RESEARCH ARTICLE

Genome-wide analysis of OSCA gene family members in Vigna radiata and their involvement in the osmotic response

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Abstract

Background: Mung bean (Vigna radiata) is a warm-season legume crop and belongs to the papilionoid subfamily of the Fabaceae family. China is the leading producer of mung bean in the world. Mung bean has significant economic and health benefits and is a promising species with broad adaptation ability and high tolerance to environmental stresses. OSCA (hyperosmolality-gated calcium-permeable channel) gene family members play an important role in the modulation of hypertonic stress, such as drought and salinity. However, genome-wide analysis of the OSCA gene family has not been conducted in mung bean.

Results: We identified a total of 13 OSCA genes in the mung bean genome and named them according to their homology with AtOSCAs. All the OSCAs were phylogenetically split 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 VrOSCAs gene pairs mainly underwent purifying selection pressure during evolution. Protein domain, motif and transmembrane analyses indicated that most of the VrOSCAs shared similar structures with their homologs. The expression pattern showed that except for VrOSCA2.1, the other 12 VrOSCAs were upregulated 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 divergent expression, which might have resulted in functionalization during subsequent evolution. The expression profiles under ABA, PEG and NaCl stress revealed a functional divergence of VrOSCA genes, which agreed with the analysis of cis-acting regulatory elements in the promoter regions of VrOSCA genes.

Conclusions: Collectively, the study provided a systematic analysis of the VrOSCA gene family in mung bean. Our results establish an important foundation for functional and evolutionary analysis of VrOSCAs and identify genes for further investigation of their ability to confer abiotic stress tolerance in mung bean.

Keywords: Mung bean (Vigna radiata), OSCA gene family, Evolutionary analysis, Expression patterns, Abiotic stress

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**Background**

Under natural environmental conditions, plants are subjected to many types of stress. Osmotic stress caused by drought and salinity is one of the key stress factors affecting plant growth and yield [1]. Osmotic stress usually disrupts the plant osmotic balance and finally causes damage to the cell membrane system [2]. In many agricultural ecosystems, water scarcity and drought could induce phosphorus deficiency, which limit crop yield significantly [3]. It has been reported that salinity stress impairs normal metabolic pathways, such as photosynthesis, respiration, mineral assimilation and biomass accumulation, thereby contributing considerably to reduced crop production [1, 4, 5]. Previous studies have found that plant responses to stress mainly include the perception and transmission of signals through various pathways and the regulation of stress-responsive gene expression, resulting in physiological and morphological modifications to resist stress [6–8]. These changes are mainly manifested in the enhancement of proline, betaine and sugar synthesis, which helps to maintain the integrity of tissue water content, and the up-regulation of key antioxidant enzymes activity to reduce the oxidation of proteins and lipids by reactive oxygen species [9–11]. During signal perception, calcium is an important second messenger in the signal transduction pathway when plants respond to stress [12, 13]. Under osmotic stress, plants induce a rapid intracellular increased concentration of free calcium ions, thereby inducing the expression of many stress-related genes to regulate plant tolerance to osmotic stress [7, 14, 15]. The increased intracellular concentration of calcium ions is mainly regulated by calcium transport systems such as calcium channels and calcium pumps [16]. Previous studies showed that stimuli-gated Ca²⁺ permeable channels served as osmosensors in bacteria and animals [17, 18], which indicated that there might be specific calcium permeable channels that function as osmosensors in plants.

In plants, OSCA is calcium nonselective cation channel protein and receptor protein for hypertonic stress [19–21]. Studies of the functional domain show that the OSCA gene family contains a calcium-dependent channel domain (DUF221) that may participate in osmotic adjustment [22, 23]. In rice, the entire OSCA gene family is characterized by the presence of a conserved DUF221 domain, which functions as an osmotic-sensing calcium channel [24]. In *Arabidopsis*, OSCA1, a hyperosmolality-gated calcium-permeable channel, was characterized as an osmosensor and mediated osmotic-stress-evoked Ca²⁺ concentration increases [20]. Studies have shown that the maize gene *ZmOSCA2.4* could enhance drought tolerance in transgenic *Arabidopsis* [25]. OSCA family members play a crucial role in plant resistance to osmotic stress. Therefore, it is important to identify and study potential genes for breeding osmotic stress-resistant varieties. Predecessors have systematically identified and analyzed the OSCA gene family in dicotyledons, including *A. thaliana* and soybean, and monocotyledon rice [20, 24, 26]. However, genome-wide analysis of the OSCA gene family has not been conducted in mung bean.

Mung bean (*Vigna radiata* (L.) R. Wilczek, 2n = 2x = 22) belongs to the papilionid subfamily of Fabaceae and is always grown in poor-soil regions because of broad adaptation ability and high tolerance to stress. Mung bean seeds are rich in protein and contain higher levels of folic acid and iron than most other legumes [27]. Completion of the mung bean genome sequence has allowed an opportunity to systematically research the OSCA gene family in mung bean [28]. In the present study, we identified putative OSCA gene family members in mung bean and analyzed their phylogeny, synthetic relationships, conserved motifs, transmembrane regions (TMs) and promoter regions containing cis-regulatory elements responsive to abiotic stress. In addition, we studied the expression profiles of OSCAs following treatment with PEG, NaCl and ABA. These findings will facilitate further research on the biological function of this gene family and provide putative gene targets for the cultivation of genetically modified osmotic stress-resistant plants.

**Results**

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

The hidden Markov model (HMM) of the 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* orthologues (Table 1). Among the 13 genes, 12 *VrOSCA* genes were distributed randomly on all 11 chromosomes except chromosomes 2, 8 and 10, while *VrOACA2.5* was located on scaffold_100. The number of amino acids of the identified *VrOACAs* varied from 592 (VrOACA2.2) to 880 (VrOACA4.1). The molecular weight (MW) of the *VrOSCA* proteins varied from 67.16 (VrOACA2.2) to 99.16 kDa (VrOACA2.5) and the isoelectric points (pI) ranged from 6.28 (VrOACA4.1) to 9.44 (VrOACA2.5).

**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, a phylogenetic tree based on the alignment of 60 full-length OSCA protein sequences was built (Additional file 1). The 60 OSCA proteins were classified into four major groups, clades 1, 2, 3, and 4. Clades 1 and 2
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 | 7          | 6435             | 775                 | 2328     | 8.91             | 88.17                  | 1     |
| VrOACA1.2 | Vradi10g01410 10 | 7          | 7609             | 773                 | 2322     | 9.22             | 88.68                  | 1     |
| VrOACA1.3 | Vradi06g03460 6 | 7          | 5939             | 760                 | 2283     | 9.08             | 87.48                  | 1     |
| VrOACA1.4 | Vradi03g00620 3 | 7          | 7957             | 640                 | 1923     | 6.68             | 72.55                  | 1     |
| VrOACA1.5 | Vradi11g08350 11 | 7          | 6738             | 863                 | 2592     | 9.06             | 98.71                  | 1     |
| VrOACA2.1 | Vradi06g14350 6 | 7          | 8460             | 721                 | 2166     | 8.53             | 81.83                  | 2     |
| VrOACA2.2 | Vradi05g11970 5 | 7          | 16,125           | 592                 | 1779     | 8.37             | 67.16                  | 2     |
| VrOACA2.3 | Vradi01g07680 1 | 7          | 11,006           | 709                 | 2130     | 8.52             | 80.50                  | 2     |
| VrOACA2.4 | Vradi04g08970 4 | 7          | 6275             | 637                 | 1914     | 6.71             | 72.90                  | 2     |
| VrOACA2.5 | Vradi0100s00520 Scaffold_100 | 5251 | 671             | 1803               | 9.03     | 67.43             | 67.95                  | 2     |
| VrOACA2.6 | Vradi05g17480 5 | 7          | 10,518           | 600                 | 2187     | 9.36             | 82.44                  | 3     |
| VrOACA2.7 | Vradi10g09440 10 | 7          | 4444             | 728                 | 2643     | 6.28             | 99.16                  | 4     |

Fig. 1 Phylogenetic tree of the OSCA gene family in mung bean, soybean, *Arabidopsis* and rice. The neighbor-joining tree was generated through the MEGA7 program using the amino acid sequences of the OSCA proteins by the neighbor-joining (NJ) method, with 1000 bootstrap replicates. The four major phylogenetic clades (1 to 4) are labelled and the OSCAs from different species are denoted by different colored backgrounds.
contained more members than clades 3 and 4 (Fig. 1). Phylogenetic analysis results showed that the OSCA gene family underwent a similar evolutionary history when comparing between the mung bean, Arabidopsis, soybean and rice genomes. Moreover, OSCA proteins derived from mung bean had a higher similarity to those from soybean (Fig. 1), demonstrating a closer phylogenetic relationship between mung bean and soybean since both belong to the Fabaceae 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 collinearity relationship of VrOSCA genes with three representative species, including one monocot (rice) and two dicots (Arabidopsis and soybean). We found that the OSCA genes of mung bean had the most homologous gene pairs with the OSCA genes of Glycine max (17), followed by Arabidopsis (7) and O. sativa (1) (Fig. 2, Additional file 2), indicating that in comparison with Arabidopsis and rice, mung bean OSCA genes show a closer phylogenetic relationship with soybean OSCA genes. This result was consistent with the phylogenetic analysis (Fig. 1), affirming the accuracy of our analysis. Some VrOSCA genes (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 (Additional file 2). These genes may play a crucial role during evolution. Meanwhile, no collinear segments of VrOSCA1.2 and VrOSCA2.2 were found in the genomes of mung bean and soybean (Additional file 2). The results indicate that large-scale expansion of OSCAs probably occur before the mung bean-soybean division, and certain VrOSCA genes might have originated from duplication of the mung bean genome after the 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 were 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 evaluate the molecular evolution. All of the Ka/Ks ratios were less than 1 (Table 2).

Conserved domain, motif and TM analyses of VrOSCA proteins
Analysis of the protein conserved domains of VrOSCAs revealed that most VrOSCAs contained three domains: late exocytosis domain (pfam13967), cytosolic domain of 10 TM putative phosphate transporter (pfam14703, DUF4463) and calcium-dependent channel domain (pfam02714, DUF221), while VrOSCA4.1 contained four domains, including two DUF221 protein domains, as shown in Fig. 4a and Additional file 3. Notably, the pfam13967 and pfam02714 protein domain are located at the N-terminus and C-terminus of all VrOSCAs, respectively, and the pfam14703 protein domain is located in the middle of pfam13967 and pfam02714 domains (Fig. 4a). These results indicate that the three domains are relatively conserved in the VrOSCA family. Meanwhile, it was discovered that the pfam13967 and

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                |
pfam02714 protein domains contained a different number of TMs, while no TMs were detected in the pfam14703 protein domain in any of the VrOSCAs (Fig. 4a, Additional file 4). All the VrOSCAs contained at least eight TMs (Fig. 4a, Additional file 4).

To further explore the potential function of VrOSCAs, we detected additional conserved motifs using the Multiple EM for Motif Elicitation (MEME) tool, and a total of 20 conserved motifs were detected (Fig. 4b, Additional file 5). Notably, all clades contained motifs 1, 2 and 4 (Fig. 4b), indicating that all genes perform the three functions. Among them, motif 1 and motif 2 were located in the calcium-dependent channel domain, and motif 4 was located in the late exocytosis domain (Fig. 4). Some conserved domains were restricted to specific clades. For example, motif 16 and motif 12 were only detected in clade 1 and clade 2, respectively (Fig. 4b), which indicated functional differences between clade 1 and clade 2. We also observed different motifs within the same clade (Fig. 4b), suggesting that there were different mechanisms of action within each clade. For example, VrOSCA1.4 in clade 1 lacked motifs 5, 14, 15, 17 and 19, whereas the other four VrOSCAs (VrOSCA1.1, 1.2, 1.3 and 1.5) in clade 1 contained these motifs.
(Fig. 4b). This phenomenon was also observed in other clades. VrOSCA4.1 in clade 4 contained the fewest motifs (Fig. 4b). The results of the conserved motif analysis were generally consistent with the phylogenetic analysis.

Expression of VrOSCA4s under ABA and abiotic stress
PEG and NaCl stress may cause similar cellular damage and lead to osmotic stress [29]. Plants adapt and respond to drought and salt stress by inducing the expression of a range of genes. ABA is an important plant hormone that regulates the expression of stress-responsive genes in plants [30]. We studied the expression profiles of the 13 VrOSCA4 genes following treatment of mung bean with ABA, PEG and NaCl for 4 h, 12 h and 24 h. Analysis of the expression profiles showed that except for VrOSCA2.1, the other 12 VrOSCA4 genes were upregulated by ABA, PEG and NaCl treatment. VrOSCA2.1 was significantly downregulated by ABA, PEG and NaCl treatment (Fig. 5, Additional file 6). The expression patterns of all the upregulated genes 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 genes were relatively higher than those of VrOSCA1.1, −1.2 and −1.3 genes under the three types of osmotic stress (Fig. 5, Additional file 6). Additionally, following ABA treatment, genes expression increased by a factor of more than 10-fold in VrOSCA1.4, −2.2, −2.3, −2.4, −2.5, −2.6 and −3.1 genes when compared to expression at 0 h of treatment. Following PEG treatment, the expression of VrOSCA1.4, −2.2, −2.4, −2.5, −2.6 and −3.1 genes increased by a factor of more than 10-fold when compared to expression at 0 h of treatment. Following NaCl treatment, the expression of VrOSCA1.4, −1.5, −2.2, −2.4, −2.5 and −3.1 genes increased by a factor of more than 10-fold when compared to expression at 0 h of treatment (Additional file 6). Among these genes, VrOSCA1.4 showed the largest fold change in relative gene expression following treatment with the three types of osmotic stress than when under the normal growth conditions (Additional file 6). These results indicate that mung bean OSCA genes respond to osmotic stress caused by ABA, PEG and NaCl treatment.

Cis-acting element analysis in the promoters 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 pathways [31]. Therefore, we analyzed the cis-acting elements involved in ABA and abiotic stress responses in the 1.5 kb promoter region of VrOSCA genes, including ABRE, DRE, MBS, TC-rich and LTR elements. We found that all the VrOSCA genes, except VrOSCA1.5, contained at least one of these cis-acting elements (Fig. 6, Additional file 7). Moreover, the cis-acting elements of the VrOSCA4s among clades were different. For example, clade 1 and clade 2 contained DRE and MBS elements associated with drought stress, but genes in clade 3 and clade 4 did not. Clade 1, clade 2 and clade 3 contained LTR elements associated with low temperature stress, while the gene in clade 4 did not (Fig. 6, Additional file 7). These results indicate that VrOSCA4 genes in different clades might respond to stress collectively. In clade 2, only VrOSCA2.2 and VrOSCA2.4 contained MBS element, only VrOSCA2.1 contained TC-rich element, and only VrOSCA2.2 contained LTR element (Fig. 6, Additional file 7). This phenomenon was also observed in clade 1. These results suggest that VrOSCA4s in the same clade may have different functions.
Discussion

Because mung bean is a broadly adapted and highly stress-tolerant crop, whole genome sequencing of mung bean is conducive to the identification of resistance genes and genetic improvement of crops. In the present study, we performed a genome-wide analysis of OSCA genes in mung bean and identified a total of 13 VrOSCA genes. The VrOSCA proteins varied substantially in sequence and physicochemical properties (Table 1), which were comparable with OSCA genes from other plant species [20, 24, 26, 32]. 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 [20, 24, 26]. Each clade included OSCA members from mung bean, Arabidopsis, soybean and rice, indicating that the OSCA family originated and diversified prior to divergence of mung bean, Arabidopsis, soybean and rice. The clade 3 and clade 4 contained fewer members but were conserved across species, indicating that OSCA members in clade 3 and clade 4 may play an indispensable role in biological processes. The different numbers of OSCAs within the mung bean, Arabidopsis, soybean and rice genomes indicate that the majority of OSCAs in the mung bean, Arabidopsis, soybean and rice genomes undergo greater genetic variation after their divergence.

Based on OSCA family member phylogenetic relationships (Fig. 1), we systematically analyzed the syntenic relationship of OSCAs in mung bean, Arabidopsis, soybean and rice (Fig. 2, Additional file 2). Large-scale expansion of OSCAs probably occurred after monocot and dicot division. Although VrOSCA2.2 and GmOSCA2.1 were clustered together (Fig. 1), VrOSCA2.2 was absent from the syntenic analysis. We did not find syntenic 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 collinearity relationship between mung bean and soybean showed that VrOSCA2.3, VrOSCA2.4 and VrOSCA2.5 had collinearity relationships with GmOSCA3, but VrOSCA2.2 did not (Additional file 2). Therefore, the duplication events of VrOSCA2.1/VrOSCA2.2 might have occurred after the divergence of 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 purifying selective pressure (Table 2). As purifying selection restricts gene divergence, the duplicated VrOSCA genes might have retained some similar functions [33]. Our results also showed that the expression patterns of VrOSCA2.3, −2.4 and −2.5 genes were similar under ABA-, PEG- and NaCl-induced osmotic stress.

Previous studies have shown that each AtOSCA protein contains 11 TMs [23, 34, 35]. In contrast, VrOSCAs contained 8–10 TMs, which indicates that VrOSCAs experienced genetic variation during evolution. To investigate the structural features of VrOSCAs, conserved domains were analyzed. The results showed that the structural domain was highly conserved (Fig. 4a) and the distribution of the pfam13967, pfam14703 and pfam02714 protein domains in VrOSCAs was consistent with that of the OSCA proteins in maize [32]. Meanwhile, all the TMs were in the pfam02714 and pfam13967 protein domains (Fig. 4a). In this study, 20
distinct conserved motifs were identified. The motifs of VrOSCA in clade 1, clade 2 and clade 3 were highly conserved and the composition patterns of the conserved motifs in these three clades were similar. However, VrOSCA1.4 in clade 4 contained fewer conserved motifs than the other VrOSCA (Fig. 4b). Moreover, the expression of VrOSCA4.1 gene increased by a factor of less than 10-fold following treatment with ABA-, PEG- and NaCl-induced osmotic stress than when under the normal growth conditions (Additional file 6), suggesting that VrOSCA4.1 may have an indirect function in the osmotic stress response [25].

In this study, the dynamic osmotic stress-responsive expression patterns of VrOSCA were analyzed. Expression profile analysis of VrOSCA 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 channel protein family, the VrOSCA genes responded to ABA-, PEG- and NaCl-induced osmotic stress, which is consistent with the response of OSCA genes in Arabidopsis and rice [24, 36]. However, VrOSCA exhibited differential expression under osmotic stress, not only among clades but also among members within the same clade, suggesting that different VrOSCA might have diverse functions. The present results showed that VrOSCA2.1 was significantly downregulated by ABA, PEG and NaCl treatment, whereas the other 12 VrOSCA genes were significantly upregulated by these three types of osmotic stress (Fig. 5, Additional file 6), suggesting that the 12 VrOSCA might be crucial mediators of the osmotic stress response and contribute to the establishment of complex signaling networks in mung bean. Upregulation of the 8 VrOSCA genes (except VrOSCA1.1, −1.2, −1.3 and −4.1) ranged from 10- to 70-fold (Additional file 6), 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 increased expression of more than 20-fold when compared to the expression levels under control conditions (0 h) (Additional file 6). Thus, VrOSCA2.2 and −2.4 may simultaneously respond to ABA, PEG and NaCl stress-response pathways, and there may be interactions in the pathways that are responsive to the three types of osmotic stress. Regardless, these genes played an important role in drought and high-salinity tolerance. Moreover, the expression of duplicated genes showed that two pairs of duplicated genes shared similar expression patterns, suggesting that the genes might have retained some essential functions during subsequent evolution. However, the duplicated genes VrOSCA2.1/VrOSCA2.2 showed divergent expression, which might have experienced functionalization after the duplication events [37]. Our work has identified genes for further characterization of their functional involvement in osmotic stress.

Analysis of promoter components of the 13 VrOSCA genes revealed 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. Only five VrOSCA genes, VrOSCA1.1, −1.2, −2.1, −1.2 and -2.6, contained DRE element (Fig. 6, Additional file 7). Furthermore, the promoters of VrOSCA genes classified in the same clade also contained different types and numbers of response elements. Therefore, different genes classified in the same clade may show functional diversity and may also have different mechanisms of action [38]. In clade 1, only VrOSCA1.4 contained MBS element associated with the drought response (Fig. 6, Additional file 7). The relative expression value of VrOSCA1.4 was significantly higher than other VrOSCA in clade 1 under PEG treatment (Additional file 6), suggesting that VrOSCA1.4 may play a more important role in the response to drought stress. In addition, the promoters of VrOSCA2.2 and −2.4 also contained MBS elements (Fig. 6). The relative expression level of VrOSCA2.2 and −2.4 increased more than 20-fold compared with the control (0 h) under PEG treatment (Additional file 6). This result indicated that genes in different clades may exhibit synergies [39]. The promoters of VrOSCA2.2 and −2.6 contained ABRE (abscisic acid responsive element) and DRE (drought, salt and cold responsive element) elements, and the relative expression levels of VrOSCA2.2 and −2.6 were more than 10-fold that of the control (0 h) under ABA, PEG and NaCl stress (Additional file 6). Therefore, the stress-inducible cis-acting regulatory elements present in the promoter play an important role in modulating the expression of genes in response to abiotic stress.

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

Methods
Identification of OSCA gene family members in the mung bean genome
The V. radiata genome database (genome assembly: Vradiata_ver6) was downloaded from EnsemblPlants (http://plants.ensembl.org/index.html). The conserved OSCA DUF221 protein domain (pfam accession
number: 02714) from the Pfam database [40] was used to build the HMM profiles (http://hmmer.janelia.org/) and query the V. radiata whole-genome protein database. Each non-redundant sequence was confirmed using the Simple Modular Architecture Research Tool (SMART) web server (http://smart.embl.de/) [41], the Conserved Domain Database (CDD) in the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi) [42] and the Pfam website (http://pfam.xfam.org/). The MW and pI of OSCA proteins were predicted with ProtParam (http://web.expasy.org/protparam/).

Conserved motifs, TMs and phylogenetic analysis
The conserved motifs and TMs of mung bean OSCA proteins were identified using the MEME program (http://meme-suite.org/meme/) and the TMHMM Server V.2.0 (www.cbs.dtu.dk/services/TMHMM/), respectively. Multiple sequence alignment was analyzed with the ClustalW program [43], and the phylogenetic tree was constructed using MEGA 7 (Molecular Evolutionary Genetics Analysis) software with the neighbor-joining (NJ) method and 1000 replicate iterations [44].

Interspecies synteny analysis and gene duplication
To analyze the relationships of orthologous OSCA genes in different species, multiple sequence alignment was used to detect the sequences of mung bean and other species with a similarity of more than 70%. Then, the Multiple Collinearity Scan toolkit (MCScanX) was adopted to analyze 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 the MCScanX program with the default parameters and plotted with Circos software [45]. The Ka (nonsynonymous substitution rate) and Ks (synonymous substitution rate) were investigated using DnaSP v5.0 software [46], and the selection pressure was calculated by the Ka/Ks ratio.

Plant materials and stress treatments
In this study, the mung bean cultivar VC1973A was used to analyze gene expression profiles following treatment with drought, salt and ABA. The seeds of cultivar VC1973A were obtained from the Chinese Academy of Agricultural Sciences. VC1973A was grown in a growth chamber at 24 °C with a photoperiod of 16 h. When the first trifoliolate leaf appeared, seedlings were treated with 20% PEG-6000, NaCl (100 mM) and ABA (100 μM) solution as described previously [47]. 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
Total RNA from the leaves was isolated using an RNA-prep Pure Plant Kit (Tiangen, Beijing, China), and first-strand cDNA was synthesized using a SuperScript™ III Reverse Transcriptase kit (Invitrogen, USA). Quantitative real-time PCR (qRT-PCR) was performed in an ABI-ViiA 7 Real-Time PCR system (Applied Biosystems, USA) with SYBR Green PCR mix (QIAGEN). PCR 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−ΔΔCT method [48]. Gene-specific primers were designed using Primer Express Software v2.0 (Additional file 8) 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. The heat map of VrOSCA gene expression was generated using TBtools (v0.6632) and was clustered hierarchically based on the expression patterns. For statistical convenience, log2 expression values were used for the expression of VrOSCAs in the heatmap.

Abiotic stress-responsive cis-regulatory element analysis in the promoter regions of VrOSCA genes
The sequences of 1.5 kb promoter regions upstream of VrOSCA genes were downloaded from EnsemblPlants (http://plantsensembl.org/index.html) (Additional file 9). The PLACE website (http://www.dna.affrc.go.jp/PLACE/?action=newplace) [49] was used to identify the putative cis-regulatory elements involved in ABA and abiotic stress responses in the promoter region.

Abbreviations
OSCA: Hyperosmolality-gated calcium-permeable channels; TM: Transmembrane regions; SMART: Simple modular architecture research tool; NCBI: National center for biotechnology information; TAIR: The arabidopsis information resource; CDD: Conserved domain database; MW: Molecular weight; pI: Isoelectric point; MEGA: Molecular evolutionary genetics analysis; MEME: Multiple EM for motif elicitation; Ka/Ks: Nonsynonymous substitution rate/synonymous substitution rate; qRT-PCR: Quantitative real-time PCR

Supplementary Information
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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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Availability of data and materials
The Arabidopsis OSCA protein sequences were collected from the Arabidopsis information source (TAIR) database (http://www.arabidopsis.org). The rice (IRGSP-1.0) were downloaded from EnsemblPlants (http://plants.ensembl.org/index.html). All the accession numbers of OSCAs were contained in the additional file 1. The genome sequences of mung bean (Vindia_rapa), soybean (Glycine_max_v2.1) and rice (IRGSP-1.0) were downloaded from EnsemblPlants (http://plants.ensembl.org/index.html). All the datasets used and analyzed during the current study are included in the published article and its additional files.

Declarations
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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