Identification of stress repressive zinc finger gene family and its expression analysis in rice under abiotic constraints

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Abstract

Background: Stress repressive zinc finger (SRZ) gene family in rice is one of the plant defense gene families that play a pivotal role in plant growth regulation and development, particularly under stressful conditions. However, there is no genome-wide survey regarding SRZ gene family in rice (OsSRZ) till date.

Results: We studied, herein, this gene family by performing a genome-wide screening and we identified 25 OsSRZ gene members using Japonica cultivar as an investigating material. Their chromosome localizations, phylogenetic relationships, genomic structures, conserved domains and promoter cis-regulatory elements were analyzed. Besides, their spatio-temporal expression profiles and expression patterns under various hormones and stress treatments were also assessed. Based on the phylogeny and domain constitution, the OsSRZ gene family was classified into five groups (I-V). Conserved domains analysis demonstrates that OsSRZ proteins contain at least one highly conserved SRZ domain. The analysis of expression patterns of the SRZ gene family reveal that OsSRZ genes display tissue-specific expression patterns at various rice developmental stages and exhibit differential responses to both phytohormones and abiotic stresses. Furthermore, q-RT-PCR analysis reveals that Os SRZ genes exhibit different expression patterns under various abiotic stresses. We notice the presence of a single specific gene considerably or strongly up-regulated for each kind of abiotic stress. Over 12 OsSRZ genes analyzed with q-RT-PCR, solely 4 genes (OsSRZ 1, 2, 10 and 11) were found to be substantially or strongly up-regulated following abiotic stress. Notably, OsSRZ 10 and 11 were up-regulated under heat stress by 7 and 5 times, respectively. However, OsSRZ2 was up-regulated by 7 and 3.5 folds under salt and cold stresses, respectively. Interestingly, OsSRZ1 was up-regulated by about 3~11 times in 24 h following artificial oxidative stress application using 1 mM H_2O_2 .

Conclusions: We deduce that some members of OsSRZ gene family function as abiotic stress marker in rice. At the genomic level and expression pattern, our genome-wide survey could provide promising and valuable insights to widen and strengthen further future investigation by leading a cutting edge research regarding the biological and molecular functions of this gene family.

1. Background

Plants are frequently subjected to various biotic and abiotic stresses during their growth and development. In order to survive, plants have evolved different resistance mechanisms in the long-term evolution process to withstand adverse environmental conditions [1]. For example, under adverse conditions, the expression levels of some genes could change, especially those of stress-related genes. Mostly, the expression changes of these genes are accompanied by the expression of other genes. Some genes are up-regulated but some others are down-regulated under stresses. A lot of genes and gene families related to stress are identified and characterized in different plant species [2–6]. Among them, some families of transcription factors including AP2/EREBP, bZIP, bHLH/MYC, MYB, HSF, WRKY, NAC and zinc finger proteins play an important role and form a complex regulatory network to relieve the effect of severe stress conditions [7–11]. Some of which, such as bZIP, WRKY, NAC form a large gene family in

plants and are widely known. In recent years, there has been more and more evidence indicate that these families are not only involved in many biotic and abiotic stresses responses, but also participate in various biological processes including seed germination, organ differentiation, flowering regulation, plant height and embryo development [6, 12–14]. However, stress repressive zinc finger (*SRZ)* gene family in plants is hardly known and less investigated.

Zinc finger protein contains a conservative zinc finger (Znf) domain, which are relatively small protein motifs that contain multiple finger-like protrusions that make tandem contacts with their target molecule [15–17]. Some of them bind zinc, but many do not; instead binding other metals such as iron or no metal at all. They were first identified as a DNA-binding motif; however, they are now recognized to bind DNA, RNA, protein or themselves [16, 18–19]. The SRZ protein contains a specific zinc finger domain like X-W-C-C-N-C-C, and X represents any amino acid [20]. More than a decade ago, a study showed that the expression of *SRZ1* was inhibited by various stresses, including water stress, salt stress, drought stress, and abscisic acid (ABA) treatment, but it was induced by salicylic acid [20]. Ectopic expression of *OsSRZ1* gene in tobacco inhibited the expression of stress-related genes such as *osmotin, NtERB10B, NtERB10C,* and reduced the cold and salt tolerance of transgenic plants [20]. *OsZFP1* (*OsSRZ2)* was induced by fungal pathogen Magnaporthe oryzae (*M. oryzae)* infection, and over-expression plants confer more resistance against *M. oryzae* [21]. OsDjA6 (Heat shock protein 40) interacts with OsSRZ2, and the expression of *WRKY45, NPR1,* and *PR5* are increased in the *OsDjA6*-RNAi rice, which increases the resistance to *M. oryzae* [22]. *SPL33* (*OsSRZ18)* encodes a eukaryotic translation elongation factor 1 alpha (eEF1A)-like protein consisting of one zinc finger domain and three EF-Tu domains [23]. Mutant *spl33* exhibits programmed cell death-mediated cell death, dwarfing and early leaf senescence [23]. Moreover, the *SPL33* loss-of-function mutant induces defense responses and enhances resistance to both *M. oryzae* and the bacterial pathogen Xanthomonas oryzae pv. oryzae by up-regulating defense-related genes [23]. A recent study showed that *LML1* encodes a conserved eukaryotic release factor 1 protein and regulates cell death and pathogen resistance by forming a conserved complex with *OsSRZ18* in rice [24].

Hence, it is very necessary and valuable to comprehend analyze the SRZ family in rice and provide a common nomenclature for this class of proteins. In this study, 25 rice *SRZ* genes (*OsSRZs)* were identified through a genome-wide survey. The gene structures, conserved SRZ zinc finger domain and phylogenetic relationship of the OsSRZs were performed. The spatio-temporal expression profiles and expression patterns of *OsSRZ* genes under various hormones treatments (ABA, GA, IAA and JA) were analyzed using Affymetrix Gene Chip microarrays. In addition, the expression analysis of the rice *SRZ* genes under multiple abiotic stresses (heat, drought and salt stress) was also assessed by RNA-seq. Real-time quantitative PCR (q-RT-PCR) was also performed to better understand the effects of abiotic stresses on the expression of *OsSRZ* gene family. To our knowledge, this is the first report focusing on the family-level identification of *OsSRZ* genes and analyzes their expression patterns. Our analysis given herein on *OsSRZ* genes at the genomic level would provide a solid basis for further functional characterization of specific genes in the family.

2. Results

2.1 Identification of rice SRZ domain-containing proteins

To characterize and identify all members of SRZ domain-containing proteins in rice using *Oryza sativa* L. japonica as an investigating material. BLASTP analysis was performed using the sequence of 4 known rice SRZ proteins (SRZ1, 2, 3 and 4). With retrieval, comparison and analysis of conservative domain, we identified 25 *SRZ* genes in rice by removing the redundant sequences. For the sake of simplicity and consistency, these genes were named as *OsSRZ1* to *OsSRZ25* according to their homology among the SRZ members in rice. The detailed information for each SRZ was presented in Table 1, including gene name, RGAP ID, chromosome location, genomic position, length of protein, molecular weights (MW), theoretical isoelectric point (Ip), and number of SRZ domain. The 25 deduced proteins possess divergent lengths, resulting in diverse Ips and MWs. The length of the protein sequences ranged from 139 (OsSRZ3) to 1000 AA (OsSRZ19). The rice SRZ genes encode proteins with predicted MWs of 15.4715 (OsSRZ3) to 110.3kDa (OsSRZ19) and theoretical Ips from 4.9166 (OsSRZ24) to 11.6554 (OsSRZ13).

2.2 Phylogenetic analysis of rice SRZ proteins

To analyze the genetic characteristics among 25 OsSRZ domain-containing protein sequences in rice, a phylogenetic analysis of the SRZ full-length AA sequences was performed by MEGA6.0 based on the NJ method. As shown in Fig. 1, all SRZ proteins were classified into five distinct groups (I-V) and displayed in the constructed phylogenetic tree based on their phylogenetic relationships. Eight *OsSRZ* gene members constitute the group V, which represents the largest group of OsSRZ domain-containing proteins, followed by group I with seven members. Both groups II and IV contain four members for each. However, a couple of OsSRZ members (OsSRZ7 and 19) belong to the group III (Fig. 1).

2.3 Chromosomal distribution of rice SRZ genes

To determine the genomic distribution of *OsSRZ* genes, they were mapped to chromosomes of the published rice genome according to their location. As depicted in Fig. 2, *SRZ* genes were distributed on 10 chromosomes over 12, with exception chromosomes 5 and 11. Among them, there were five genes (*OsSRZ3, 7, 12, 16* and *18)* on chromosome 1, four genes (*OsSRZ4, 12, 20* and *21)* on chromosome 4 and three genes were distributed on chromosomes 2, 6 and 7 and two genes on for each of chromosomes 3 and 9. A single gene was located on each of chromosomes 8, 10 and 12.

2.4 Exon-intron structure analysis of rice SRZs

To gain more insight s about *OsSRZ* gene family structure and/or function and better understand their evolutionary origin and putative functional diversification, the full-length encoding sequence of SRZ were compared with the corresponding genomic DNA sequences to determine the numbers and positions of

exons-introns within each *OsSRZ* gene using GSDS. Our results show that the number of exons-introns was different between rice SRZ members (Fig. 3). The length of the genomic sequence ranged from 1,242 bp (*OsSRZ11)* to 12,131 bp (*OsSRZ6)* and almost all members possess a sequence length less than 9 kb, except *OsSRZ6.* Most rice SRZ members contain three or more exons, except *OsSRZ1*-3, *11,* and *16.* Furthermore, these members such as *OsSRZ15, 18-19, 21,* and *24* contain the largest number of exons, where the number of exons was above 10 (Fig. 3).

2.5 SRZ domain analysis of OsSRZ protein

To explore the domain of SRZ, the sequences of full-length rice SRZ protein were deduced and their domain organization was investigated. The total 25 potential conserved sequence motifs were identified by MEME (see material and methods). To highlight, we specifically analyzed the SRZ motifs and marked its location. Our data analysis show that each SRZ member contains at least one conserved zinc finger domain (X-W-C-C-N-C-C, X stands for AA) also known as a small protein motifs consisting of 21 AAs (Fig. 4), which we named it the SRZ motif (or domain) for convenience; and its distribution location and number also showed diversity and difference (Fig. 4). Most of the members contain one SRZ motif, and a few members contain two or three SRZ motifs. We observed that six members OsSRZ9, 12, 14, 22–24 contain two SRZ motifs, and the seven members including OsSRZ1–3, 5, 8, 10 and 14 contain three SRZ motifs. Interestingly, the number of SRZ motif contained in all members of group I was not less than 3. In addition, both OsSRZ4 and 6 contain four SRZ motifs (Fig. 4).

2.6 Cis-elements in the promoter regions of *OsSRZ* genes.

The cis-elements of the promoter are involved in gene regulation by interacting with their corresponding trans-regulatory factors. Therefore, further studies on the putative cis-elements would provide valuable information for the expression of *OsSRZ* genes. So the promoter regions of 25 *OsSRZ* genes were retrieved and submitted to PlantCARE database for cis-elements identification (Tables 2 and S2). In total 31 cis-elements were identified. As expected, the conventional promoter elements (TATA-box and CAAT-box) were detected in all the *OsSRZ* promoter regions. The remaining 29 cis-acting elements can be divided into four groups. Twelve cis-elements are light responsive, including SP1, G-Box, Box 4, GAG-motif, GT1-motif, I-box, AAGAA-motif, Box I, circadian, ATCT-motif, ACE and TCT-motif. Five cis-elements are hormone responsive such as TCA-element, ABRE, CGTCA-motif, TGACG-motif and GARE-motif. Six cis-elements are well-known as stress responsive elements: HSE, TC-rich repeats, GC-motif, MBS, LTR and ARE. The fourth group contains 6 cis-element including Skn–1_motif, O2-site, GCN4_motif, CAT-box, A-box and 5UTR Py-rich stretch. Skn–1_motif and GCN4_motif are required for endosperm expression, CAT-box is required for meristem expression and O_2-site is involved in zein metabolism regulation. This suggests a potential prominent role for the *OsSRZ* gene family in the response and defense of rice seedlings against abiotic stresses such as heat, drought and salt.

2.7 Tissue-specific expression of SRZ genes in rice

To investigate the spatial and temporal expression patterns of all the identified *OsSRZ* genes, we used microarray data which were retrieved from RXP_0001 of the gene expression levels of 10 tissues from various developmental stages of rice (Table S3). The heatmap indicates that the 25 *OsSRZ* detected genes are involved in various biological processes and are expressed in the majority of tissues, but their specific individual expression levels were diverse and differentially (Fig. 5).

As shown in Fig. 5, most of the *OsSRZ* members show low expression in roots, leaves and leaf sheaths, and high expression in embryo and endosperm. A similar expression pattern was observed for 10 *OsSRZ* genes (*OsSRZ5, 10-11, 14-15, 18-20* and *22-23),*, those show relatively higher expression levels in embryos, but lower expression in roots, leaves and leaf sheaths (Fig. 5). With a slight difference to the precedent 10 genes, *OsSRZ3-4, 12* and *21* display high expressions in leaves, but low in endosperm. *OsSRZ1* and *17* were constitutively expressed to a high level in inflorescence, pistil and embryos, but with low transcript abundance in leaves, root and leaf sheaths. The other *OsSRZ* genes were specifically expressed in one or several organs, such as *OsSRZ5-7, 9* and *13.* Interestingly, two members (*OsSRZ2* and *3)* were highly expressed in leaf sheaths and uniquely *OsSRZ25* was highly expressed in root and embryos.

2.8 Expression of *OsSRZ* genes in response to plant hormones

Plant hormone plays a major role in plant growth and development and in response to abiotic adversity. To examine the effects of various hormones on the expression of rice SRZ gene family, microarray data from RXP_1006, RXP_1007, RXP_1008 and RXP_1012 was used to analyze the transcriptional levels of all the SRZ genes under four hormone treatments (Table S4). The heat map shows the expression levels of 25 *OsSRZ* genes under ABA, GA, IAA and JA treatments (Fig. 6).

Under 50 μM ABA treatment (Fig. 6), eight *SRZ* genes (*OsSRZ1, 10, 15-16, 20–22* and *24)* were induced at 6 h of treatment, while six other members (*OsSRZ2, 5-6, 8, 12* and *17)* were down-regulated. The changes in five genes (*OsSRZ9, 11, 13, 19* and *25)* expression levels were mild within 12 h of ABA treatment. However, a single gene (*OsSRZ23)* was severely inhibited after 12 h of ABA application.

Following the application of 10 μM GA (Fig. 6), the changes of most *SRZ* genes expressions were not obvious, and a few genes were induced. *OsSRZ2-3, 17* and *25* were up-regulated at 6 h, subsequently *OsSRZ2–3* and *17* were down-regulated at 12 h. Only two genes (*OsSRZ8* and *21)* were inhibited at 12 h of GA hormone application.

Upon 10 μM IAA treatment (Fig. 6), similarly eight *OsSRZ* genes (*OsSRZ1, 10, 13-15, 20-22)* were up-regulated after 6 h of IAA treatment; however, six members (*OsSRZ2-3, 5, 7-8* and *17)* were considerably down-regulated. Nevertheless, there was no significant change in the expression levels of *OsSRZ4, 6, 9, 11, 19, 23* and *25.*

When treated with 100 μM Jasmonic acid JA (Fig. 6), the expression of a large number of *OsSRZ* genes (*OsSRZ1, 3, 13, 15-16, 20-22* and *24)* were rapidly enhanced, and some of them reached a peak after solely 6 h of treatment and they were maintained at a high level for the remaining time of experiment (18 h). In contrast, the expression of six genes (*OsSRZ2, 5, 8, 12, 17,* and *25)* were severely inhibited within 6 h in the presence of 100 μM JA and were maintained at a low level for the supplementary 6 other hours (total 12 h). In addition, the expression changes of six genes (*OsSRZ4, 6, 9, 11, 14* and *19)* were moderately changed within 12 h under JA treatment (Fig. 6).

2.9 Expression profiles and differential gene expression (DGE) of OsSRZ genes family under various abiotic stresses

2.9.1 Heat stress

In this section, we aimed to understand whether these *OsSRZ* genes may play any crucial role in the protection against abiotic stress in rice. To unravel the relative expression levels and better distinguish the functions of *SRZ* gene family and their potential implications in various abiotic stresses, we investigated their expression profiles under heat, drought and salt (Table S5).

Following temperature treatment of 45 °C (Fig. 7), the analysis of expression data show that three genes (*OsSRZ10, 13* and *16)* were up-regulated in just 1 h of heat treatment, but they were down-regulated later as measured after 12 h exposure of two-week old rice seedlings to heat stress. Two members (*OsSRZ20* and *24)* were up-regulated after 3 h exposure to 45 °C and were down-regulated after 6 h (Fig. 7a). A similar expression pattern was observed where three *OsSRZ* gene members (*OsSRZ1, 2* and *3)* were decrease in their expression levels within 24 h exposure to 45 °C, especially strongly inhibited at 12 or 24 h (Fig. 7a). Six genes (*OsSRZ5-6, 11, 14, 18* and *24* displayed a decrease in their expressions levels either after 1 or 3 h subjection to temperatures of 45 °C (Fig. 7a). Furthermore, the changes in the expression for the following *OsSRZ* genes (*OsSRZ4, 9, 15, 17, 19-20* and *23)* were mild and maintained at a low level (Fig. 7a).

Furthermore, we assessed the expression levels of 12 randomly selected genes from the five groups (I-V) to quantitatively determine their potential involvement in the thermotolerance process in rice following exposure to high temperature treatment of 45 °C (Fig. 7b) and very likely in other kinds of abiotic stress such as drought, salt, cold and oxidative. Among them, seven *OsSRZ* genes (*OsSRZ1, 4, 6, 8, 9-11)* were interestingly induced within 6 h heat treatment, *OsSRZ8, 10* and *11* reached their peaks in either 12 or 24 h; conversely, *OsSRZ4* and *6* were down-regulated during this time (12 and 24 h). However, four *OsSRZ* genes (*OsSRZ2, 3, 7* and *12)* were drastically lost their activity with increasing the time of heat stress treatment from 1 to 24 h (Fig. 7b).

2.9.2 Drought stress

After application of 20% PEG at the root level in the hydroponic solution to exert drought stress for two-week old rice seedlings, the analysis of expression data show that most of the *OsSRZ* genes exhibited reduction in their transcripts during the first 1 h of drought (Fig. S1a), but were subsequently up-regulated after 3 and 6 h (Fig. S1a). Hence, six genes (OsSRZ*2, 5-6, 8, 12* and *17)* were down-regulated within 1 h of PEG application, and they highly expressed following 6 h of drought. For rice seedlings endured 12 h drought stress, most of the *OsSRZ* genes, especially *OsSRZ3, 5, 10, 13* and *15* displayed a decline in their expression levels, except *OsSRZ8* (Fig. S1a). Notably, only two genes (*OsSRZ2* and *15)* were significantly inhibited after 24 h of treatment with 20% PEG (Fig. S1a). Besides, another gene (*OsSRZ16)* behaves differently and shows a high expression level within 1 h of drought but had a reduced transcription level for the control (0h) and after 3 h of drought treatment.

When rice seedlings were subjected to 20% PEG supplied in their hydroponic culture medium, most *OsSRZ* genes were predominantly, down-regulated within 1 h of drought, except for OsSRZ1 and 3. The *OsSRZ3* gene was downregulated at 3 h, and then up-regulated to reach its peak at 6 h of drought, then strongly down-regulated back at 12 h (Fig. S1b). Strikingly, the expression of *OsSRZ12* was severely inhibited with initiating the drought treatment and then showed a low expression level thereafter (Fig. S1b). In addition, four genes (*OsSRZ2, 6, 10-11)* display the maximum of their expression levels at the middle of the stress period (12 h) therefore they were dramatically inhibited by the end of the drought stress treatment (24 h).

2.9.3 Salt stress

Following application of 200 mM NaCl (*Fig. S2a)*,, the analysis of transcript expression data showed that most of the *OsSRZ* genes were mostly induced by NaCl treatment within the starting 6 h; however, they were down-regulated at 12 h of salt exposure. Three genes (*OsSRZ10, 13* and *16)* were strongly expressed within 1 h of salt application, and they were down-regulated later after 24 h. In contrast, *OsSRZ2, OsSRZ8* and *OsSRZ14* were moderately expressed within 1 h, and they were further up-regulated at 6 and 24 h. Four genes (*OsSRZ5, 8, 12* and *17)* were significantly induced after 24 h of NaCl treatment and were maintained at a high level. At 3 h, *OsSRZ2* and *16* were maximally expressed and reached their peak (Fig. S2a). In addition, the expression levels of seven *OsSRZ* genes (*OsSRZ4, 6-7, 9, 15, 19,* and *23)* did not significantly change and they were slightly induced at 1 h of NaCl treatment and subsequently maintained at a relatively stable expression level for the other 23 h lasting of NaCl treatment.

When rice seedlings are challenged with 200 mM NaCl, two *OsSRZ* genes (*OsSRZ2* and *11)* were obviously and strongly up-regulated after 12 h of salt stress, but *OsSRZ11* was down-regulated during the last stage of salt (24 h) (Fig. S2b). *OsSRZ3* and *12* were severely suppressed at 6 h and maintained at a low level during the next 18 h of salt duration. Four genes (*OsSRZ5,7–9)* showed a similar expression pattern (Fig. S2b). Notably, they were up-regulated after exposure for just 1 h to 200 mM NaCl; however, they were down-regulated 2 h latter (total 3 h of salt) and remained thereafter with low transcript abundance (Fig. S2b).

2.9.4 Cold and oxidative stresses

To widen and strengthen our investigation and gain more insights about the dynamics of the *OsSRZ* genes family under different abiotic stresses and the changes in their expression levels in rice, we performed uniquely qRT-PCR without heatmap analysis for 12 *OsSRZ* genes randomly selected from the 5 groups (I-V) previously identified, under cold and oxidative stress.

Accordingly, during cold stress (Fig. S3), most *SRZ* genes were down-regulated from 1 to 24 h except *OsSRZ2,* which was obviously up-regulated after 6 h treatment of two-week old rice seedlings at 4 °C, then maintained at a high level at 12 and 24 h (Fig. S3). This suggests very likely that the *OsSRZ2* could responsible for the cold tolerance and defense against cold hardening in rice at earlier stage of growth (two-week old seedling). Paradoxically, *OsSRZ7* and *12* were inhibited following just 1 h exposure to low temperature (4 °C) and showed the lowest transcript abundance at 24 h (Fig. S3).

In the same regard, once two-week old rice seedlings were challenged with 1 mM H_2O_2 (Fig. S4) most of the *OsSRZ* genes were considerably induced during the three first hours of oxidative stress (1 and 3 h), especially *OsSRZ1, 8* and *10* (Fig. S4). It is obvious that *OsSRZ1* was strikingly induced after just 1 h exposure to oxidative stress (Fig. S4) and reached its peak after 6 h of oxidative stress, then maintained at a high level after 24 h (Fig. S4). This strongly suggests that this gene is a potential candidate for the resistance against oxidative stress and may potentially help scavenging reactive oxygen species (ROS) to abolish their deleterious effects on the living cells, at least, for young rice plants studied herein. Conversely, *OsSRZ12* was dramatically inhibited at the beginning of cold (1 h), and then was maintained at a low level for the 23 h of low temperature treatment (Fig. S4). A similar expression pattern was observed between *OsSRZ2-3,* which were up-regulated at the beginning of stress as evaluated at 3 h and 6 h (Fig. S4), but they were down-regulated later as assessed at 12 h and showed their lowest expression levels at the end of experiment that lasted 24 h (Fig. S4).

In compilation, we evaluated the percentages of either up-regulated (Fig. 8; two upper pie charts A and B) or down regulated (Fig. 8; two bottom pie charts C and D). *OsSRZ* genes in two-week old rice seedlings endured various abiotic stresses for 12 or 24 h. The percentages were assessed based on the gene expression levels of 12 randomly selected genes from the 5 previously identified groups (I-V). As shown in Fig. 8, most of the up-regulated *OsSRZ* genes are implicated in oxidative stress. Hence, 25 and 50% genes were up-regulated at 12 and 24 h of oxidative stress, respectively (Fig. 8; upper two panels A and B). A new aspect of this study is that, except cold stress, the 12 investigated *OsSRZ* genes were almost evenly shared in controlling the various applied abiotic stresses for 12 h (Fig. 8; upper left pie chart A). The same was also observed for down regulated genes (Fig. 8; bottom left pie chart C). Except for cold stress, we recorded only 5% up-regulated genes versus 28% were down regulated following 12 h cold stress treatment (Fig. 8; upper and bottom left pie charts A and C). However, after 24 h of stress applied on two-week old rice seedlings, a new reshuffling and/or redistribution of the *OsSRZ* gene family members between the various kinds of stress was observed (Fig. 8; upper and bottom right pie charts B and D). Hence, we recorded an increase in the *OsSRZ* genes number controlling heat and oxidative stress (Fig. 8

upper right pie chart). We notice an increase by 8% for heat and a doubled genes percentage (2 * 25% = 50%) for oxidative stress and the complete disappearance of up-regulated genes in response to drought after 24 h. This might be explained by the fact that after a single day (24 h) of challenging with 20% PEG, rice seedlings could acquire adaptability to drought through adjusting some metabolic and very likely photosynthetic processes such as stomatal closure and/or decreased transpiration rate to acclimate the artificial drought stress provoked by PEG application. In this case, the OsSRZ did not sense stress situation to be up-regulated as observed earlier at 12 h of drought (25% of genes were up-regulated, Fig. 8A).

However, the downregulated *OsSRZ* genes following heat and oxidative stresses were considerably smaller than those up-regulated following the same two kinds of stress (Fig. 8; compare upper and bottom right pie charts). Notably, the percentage of *OsSRZ* genes down-regulated following heat and oxidative stresses was 17 and 7%, respectively (Fig. 8; bottom right pie chart). The reverse was observed for cold stress, where we notice an up-regulation of 5% and down regulation of 26% of the 12 *OsSRZ* genes used in our screening using qRT-PCR analysis. Ultimately, it seems that the *OsSRZ* gene family plays crucial role in response to oxidative and heat stress as reflected by the dynamics of these genes under these two kinds of stress. On light of our results, this *OsSRZ* genes family appears less involved in salt stress and with less impact on drought and cold stresses.

Our gene expression results summarized in Fig. 9 display the dynamics of this *SRZ* gene family under abiotic stresses. We aimed from the schematic diagram to highlight the most important *OsSRZ* genes involved in a such kind of abiotic stress studied herein. Interestingly, we notice the presence of a single specific gene considerably or strongly up-regulated for each kind of abiotic stress. This suggests that this gene could function as an indicator or marker of that abiotic stress under which it is found to be substantially up-regulated among the *OsSRZ* genes family. Over 12 *OsSRZ* genes analyzed with q-RT-PCR, 4 genes only (*OsSRZ* 1, 2, 10 and 11) were found to be substantially and/or strongly up-regulated following abiotic stressful situation. Hence, genes *OsSRZ* 10 and 11 were up-regulated under heat stress by 7 and 5 times, respectively (Fig. 7B). However, *OsSRZ2* was up-regulated by 7 and 3.5 folds under salt and cold stresses as displayed in histograms of Figs. S2B and S3, respectively. Interestingly, *OsSRZ1* was up-regulated by about 3~11 times in 24 h following artificial oxidative stress application using 1 mM H_2O_2 (Fig. S4).

Eventually, our investigation shows that an active and diversified dynamics of *OsSRZ* genes family under abiotic stress in rice seedlings. It seems also that this genes family functions more than as stress markers and it could be involved in other metabolic and molecular processes. Thus, it needs to be more and deeply investigated to obviously unravel the biological function(s) of each gene member of the *OsSRZ* gene family.

3. Discussion

Rice represents the staple food for more than half of the global population and is one of the most important crops worldwide actually. A rise in temperature up to 5 °C by the end of this century as predicted by the International Panel for Climate Change and an increase in the frequency and severity of extreme weather events [25], could seriously affect crop yield, quality, stability of food supply and international food security [26–37]. Thus, increasing the crops resistance to severe environmental conditions would constitute a positive and effective precaution against the risks that could be caused by the above weather and climate changes predictions. The SRZ family is a large family in plant and plays an important role in response to adversities [20, 22–23].

The abundance and availability of biological information resources has facilitated the discovery and identification of gene families, which is of great significance for the excavation of potential functional genes [28–29]. In this study, 25 rice SRZ genes were identified and investigated at the genomic level and their expression patterns were analyzed under different abiotic stresses and phytohormones treatments. Compared to some other large gene families such as WRKY, NAC, bZIP, etc., with relatively high genes members 102, 151 and 89 in the rice, respectively [30–32]. The number of SRZ family members is much less (only 25 genes). Our current study shows that each gene member among the 25 rice SRZ proteins contains at least one conserved zinc finger domains consisting of 21 AA. This conserved domain is called SRZ domain for convenience (Fig. 4A). However, it was observed that among the 25 genes the putative amino acid sequence similarities are not high except for the SRZ domain and their exon/intron structures are classified into diverse types (Fig. 4B). The length of the amino acid span was also very large, ranging from 139 (OsSRZ3) to 1000 (OsSRZ19). Interestingly, OsSRZ3 contained three SRZ domains, while OsSRZ19 contained only one. Similarly, OsSRZ1 (173 AA) and OsSRZ2 (145 AA) possess an AA sequence length smaller than 200 and contain three SRZ domains, while OsSRZ15 (682 AA), OsSR18 (655 AA) and OsSR21 (656 AA) own a genomic sequences length more than 650 AA, but contain only one SRZ domain (Fig. 4). These data corroborate that this SRZ genes family exhibits diversity in evolution and function as previously reflected in our results (Figs. 1 and 2).

Both OsSRZ18 and LML1 are involved in the regulation of programmed cell death and they interact between each other. Their deletion mutation causes dwarfing and premature senescence in rice [23–24]. This suggests a prominent role for the SRZ family in the regulation of growth and development in rice plants [23–24]. However, the expression profiles of rice SRZ genes family (*OsSRZ)* have not yet been previously demonstrated. In our present study, the transcript profiles of 25 *OsSRZ* genes were locally investigated in different organs of rice plant (Fig. 5). Most genes members have shown low expression in roots and sheaths, high expression in embryos and endosperms, and some members including *OsSRZ2-4, 12* and *21* were highly expressed in leaves (Fig. 5). Interestingly, it is worthy to note that none of the highly expressed genes in leaves belongs to the third (III) group. In addition, only two genes (*OsSRZ13* and *25)* were highly expressed in the root and solely *OsSRZ25* was highly expressed in the ovary. Similarly, the highly expressed genes in the leaf sheath were only *OsSRZ2* and *3,* both of them belong to the first group. These *OsSRZ* genes family exhibited abundant expression characteristics in different organs. This strongly suggests their potential and differential biological functions in rice seedlings growth and development processes.

Plant hormones were previously reported to bear main regulators of growth and development and nowadays they are widely involved in adversity responses [33]. Our above investigation shows that most of the 25 *OsSRZ* members were either inhibited or induced to different levels under various hormonal treatments (ABA, GA, IAA and JA; Fig. 6), and most of them harbor hormone-responsive cis-elements in their promoters. This predicts that the *OsSRZ* genes may have distinct roles in different phytohormone physiological and molecular processes. Typically, ABA seems responsible for plant defense against abiotic stresses such as heat, drought, salinity, cold, and wounding which are known to trigger an enhancement in ABA accumulation levels and signaling pathways [34–35]. Notably, *OsSRZ1, 10, 15-16, 20-22* and *24* were substantially induced by ABA, but *OsSRZ2, 5-6, 8, 12* and *17* were down-regulated (Fig. 6). This reveals that these two gene groups play very likely a diametrically opposite role in ABA signaling pathway. However, JA and SA play major roles in plant resistance and protection against pathogen infection through increasing their levels in the plant organs [36]. Under JA treatment, most of OsSRZ members were significantly induced or inhibited and suggests that the *OsSRZ* genes family could mainly rely on the JA pathway to strengthen the plant resistance to pathogens. OsDjA6 (Heat shock protein 40) interacts with OsSRZ2, and the OsDjA6-RNAi rice has enhanced resistance to the blast fungus *Magnaporthe oryzae* [22]. Consistently, this indicates that *SRZ2* may potentially constitute a negative regulator for *M. oryzae* infection. Confusingly, *OsSRZ2* was also induced by *M. oryzae* infection, and its over-expressed rice plants enhance and strengthen resistance against blast fungus *M. oryzae* [21]. Our actual study, *OsSRZ2* was significantly inhibited by JA treatment (Fig. 6), suggesting that it may act as a negative regulator in JA signaling pathways to modulate plants response to pathogen infections. Similarly, *OsSRZ18* was also inhibited by the same JA treatment (Fig. 6) and the *OsSRZ18* defective (loss-of-function) mutant induces defense responses and enhances resistance to both fungal pathogen *M.oryzae* and bacterial pathogen *Xanthomonas oryzae* pv. Oryzae [23]. Other than *OsSRZ2* and *OsSRZ18,* some *OsSRZ* members including *OsSRZ5, 8, 12, 17* and *25* were also inhibited, while *OsSRZ1, 3, 13, 15-16, 20-22* and *24* were induced by JA treatment (Fig. 6). This indicates that these *OsSRZ* members possess and/or exert contrasting effects on the JA signaling pathway; some act as negative and others as positive regulators in rice. Conversely, the changes in the expression level of *OsSRZ* genes were not obvious following treatments with GA and IAA, except for few members (Fig. 6).

Various biotic and abiotic stresses, including high temperatures, drought, salinity, and pathogens could trigger a number of physiological and molecular responses to adapt severe situations and activate appropriate mechanisms to withstand adverse environmental stimuli (biotic and/or abiotic). Herein, we demonstrate that each gene member among the 25 *OsSRZ* genes possesses at least one of the stress response cis-elements (HSE, TC-rich, LTR, MBS and ARE) in their promoter regions. This signifies their potential crucial roles in stress response for rice seedlings. Under heat treatment, five genes (*OsSRZ1, 8-11)* were up-regulated at different time periods and all of them contained HSE elements in their promoters. Interestingly, it was found that the promoter regions of genes *OsSRZ3* and *12* did not contain HSE elements, and their expressions were significantly inhibited upon heat stress (Fig. 7). Among the twelve *OsSRZ* genes investigated with q-RT-PCR, only *OsSRZ2* was significantly induced at low temperatures, 4 °C (Fig. S3), although its promoter region does not contain LTR elements. This suggests

that SRZ family could be involved in hardening cold as a negative regulator. Similarly, most SRZ genes showed a decline in their expression levels following 1 and 3 h under drought stress, except *OsSRZ3, 10-11* (Fig. S1). Nevertheless, *OsSRZ2* was significantly induced following salt stress treatment and showed a high expression level, suggesting its important role in response to salt stress. Particularly, a large proportion of SRZ genes were up-regulated upon oxidative stress at 1 and 3 h, especially *OsSRZ1, 8* and *9* (Fig. S4). In addition, we found that *OsSRZ12* was always suppressed by heat, cold, drought, salt or oxidative stress and the expressions of these twelve genes in rice were down-regulated by at least one type of abiotic stress. Under different abiotic stresses, the rice SRZ family exhibited a diverse expression pattern and particular dynamics (Fig. 8). This suggesting that this gens family possesses complex mechanisms in response to abiotic stress.

In this study, a comprehensive bioinformatics and genomic investigation and analysis for the 25 *OsSRZ* genes were conducted, including gene structure, conserved *OsSRZ* zinc finger domain, phylogenetic and cis-element prediction. The transcripts of some *OsSRZ* genes could be either induced or inhibited by hormones, abiotic stresses, or differentially expressed in different organs. This strongly indicates that these *OsSRZ* members may possess various physiological and molecular functions in rice. Altogether, our findings herein would establish a solid background for further functional characterization of *OsSRZ* genes, which might provide valuable information for further investigations through molecular and computational analysis using bioinformatics tool to better and deeply understand the biological function of this gene family.

4. Conclusions

25 rice *SRZ* genes were identified through a genome-wide analysis; all of them contained conserved SRZ-motifs and were classified into five groups. *SRZ* genes exhibited different expression patterns under plant hormones and abiotic stresses and their expressions were significantly inhibited by at least one type of abiotic stresses. A new aspect of this study is that it represents the first report focusing on the family-level identification of the *OsSRZ* genes and analyzes their expression patterns in rice. This would provide a solid basis for further functional characterization of specific genes in this genes family and may help identifying some promising genes among them responsible for such physiological traits, which may enhance the adaptability and resistance of rice crops to severe environmental conditions (abiotic and biotic) and/or could be used in future breeding programs for enhancing rice yield.

5. Material And Methods

5.1 Plant material and stress treatment

Oryza sativa L. Nipponbare seedlings (IRGC Acc. No. 136196) were obtained from International Rice Research Institute, grown in greenhouse at 28 °C under a 14/10 h day/ night photoperiod. Chao Zhang, listed in the authorship of current study undertook the formal identification of the rice material used here.

Voucher specimen of this material has not been deposited in any publicly available herbarium. 2-week-old seedlings were treated by various abiotic stresses as described by Byun [37].

For heat stress and cold stress, seedlings were incubated at 45 °C and 4 °C, respectively [21, 37]; for drought, salt and oxidative stress, plants were transferred to hydroponic solution containing 20% PEG (polyethylene glycol), 200 mM NaCl (sodium chloride), and 1 mM H_2O_2 (hydrogen peroxide), respectively [38]. In all cases, untreated seedlings were used as controls. The seedlings were sampled respectively at different time intervals after treatments. Shoots (including stem and leaf tissue) were harvested at 0, 1, 3, 6, 12 and 24 h after heat, salt and drought, respectively. Harvested shoots were quickly frozen in liquid nitrogen and stored at −80 °C for further analysis.

Plant hormone treatments were applied on two-week old rice seedlings for different time periods ranging from 0 to 12 h with different concentrations. The phytohormones used concentrations herein were as follows: 50 μM abscisic acid (ABA), 10 μM gibberellin acid (GA), 10 μM of indole–3-acetic acid (IAA) and 100 μM Jasmonic acid (JA).

5.2 Identification of SRZ domain-containing proteins in rice

We used 4 known rice SRZ sequences [20] as queries to perform BLAST searches against EnsemblPlants database (http://plants.ensembl.org/index.html/) and NCBI database (https://www.ncbi.nlm.nih.gov/) to identify potential members of the SRZ gene family in rice. Next, the Conserved Domain Database (CDD) [39] and MEME Suite version (http://meme-suite.org/) [40] was used to confirm whether the returned sequences from such searches encode zinc finger domain.

5.3 Chromosomal localization and phylogenetic analyses of OsSRZ family

We used MapChart [41], to draw and annotated the SRZ genes on chromosomes according to the physical positions of the *SRZ* genes. We used EnsemblPlants to perform multiple sequence alignments of full-length protein sequences and used MEGA6.0 [42], to carry out phylogenetic analyses of the SRZ proteins based on amino acid sequences using the neighbor joining (NJ) method. The phylogenetic trees were constructed using NJ methods with p-distance methods, pairwise deletion of gaps, default assumptions and support for each node was tested with 1000 bootstrap replicates.

5.4 Gene structure and motif organization analysis of OsSRZ family

Based on genomic sequence and CDS sequence information, exon-intron substructure map was constructed by the Gene Structure Display Server (GSDS2.0), web-based bioinformatics tool (http://gsds.cbi.pku.edu.cn/) [43]. Conserved motifs of SRZ proteins were analyzed using MEME Suite version with the parameters set as follows: minimum width of 8, maximum width of 45, the maximum

number of motifs was set to 10 and the number of repetitions is not limited. All other parameters were set at default.

5.5 Cis-Element prediction for *OsSRZ* gene promoter

To identify the putative cis-regulatory elements that exist in the *OsSRZ* genes, the genomic sequences, with 2000 bp upstream from the translational start codons, were used to search in the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

5.6 Publicly available microarray data analyses and RNA-seq

Microarray-based spatio-temporal expression profiles of *OsSRZs* in various tissues and organs, at different developmental stages as well as under hormone treatment were analysed using publicly available microarray. The data of expression of SRZ family in rice were retrieved from RXP_0001, RXP_1006, RXP_1007, RXP_1008 and RXP_1012 on Rice Expression Profile Database (http://ricexpro.dna.affrc.go.jp/). The data of expression of *SRZ* gene family in response to different abiotic stresses was performed by RNA-seq and it has been uploaded to the database SRA (SRP190858) of NCBI. Filtering of the reads were done using new generation sequencing (NGS) toolkit, and normalized using the FPKM method. The heatmaps were generated using MultiExperiment Viewer (MeV) 4.6.

5.7 RNA extraction and Real-time quantitative PCR

Total RNA was extracted from the materials mentioned above using TRIzol reagent kit (Invitrogen, Carlsbad, CA, US) according to the manufacturer's specification. The RNA was sequentially treated with DNase I (Promega,WI, USA) at 37°C for 15 min in order to remove the trace remaining of genomic DNA. The concentration of RNA was determined using a NanoDrop 2000 micro volume spectrophotometers (Thermo Scientific, USA), and the integrity was estimated using agarose gel electrophoresis stained with ethidium bromide. According to the transcripts sequences of *OsSRZ* genes in Nipponbare, the primer pairs (*Table S1)* used for real-time quantitative PCR (RT-qPCR) were designed using Primer 5.0 design tool and synthesized by Tsingke (Tsingke Biotech. Corp.). The amplified fragment lengths were between 80 bp and 250 bp, and selected the annealing temperature between 55 °C and 60 °C. The 18S rRNA was used as the reference gene. Real-time PCR was conducted using LightCycler 480 Real-time PCR Instrument (Roche, Swiss) with 10 μl PCR reaction mixture that included 0.5 μl of cDNA, 5 μl of 2 × LightCycler 480 SYBR Green I Master (Roche, Swiss), 0.3 μl of forward primer, 0.3 μl of reverse primer and 3.9 μl of nuclease-free water. Reaction procedure were incubated in a 96-well optical plate (Roche, Swiss) at 95 °C for 10 min, followed by 45 cycles of 95 °C for 10 s, 58 °C for 30 s. Three technical replicates were performed for each sample. The expression values of *OsSRZ* genes tested were normalized with the internal reference gene, and the relative expression levels in seeding and in response to various stress were calculated with $2^{-\Delta CT}$ and $2^{-\Delta\Delta CT}$ methods as described previously [44].

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this article, and its supplementary information files. The funding agencies played no role in the design of the study, data collection, analysis, and interpretation or writing the manuscript.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

CZ, YNT, BHF and WPW conceived and designed the experiments; CZ, YNT, NL, MQ and ZXH performed the experiments; CZ, YNT, NL JE and QSX analyzed the data; BHF, QSX and WPW contributed reagents/materials/analysis tools; CZ, JE, MQ and YNT wrote the paper; BHF, JE and WPW revised and approved the final version of the paper. All authors read and approved the final manuscript.

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Supplementary Files Legend

Additional file 1:

Fig. S1 Expression profiles of OsSRZ genes in response to drought stress. A: Heat-map determined from RNAseq data shows the hierarchical clustering of the relative expression of 23 *OsSRZ* genes (OSSRZ21 and *OsSRZ25* were not detected) in response to 20% PEG in the solution medium. Log2 ratios of expression were used to generate the heat map. Microarray data under the series accession number SRP190858 was obtained from the SRA Database of NCBI. Red indicates high expression, while green signifies low expression under drought stress. *B:* Expression levels of 12 genes checked with qRT-PCR at different time intervals ranging from 0 to 24h. Each bar data is the average of 3 biological replicates (±SE).

Additional file 2:

Fig. S2 Expression profiles of OsSRZ genes in response to salt stress. A: Heat-map determined from RNAseq data shows the hierarchical clustering of the relative expression of 23 OsSRZ genes (OSSRZ21 and *OsSRZ25* were not detected) in response to 200 mM NaCl. Log2 ratios of expression were used to create the heat map. Microarray data under the series accession number SRP190858 was obtained from the SRA Database of NCBI. Red indicates high expression, while green signifies low expression following salt stress treatment. *B:* Expression levels of 12 genes checked with qRT-PCR at different time intervals ranging from 0 to 24h. Each bar data is the average of 3 biological replicates (±SE).

Additional file 3:

Fig. S3 Expression profiles of 12 rice SRZ genes in response to cold stress. Gene expression levels of OsSRZ genes detected with q-RT-PCR using two-week old rice seedling endured low temperature stress (4 °C) for different time periods ranging from 0 to 24 h. q-RT-PCR data were analyzed using the $2^{\triangle\triangle Ct}$ method. Each bar data is the average of three biological replicates

Additional file 4:

Fig. S4 Expression profiles of 12 rice SRZ genes in response to oxidative stress. Gene expression levels of *OsSRZ* genes detected using q-RT-PCR following application of 1 mM H_2O_2 in the medium solution of two-week old rice seedlings for different durations ranging from 0 to 24 h. q-RT-PCR data were analyzed using the $2^{\triangle\triangle Ct}$ method. Each bar data is the average of three biological replicates.

Additional file 5:

Table S1. Primers sequence of SRZ gene in rice (OsSRZ)

Additional file 6:

Table S2. Cis-Element prediction for SRZ gene (OsSRZ) promoter

Additional file 7:

Table S3. The data of tissue-specific expression of SRZ genes in rice (OsSRZ)

Additional file 8:

Table S4. Expression data of rice SRZ genes (OsSRZ) under different plant hormone treatment

Additional file 9:

Table S5. Expression data of rice SRZ genes (OsSRZ) in response to different abiotic stresses

Tables

Table 1 List of SRZ genes in rice (OsSRZ).

Gene name	RGAP LOC_	Chr	Genomic position	Protein length	Protein weight	Protein pI	Number of SRZ domain
OsSRZ1	LOC_Os02g10920	2	5798886-5806501	173	18383	8.2993	3
OsSRZ2	LOC_Os06g04920	6	2162622-2169683	145	15945	8.5559	3
OsSRZ3	LOC_Os01g37460	1	20935789-20937436	139	15471.5	8.2712	3
OsSRZ4	LOC_Os04g02000	4	617514-622710	343	36217.5	8.8093	4
OsSRZ5	LOC_Os03g50120	3	28588615-28593641	504	55748.7	8.1441	3
OsSRZ6	LOC_Os07g22024	7	12313548-12325679	647	73937.5	6.4265	4
OsSRZ7	LOC_Os01g07070	1	3322774-3328256	500	47499	8.7025	1
OsSRZ8	LOC_Os03g50430	3	28774588-28777241	523	59258.1	8.4863	3
OsSRZ9	LOC_Os07g19560	7	11592341-11596865	394	43291.1	8.1479	2
OsSRZ10	LOC_Os08g41010	8	25937086-25942445	347	37422	9.0033	3
OsSRZ11	LOC_Os06g22700	6	13201039-13202281	318	35714.1	10.6324	1
OsSRZ12	LOC_Os01g59980	1	34678036-34682189	950	103570	7.0996	2
OsSRZ13	LOC_Os02g17470	2	10054334-10056434	471	53994.7	11.6554	1
OsSRZ14	LOC_Os07g30820	7	18241609-18246904	288	30221.8	8.9509	3
OsSRZ15	LOC_Os04g58140	4	34626345-34631340	682	74861.5	8.6679	1
OsSRZ16	LOC_Os01g18100	1	10137215-10140159	350	38055.9	9.8306	1
OsSRZ17	LOC_Os12g22620	12	12780442-12784337	498	54871.2	8.2295	2
OsSRZ18	LOC_Os01g02720	1	929883-934914	655	71259.9	6.0925	1
OsSRZ19	LOC_Os02g07070	2	3623608-3631551	1000	110261	8.3865	1
OsSRZ20	LOC_Os04g41470	4	24581515-24588736	589	66552.6	4.9119	1
OsSRZ21	LOC_Os04g50870	4	30085471-30093459	656	71696.3	5.9654	1
OsSRZ22	LOC_Os06g09170	6	4612249-4615892	352	38620.8	8.1182	2
OsSRZ23	LOC_Os09g12800	9	7347438-7354032	479	53302.4	8.4597	2
OsSRZ24	LOC_Os09g33876	9	20001090-20006564	588	66445.3	4.9166	2
OsSRZ25	LOC_Os10g26164	10	13557781-13562107	411	44780.3	5.3734	1

Table 2 Cis-elements identified in the promoters of more than ten OsSRZ genes.

Cis-elements	NO. of genes	Functions of cis-elements	Type of cis-elements
CAAT-box	25	common cis-acting element in promoter and enhancer regions	
TATA-box	25	core promoter element around -30 of transcription start	
Circadian	24	cis-acting regulatory element involved in circadian control	Light responsive
Skn-1_motif	23	cis-acting regulatory element required for endosperm expression	Endosperm expression
Sp1	22	light responsive element	Light responsive
ARE	21	cis-acting regulatory element essential for the anaerobic induction	Stress responsive
G-Box	21	cis-acting regulatory element involved in light responsiveness	Light responsive
MBS	20	MYB binding site involved in drought-inducibility	Stress responsive
Box 4	18	part of a conserved DNA module involved in light responsiveness	Light responsive
HSE	18	cis-acting element involved in heat stress responsiveness	Stress responsive
TC-rich repeats	17	cis-acting element involved in defense and stress responsiveness	Stress responsive
ABRE	16	cis-acting element involved in the abscisic acid responsiveness	Hormone responsive
CGTCA-motif	16	cis-acting regulatory element involved in the MeJA-responsiveness	Hormone responsive
TCA-element	16	cis-acting element involved in salicylic acid responsiveness	Hormone responsive
O2-site	15	cis-acting element involved in zein metabolism regulation	Zein metabolism regulation
TGACG-motif	15	cis-acting regulatory element involved in the MeJA-responsiveness	Hormone responsive
GAG-motif	14	part of a light responsive element	Light responsive
GCN4_motif	14	cis-regulatory element involved in endosperm expression	endosperm expression
GT1-motif	14	light responsive element	Light responsive
I-box	14	part of a light responsive element	Light responsive
AAGAA-motif	13	unknown function	Unknown function
Box I	13	light responsive element	Light responsive
5UTR Py-rich stretch	12	cis-acting element conferring high transcription levels	Enhanced Expression
CAT-box	12	cis-acting regulatory element related to meristem expression	Meristem expression
GARE-motif	12	gibberellin-responsive element	Hormone responsive
GC-motif	12	enhancer-like element involved in anoxic specific inducibility	Stress responsive
LTR	12	cis-acting element involved in low-temperature responsiveness	Stress responsive
TCT-motif	12	part of a light responsive element	Light responsive
A-box	11	sequence conserved in alpha-amylase promoters	
ACE	11	cis-acting element involved in light responsiveness	Light responsive
ATCT-motif	11	part of a conserved DNA module involved in light responsiveness	Light responsive

Figures

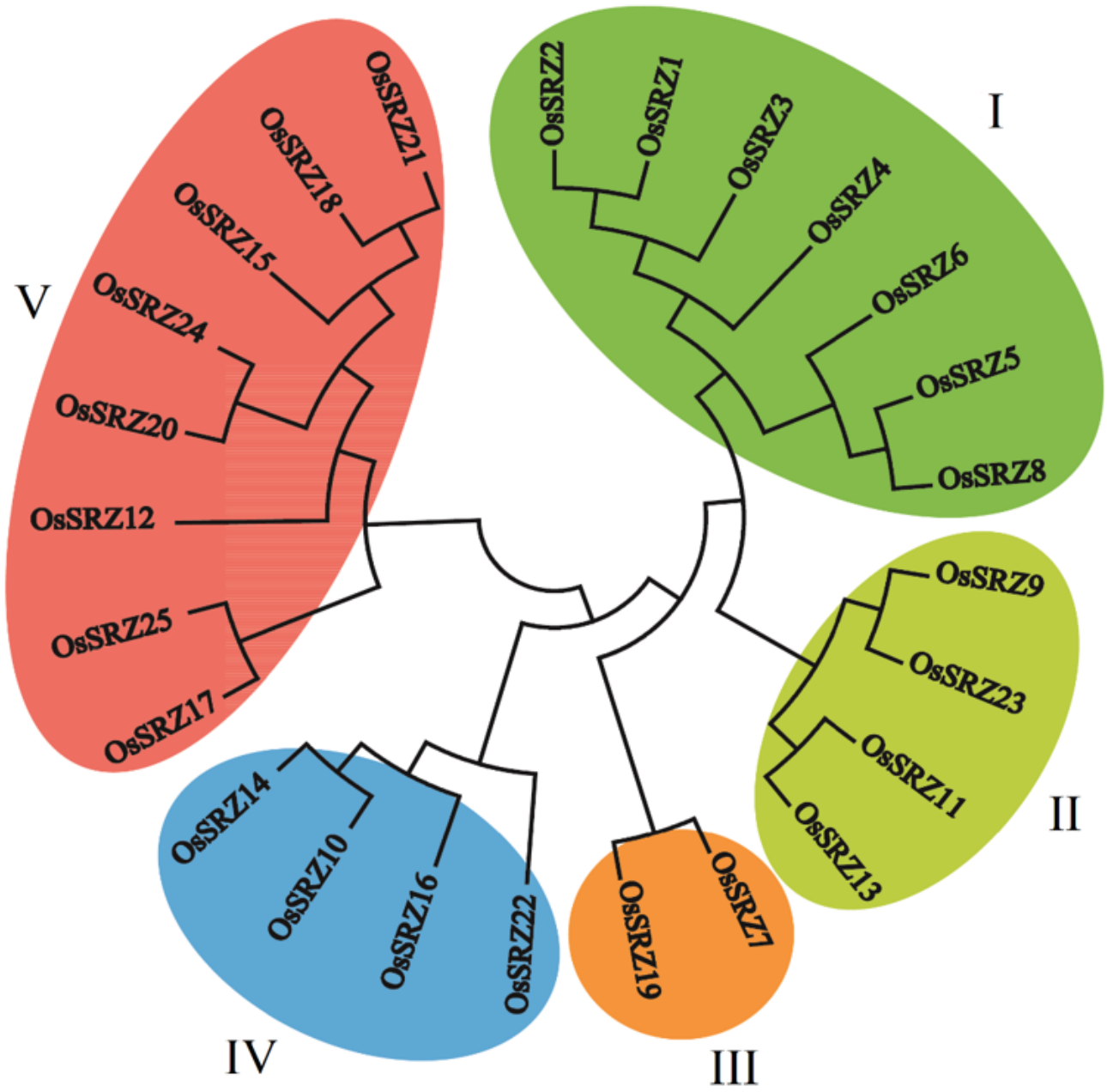

Figure 1

Phylogenetic tree for rice SRZ gene family (OsSRZ). The phylogenetic tree was constructed using the neighbor-joining (NJ) method as implemented in MEGA6.0 from a SRZ protein sequence alignment. The proteins on the tree were divided into four clades. The 25 OsSRZ genes were divided into five groups. Groups I and II belong to the same clade.

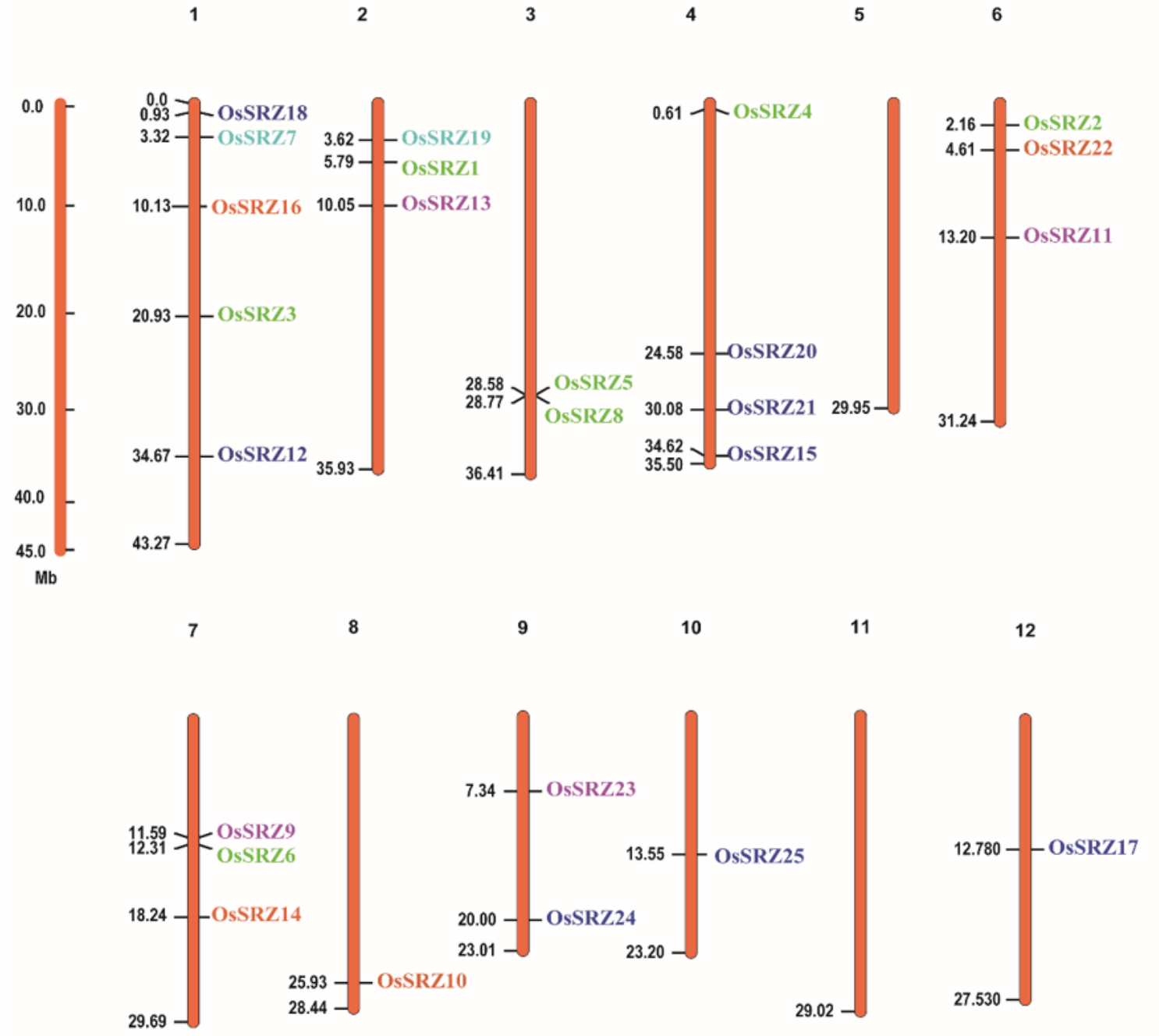

Figure 2

Chromosomal distribution of OsSRZ genes on overall 12 rcie chromosomes. Chromosomal mapping of SRZ genes was based on the physical position (Mb) in 12 rice chromosomes. The chromosome number is indicated at the top of each bar. The positions of the OsSRZ genes in the chromosomes were obtained from Ensembl Plants database (http://plants.ensembl.org/index.html/). The number in the end of each bar represents the length of the chromosome. The genes members of each group are shown in the same color. The first bar on the left without numbering represents the scaling bar.

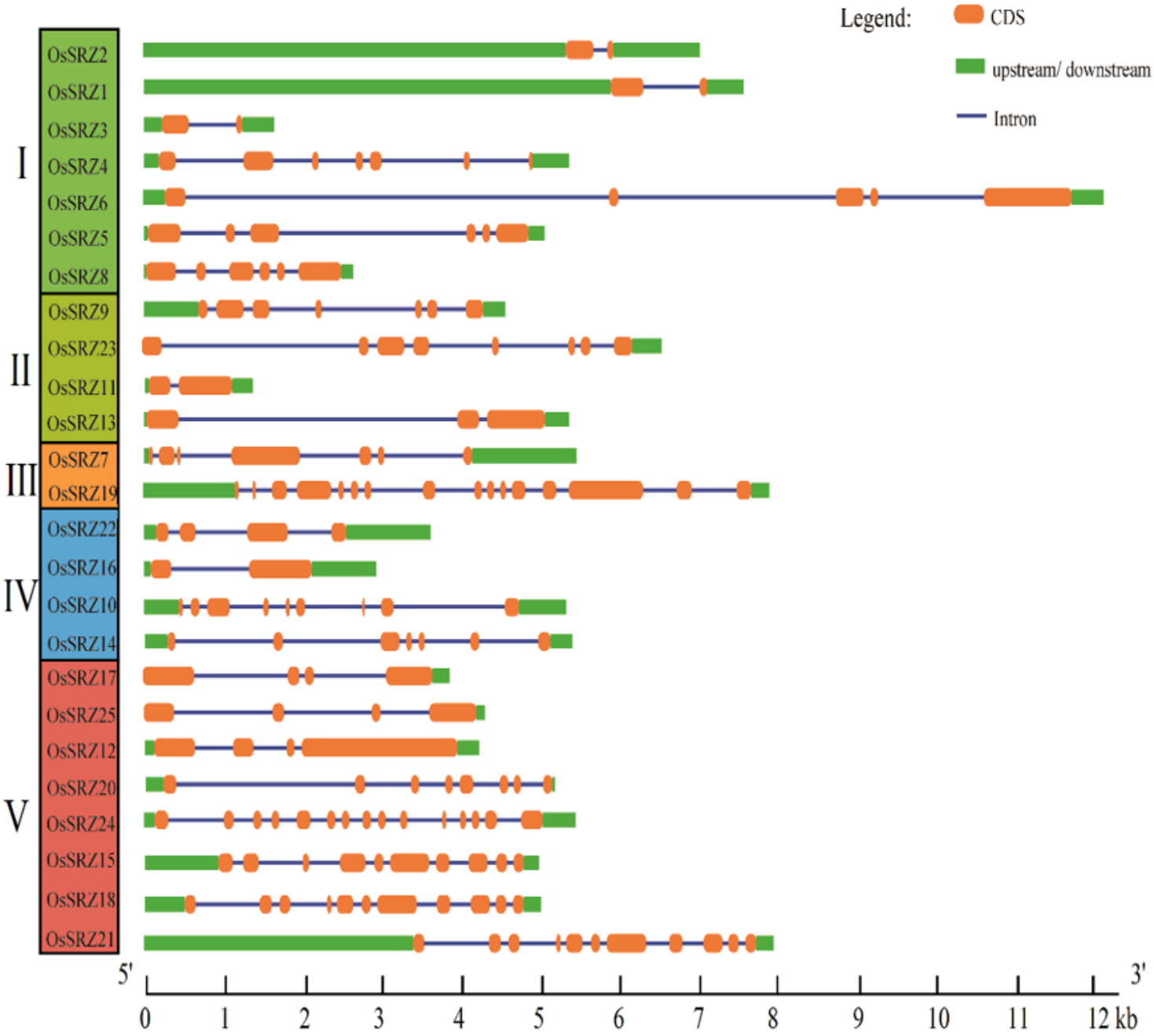

Figure 3

Exon-intron structures for the 25 OsSRZ genes. The exons, introns and untranslated regions (UTRs) are represented by orange rectangles, blue lines and green rectangles, respectively. the X-axis represents the genome length for each OsSRZ gene in kilobase (Kb).

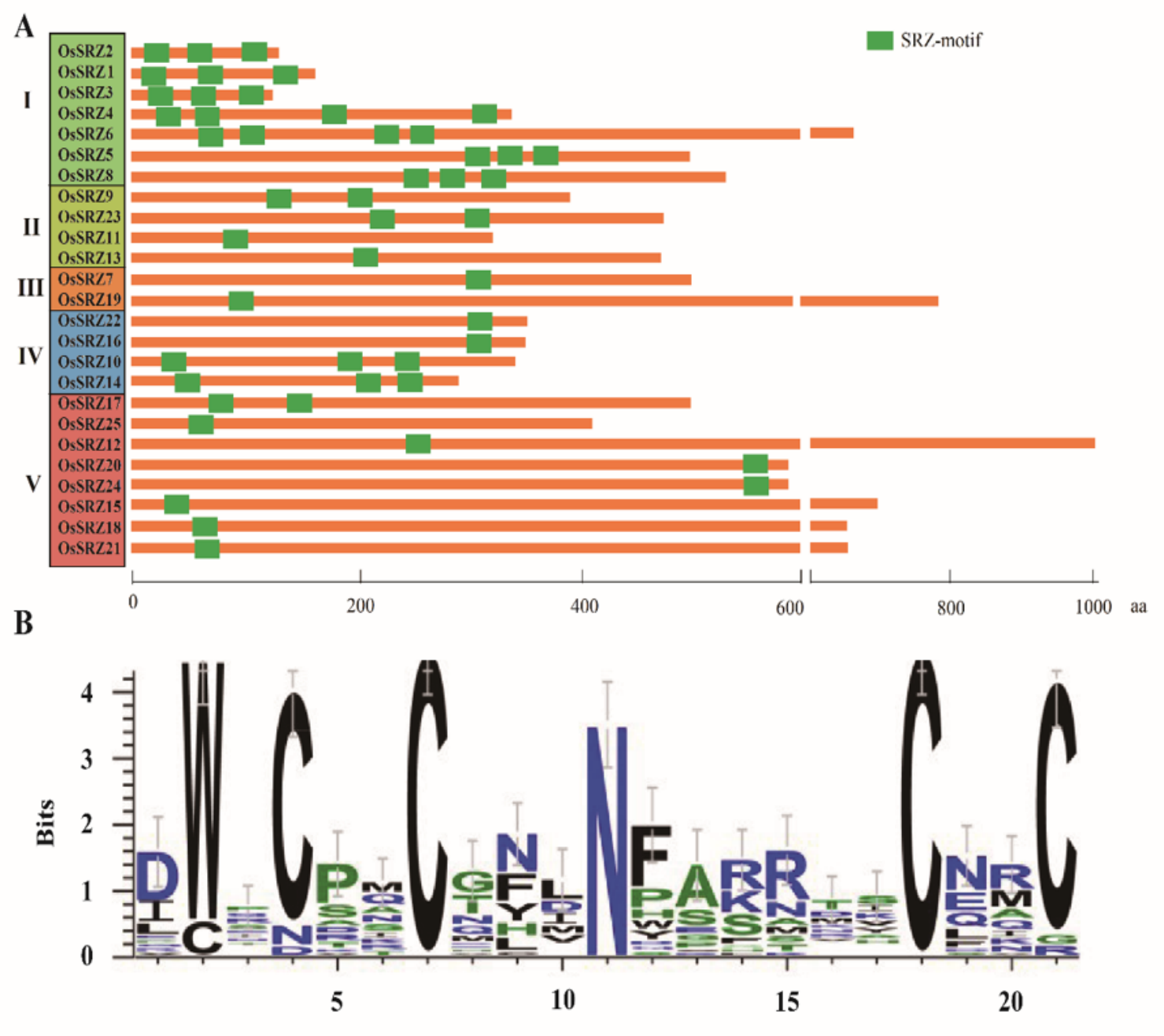

Figure 4

a) Conserved SRZ domains in 25 OsSRZ genes family. b) Logos of the protein alignment of SRZ domain are shown. The x-axis represents the conserved sequences of the domain. The y-axis displays the scale of the relative entropy, which reflects the conservation rate of each amino acid.

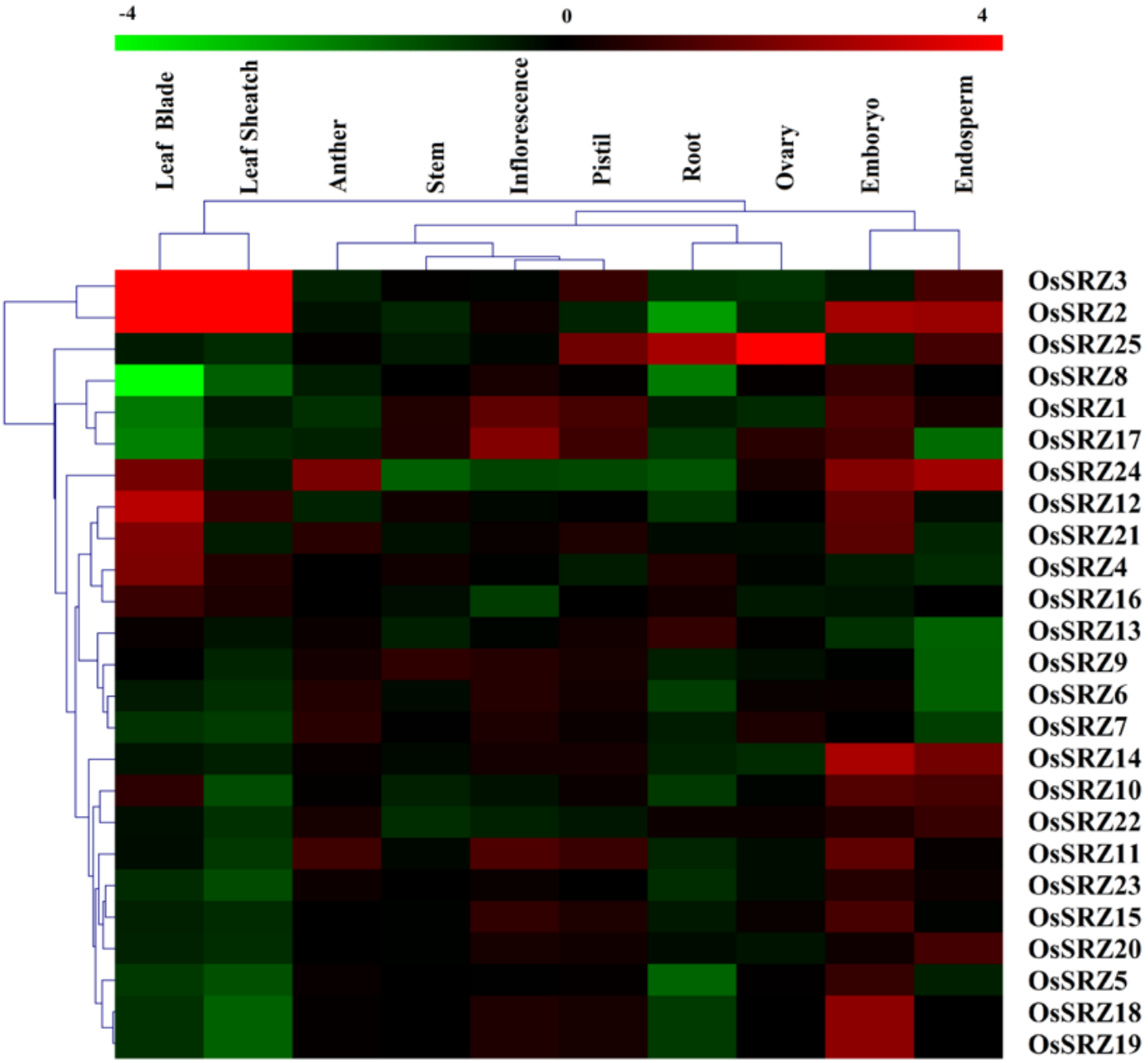

Figure 5

Organ-specific expression patterns of SRZ genes in rice. A heat-map shows the hierarchical clustering of the relative expression of 25 OsSRZ genes across 10 different tissues. Log2 ratios of expression were used to produce the heat map. Microarray data under the series accession number RXP_0001 was obtained from the Rice Expression Profile Database. Red indicates high expression, while green signifies low expression.

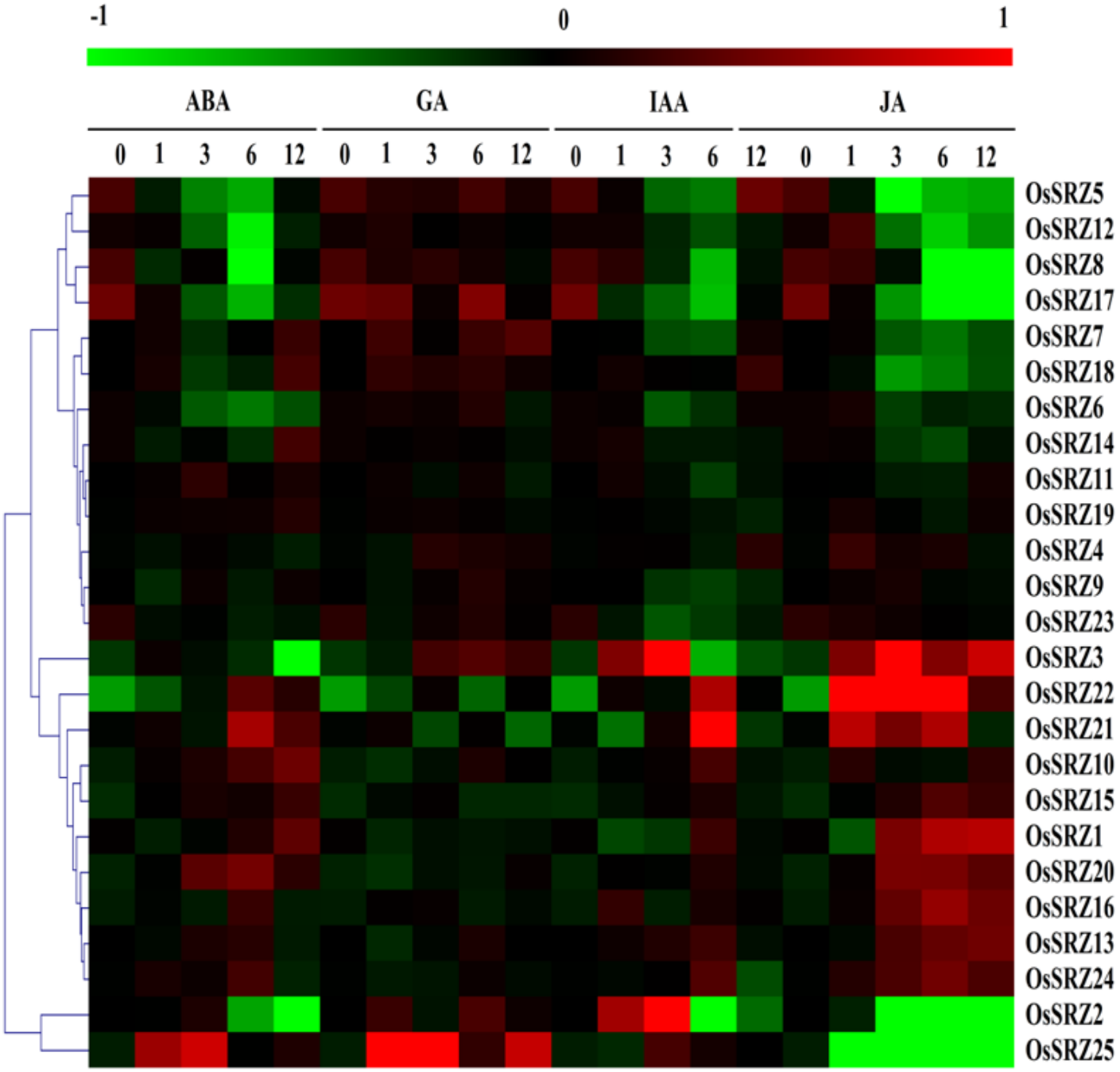

Figure 6

Expression profiles of SRZ genes under treatments of plant hormones in rice. A heat-map shows the hierarchical clustering of the relative expression of 25 OsSRZ genes under four hormonal treatments (ABA, GA, IAA and JA). Log2 ratios of expression were used to produce the heat map. Microarray data under the series accession number RXP_1006, RXP_1007, RXP_1008 and RXP_1012 was obtained from the Rice Expression Profile Database. Red indicates high expression, while green signifies low expression.

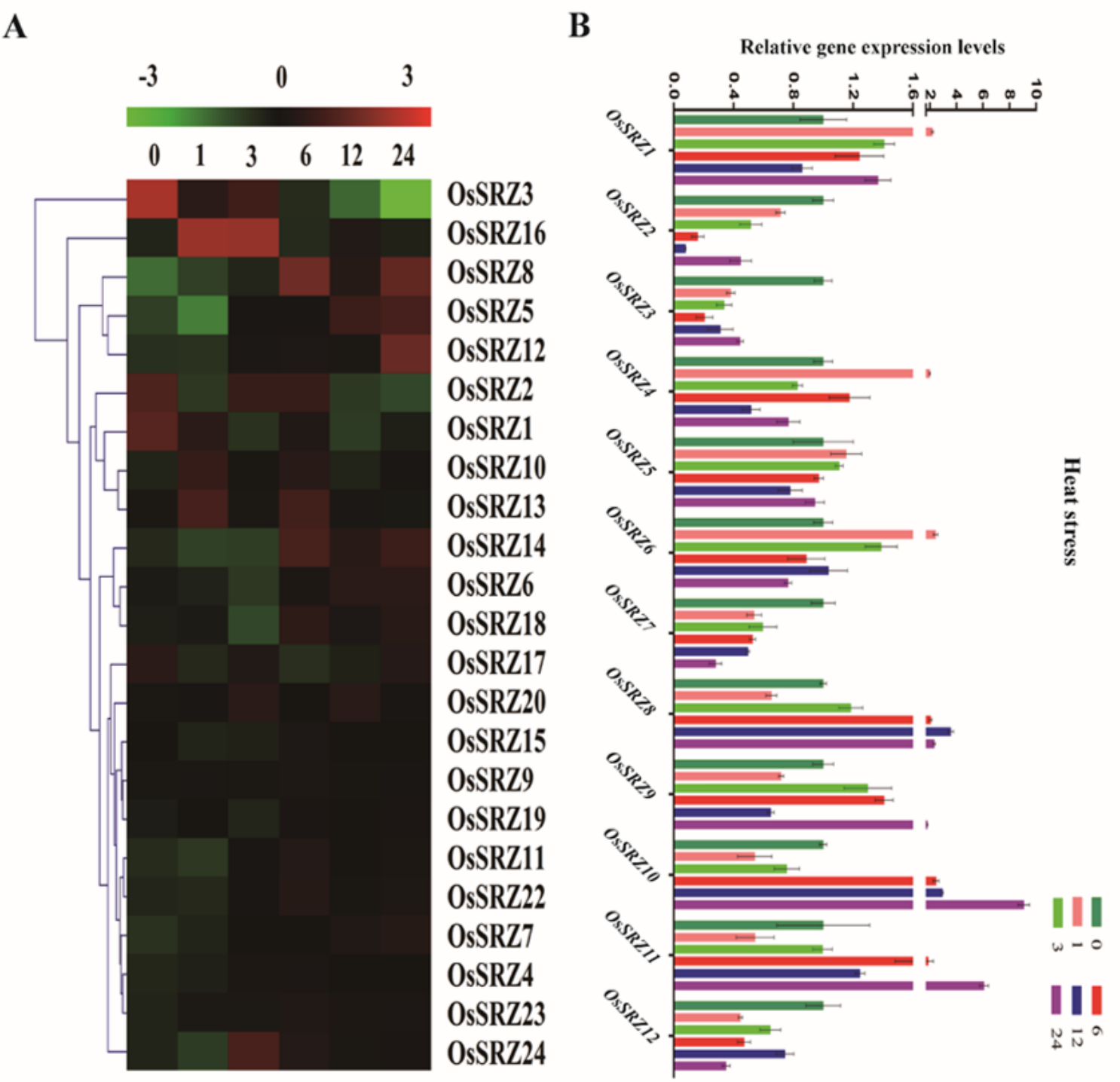

Figure 7

Expression profiles of OsSRZ genes in response to heat stress. a Heat-map determined using RNA-seq data shows the hierarchical clustering of the relative expression of 23 OsSRZ genes (OsSRZ21 and 25 were not detected) in response to heat (45 °C) for different time periods ranging from 0 to 24 h. Log2 ratios of expression were used to generate the heat-map. Microarray data under the series accession number SRP190858 was obtained from the SRA Database of NCBI. Red indicates high expression, while green signifies low expression. b Expression levels of 12 genes checked with q-RT-PCR at different time intervals ranging from 0 to 24 h. Each bar data is the average of 3 biological replicates (±SE).

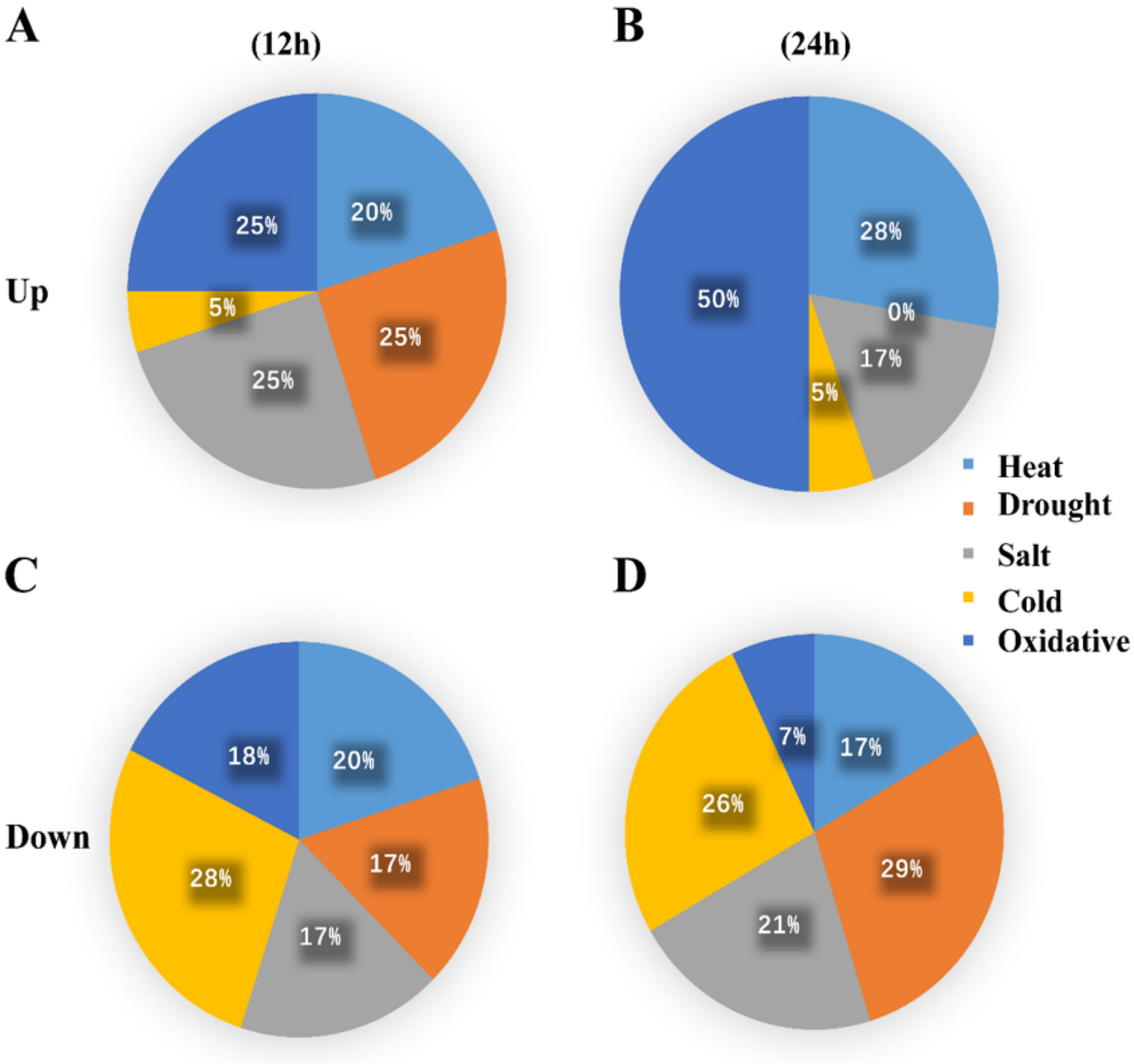

Figure 8

Distribution of OsSRZ gene family based on the gene expression level assessed by q-RT-PCR, on 12 OsSRZ genes, after 12 and 24 h exposure of two-week old rice seedlings to different abiotic stresses. The two upper pie charts represent the percentage of up-regulated (up) OsSRZ genes after 12 (a) and 24 h (b) for each kind of stress. The two bottom pie charts display the percentage of down regulated (down) OsSRZ genes after 12 (c) and 24 h (d) for each kind of stress.

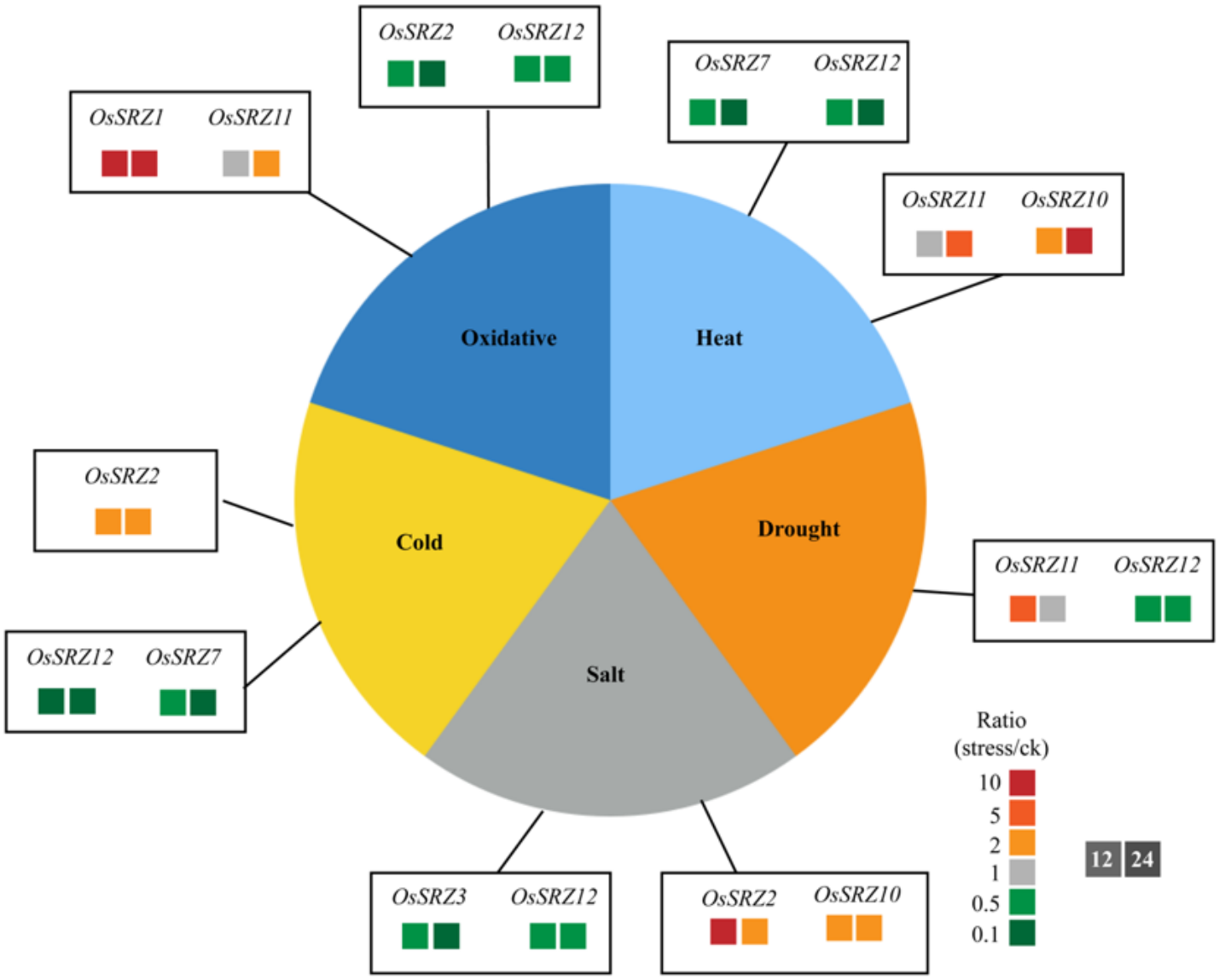

Figure 9

Schematic diagram summarizing the differentially expressed genes (DEGs) of the OsSRZ family after 12 and 24 h exposure of two-week old rice seedlings to various abiotic stresses. For Each kind of stress, at least a single gene was moderately or highly was up-regulated during the 24 h of stress duration. These genes could function as specific abiotic stress markers.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplTables.xlsx
- SupplFigures.docx