer members but conserved during species, indicating that OSCA member in clade3 and clade4 may perform an indispensable role in biological processes. The different number of OSCAs within the mung bean, *Arabidopsis*, soybean and rice indicates that the majority of OSCAs in mung bean, *Arabidopsis*, soybean and rice genomes undergo greater genetic variation after their divergence.

On the basis of OSCA family members phylogenetic relationships (Fig. 1), we systematically analyzed the synteny relationship of OSCAs in mung bean, *Arabidopsis*, soybean and rice. (Fig. 2, Additional file 2). Large-scale expansion of *OSCAs* probably occurred after monocots and dicots division. Despite *VrOSCA2.2* and *Glyma.04G048800* were clustered together (Fig. 1), *VrOSCA2.2* was absent from the synteny analysis. We did not find the synteny blocks related to *VrOSCA1.2* and *VrOSCA2.2*. To elucidate the expanded mechanism of the *OSCA* gene family in mung bean, gene duplication events were investigated (Fig. 3, Table 2). We identified a total of 3 duplicated *VrOSCA* gene pairs, including *VrOSCA2.1/VrOSCA2.2, VrOSCA2.3/VrOSCA2.4* and *VrOSCA2.4/VrOSCA2.5*. The duplication events of

VrOSCA2.1/VrOSCA2.2 might occur after the divergence of the mung bean and soybean, while *VrOSCA2.3/VrOSCA2.4* and *VrOSCA2.4/VrOSCA2.5* duplicated prior to the divergence of mung bean and soybean. Ka/Ks ratios for the duplicated *VrOSCA* gene pairs were less than 1, suggesting that the duplicated *VrOSCAs* might have experienced a purifying selective pressure (Table 2). As the purifying selection restricts the gene divergences, the duplicated *VrOSCA* genes might have retained some similar functions [27]. Our results also showed that the expression pattern of *VrOSCA2.3*, *-2.4* and – 2.5was similar under ABA-, PEG- and NaCl-induced osmotic stresses.

Previous studies have shown that OSCA protein contained 11 transmembrane helices (TMs)in *Arabidopsis* and 6-10TMs in rice [18, 28]. In contrast, VrOSCAs contained 8–10 TMs, which indicates that VrOSCAs had experienced greater genetic variation during evolution. To investigate the structure features of VrOSCAs, conserved domain was analyzed. The results showed that the structural domain was highly conserved (Fig. 3a) and the distribution of protein domain pfam13967, pfam14703 and pfam02714 in VrOSCAs is consistent with that in OSCA proteins in maize [26]. Meanwhile, all the TMs were located in protein domain pfam 02714 and pfam13967. In this study, 20 distinct conserved motifs were identified. The motifs of VrOSCAs in clade1, clade2 and clade3 were highly conserved and the composition patterns of the conserved motifs in these three clades were similar. However, VrOSCA4.1 in clade 4 contained fewer conserved motifs than other VrOSCAs. Moreover, the expression level of *VrOSCA4.1* showed little difference under ABA-, PEG- and NaCl-induced osmotic stresses, suggesting that *VrOSCA4.1* may have a indirect function in osmotic stress response [19].

Analysis of promoter components of the 13 *VrOSCA* genes showed they contained variable types of core components associated with ABA responsiveness (ABRE) and stress responsiveness (DRE, MBS, LTR and TC-rich). For example, all the genes contained ABRE core components that play a crucial role in ABA-dependent gene expression, except *VrOSCA1.5* (Fig. 5, Additional file 5). Similarly, the dehydration response elements DRE is involved in ABA-independent gene expression in response to osmotic stress [10]. Only five *VrOSCA* genes, *VrOSCA1.1*, *-1.2*, *-2.1*, *-1.2* and*-2.6*, contained DRE. Furthermore, the promoters of *VrOSCA* genes classified in the same clade also contained different types and numbers of response elements, so different genes classified in the same clade may show functional diversity and have different action mechanisms. Moreover, genes in different clades may exist synergies [29, 30].

In this study, the dynamic osmotic stress responsive expression patterns of *VrOSCAs* were analyzed. Expression profiles analysis of *VrOSCAs* can help us to understand their possible functions in osmotic stress and offer crucial clues for functional assessment. As members of the OSCA hyperosmotic calcium channels proteins family, the *VrOSCA* genes responded to ABA-, PEG- and NaCl-induced osmotic stresses, consistent with the *OSCA* members in *Arabidopsis* and rice [18, 31]. However, *VrOSCAs* exhibited differential expression under the osmotic stresses, not only amongst clades but also amongst members within the same clade, suggesting that these *VrOSCAs* might have diverse functions. The present results showed that *VrOSCA2.1* was significantly down-regulated under ABA, PEG and NaCl treatments, whereas the other 12 *VrOSCA* genes were significantly up-regulated under the three osmotic stresses (Fig. 6, Additional file 6), suggesting that the 12 *VrOSCAs* might be crucial mediators of osmotic stress response and contribute to the establishment of complex signaling networks in mung bean. Up-regulation of the 8 *VrOSCA* genes (except *VrOSCA1.1*, *-1.2*, *-1.3* and – 4.1) ranged from 10 to 70-fold, which indicated that these genes responded positively to osmotic stress. *VrOSCA2.2* and – 2.4 responded strongly to ABA, PEG and NaCl stress, and showed more than 20-fold expression levels than the control (0 h). Thus, *VrOSCA2.2* and – 2.4 may simultaneously response to ABA, PEG and NaCl stress-response pathways and there may be interaction in the pathways responsive to the three stresses. Regardless, these genes played an important role in drought and high-salinity tolerance. Moreover, the expression of duplicated genes showed two pairs of duplicated genes shared similar expression patterns, suggesting they might retain some essential functions during subsequent evolution. However, the duplicated genes *VrOSCA2.1/ VrOSCA2.2*showed divergence expression, which might experience functionalization after the duplication events [32]. Our results provided promising genes for further characterization in their functional involvement in osmotic stress.

Conclusions

In conclusion, a total of 13 *OSCA* genes were identified in the mung bean. The comprehensive analysis of the VrOSCA gene family provided important information such as phylogenetic relationship, duplication events and expansion profile. These findings lay an important foundation for understanding the molecular evolution of the OSCA family in mung bean and providing candidate genes for further study of abiotic stress tolerance in mung bean.

Methods

Identification of OSCA family numbers in the Mungbean Genome

The *V. radiata* genome database (genome assembly: Vradiata_ver6) was downloaded from EnsemblPlants (http://plants.ensembl.org/index.html). The conserved OSCA domain DUF221 (pfam accession number: 02714) from the Pfam database [33] was used to build the Hidden Markov Model (HMM)profiles (http://hmmer.janelia.org/) and query the *V. radiata* whole-genome protein database. Each non-redundant sequence was confirmed using the SMART web server (http://smart.embl.de/) [34], the Conserved Domain Database in National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) [35] and Pfam website (http://pfam.xfam.org/). The molecular weight (MW) and isoelectric point (PI) of OSCA proteins were predicted with ProtParam (http://web.expasy.org/protparam/).

Conserved motifs, transmembrane regions and phylogenetic analysis

The conserved motifs and transmembrane region (TMs) of mung bean OSCA proteins were identified using the MEME program (http://memesuite.org/tools/meme) and the TMHMM Server V.2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/), respectively. Multiple sequence alignment was analyzed with ClustalW program [36] and the phylogenetic tree was constructed using MEGA 7 software with neighbour-joining (NJ) method and 1000 replicates iterations [37].

Interspecies Synteny Analysis And Gene Duplication

In order to analysis the relationships of orthologous *OSCA* genes in different species, multiple sequence alignment was used to detect sequences of mung bean and soybean with the similarity of more than 70%. Then, Multiple Collinearity Scan toolkit (MCScanX) was adopted to analysis the collinear block with the default parameters. Finally, the linear analysis map was illustrated using Dual Synteny Plotter software (https://github.com/CJ-Chen/TBtools).Duplicated gene pairs were analyzed using MCScanX program with the default parameters and plotted with the Circos software [38]. The Ka (non-synonymous substitution rate) and Ks (synonymous substitution rate) were investigated by DnaSP v5.0 software [39], and the selection pressure was calculated by the Ka/Ks ratio.

Abiotic stress responsive cis-elements analysis in the promoter regions of VrOSCA genes

The PLACE website (http://www.dna.affrc.go.jp/PLACE/signalscan.html) [40] was used to identify putative cis-elements involved in ABA and abiotic stress response in the 1.5 kb upstream promoter region from the transcription start site.

Plant materials and stress treatments

In this study, the mung bean cultivar VC1973A was used to analyze gene expression profiles under drought, salt and ABA treatments. The seeds of cultivars VC1973A were obtained from the Chinese Academy of Agricultural Sciences. The VC1973A were grown in a growth chamber at 24 °C with a photoperiod of 16 h. When the appearance of the first trifoliolate leaf, seedling was treated with 20% polyethylene glycol-6000(PEG-6000), NaCl (100mM) and abscisic acid

(ABA) (100 µM) solution as described previously, respectively [41]. The leaves were collected at 0 h, 4 h, 12 h and 24 h and stored at -80 °C.

Expression profile analysis of *VrOSCA* genes under stress treatments

The total RNA from the leaves was isolated using an RNAprep Pure Plant Kit (Tiangen, Beijing, China), and the first-strand cDNA was synthesized using SuperScript™ III Reverse Transcriptase kit (Invitrogen, USA). Quantitative real-time PCR (qRT-PCR) was performed in ABIViiA 7 Real-Time PCR system (Applied Biosystems, USA) by SYBR Green PCR mix (QIAGEN). The PCR reaction was performed with the following conditions: 95°C for 2 min followed by 40 cycles of 94°C for 10 s and 59°C for 10 s. The relative expression level of *VrOSCA* genes was calculated by the $2^{-\Delta\Delta CT}$ method [42]. Gene-specific primers were designed using Primer Express Software v2.0 (Additional file 7) and synthesized commercially (HUADA Gene, Beijing, China). The *V. radiata* actin gene (GenBank: AF143208.1) was used as an endogenous control for qRT-PCR. Each experiment was repeated using different cDNAs from three biological replicates.

Supplementary information

Additional file 1. The OSCAs amino acid sequences in mung bean, soybean, *Arabidopsis* and rice.

Additional file 2. Synteny analysis of OSCA genes between mung bean and other plant species.

Additional file 3. The position of the transmembrane region of VrOSCAs.

Additional file 4. Sequences of the 20 motifs in the VrOSCAs.

Additional file 5. Positions of abiotic stress-responsive cis-acting elements in the 1.5 kb upstream promoter of *VrOSCA* genes.

Additional file 6. Expression patterns of *VrOSCA* genes in response to ABA, PEG and NaCl treatments.

Additional file 7. PCR primers used for qRT-PCR in this study.

Abbreviations

OSCA: hyperosmolality-gated calcium-permeable channels; NCBI:National Center for Biotechnology Information; TAIR:The Arabidopsis Information Resource; CDD:Conserved Domain Database; SMART:Simple Modular Architecture Research Tool; MEGA:Molecular Evolutionary Genetics Analysis; MEME:Multiple EM for Motif Elicitation; GSDS:Gene Structure Display Server; Ka/Ks:Non-synonymous substitution rate/synonymous substitution rate; qRT-PCR:Quantitative real-time PCR

Declarations

Availability of data and materials

The *Arabidopsis* OSCA protein sequences were collected from the *Arabidopsis* information source (TAIR) database (http://www.arabidopsis.org). The genome sequences of mung bean, *soybean* and rice were downloaded from EnsemblPlants (http://plants.ensembl.org/index.html). The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LY and BX conceived and designed the study. LY, RW, XC and FL conducted the experiments and analyzed the data. LY and XC wrote the manuscript. RW, MZ and BX revised the manuscript. All authors have read and approved the final version of the paper.

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