

Transcription dynamics of *Saltol* QTL localized genes encoding transcription factors, reveals their differential regulation in contrasting genotypes of rice

Kamlesh K. Nutan¹ · Hemant R. Kushwaha² · Sneha L. Singla-Pareek³ · Ashwani Pareek¹

Received: 6 July 2016 / Revised: 2 October 2016 / Accepted: 10 October 2016 / Published online: 15 November 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Salinity is one of the major environmental factors affecting the growth and yield of rice crop. Salinity stress response is a multigenic trait and numerous approaches have been used to dissect out the key determinants of complex salt tolerance trait and their regulation in plant. In the current study, we have investigated expression dynamics of the genes encoding transcription factors (SalTFs) localized within a major salinity tolerance related QTL—‘*Saltol*’ in the contrasting cultivars of rice. SalTFs were found to be differentially regulated between the contrasting genotypes of rice, with higher constitutive expression in the salt tolerant landrace, Pokkali than the cultivar IR64. Moreover, SalTFs were found to exhibit inducibility in the salt sensitive cultivar at late duration (after 24 h) of salinity stress. Further, the transcript abundance analysis of these SalTFs at various developmental stages of rice revealed that low expressing genes may be involved in developmental responses, while high expressing genes can be linked with the salt stress response. Grouping of these genes was well supported by *in silico* protein–protein interaction studies and distribution of single-nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) in the promoter and genic regions of these

genes. Taken together, we propose that out of 14 SalTFs, eight members are strongly correlated with the salinity stress tolerance in rice and six are involved in plant growth and development.

Keywords Contrasting genotypes · Gene expression · QTL · Rice · Salinity stress · *Saltol* · SalTFs

Introduction

Plants being sessile, their growth and yield are strongly influenced by abiotic stresses such as drought, high salinity and extremes of temperature. Among all the abiotic stresses, salinity leads to a major loss in plant productivity (Chinnusamy et al. 2005; Pareek et al. 2010). Additionally, one fifth of irrigated arable land in the world has been reported to be adversely influenced by high soil salinity (Negrão et al. 2011). Salinity stress response, like response towards other abiotic stresses, such as drought (Kantar et al. 2011; Akpinar et al. 2012, 2013), is a multigenic trait involving a large number of genes operating in close co-ordination (Bohnert et al. 2006; Sreenivasulu et al. 2007; Kumari et al. 2009; Nongpiur et al. 2012, 2016). To facilitate the development of new crop varieties with high salinity tolerance, a clear understanding of the genetic mechanisms controlling salt tolerance is imperative.

Rice (*Oryza sativa* L.) is a glycophyte and major source of nutrition for most of the Asian and African countries representing nearly one third of the people living on earth. Most rice types, except some of the *indica* genotypes, such as Pokkali, are sensitive to salt (Yeo et al. 1990). Sensitivity of rice towards salinity stress varies with its growth stages, where high sensitivity is reported at the young seedling and reproductive stage, contributing ultimately to the loss in productivity in salt-affected soils (Lutts et al. 1995, Lafitte et al. 2007). Numerous evidence have clearly established salinity

Electronic supplementary material The online version of this article (doi:10.1007/s10142-016-0529-5) contains supplementary material, which is available to authorized users.

✉ Ashwani Pareek
ashwanip@mail.jnu.ac.in; ashwanipareek@gmail.com

¹ Stress Physiology and Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

² Synthetic Biology and Biofuel, International Center for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India

³ Plant Stress Biology, International Centre for Genetic Engineering and Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India

response in crop plants to be controlled by many genes localized on different chromosomes (Chinnusamy et al. 2005; Sahi et al. 2006; Kumari et al. 2009; Lakra et al. 2013) or by multiple genes clustered on a particular segment of chromosome, called quantitative trait loci (QTL) (Bonilla et al. 2002). Several QTLs related to salinity tolerance have been identified in rice (Gu et al. 2000; Prasad et al. 2000; Bonilla et al. 2002; Gregorio et al. 2002; Lin et al. 2004; Sabouri et al. 2009; Thomson et al. 2010; Islam et al. 2011; Ghomi et al. 2013). Among them, a major QTL called *Saltol* (9.3–16.4 Mb) has been mapped, flanked by SSR markers RM1287 and RM6711, on the short arm of chromosome I of rice (Bonilla et al. 2002; Gregorio et al. 2002; Thomson et al. 2010). This QTL accounted for 64.3 to 80.2 % of the phenotypic variation in salt tolerance (Bonilla et al. 2002).

Response towards various abiotic stress conditions varies with the genotypes of the same species, thus necessitating the analysis of contrasting genotypes which can potentially reveal the crucial details about the specialized physiological and/or molecular mechanisms contributing towards the stress response. Several reports in rice (Karan et al. 2009; Kumari et al. 2009; Walia et al. 2009, do Amaral et al. 2016), wheat (Budak et al. 2013), sorghum (Gelli et al. 2014), barley (Gao et al. 2013; Witzel et al. 2014), watermelon (Fan et al. 2014) and *Brassica* (Kumar et al. 2009; Sharma et al. 2015) have distinctly highlighted the usefulness of this method, thus giving insight into how the differential regulation of genes might be responsible for the stress-sensitivity or tolerance, as observed in the contrasting genotypes. Rice species show a lot of genetic diversity, even in salinity stress response, where traditional landrace of rice, namely Pokkali, has been found to be tolerant, while IR64 has been shown to be sensitive (El-Shabrawi et al. 2010). Studies carried out with above objectives in mind, using rice seedlings of salt sensitive genotype IR29 and salt tolerant recombinant inbred line FL478 (derived from Pokkali and IR29), have shown more favourable physiological parameters for salinity tolerance, like less necrosis, higher K^+/Na^+ ratio, increased anthocyanin and chlorophyll content in the latter (Senadheera et al. 2009; Walia et al. 2009). Similarly, in another report from our group, morphological and physiological analysis in 4-day-old rice seedlings under salinity stress revealed higher chlorophyll and proline contents, increased shoot growth and lesser membrane injury thus confirming higher tolerance ability of Pokkali over IR64 (Kumari et al. 2009). Owing to the high sensitivity towards various abiotic stresses, most of these studies focused on analysis of stress response at the seedling stage (Walia et al. 2007, 2009; Kumari et al. 2009; Thomson et al. 2010).

Recent work from our lab has exploited IR64 and Pokkali rice for finding out candidate genes for salinity tolerance (Karan et al. 2009; Kumari et al. 2009; Kumar et al. 2012a). In fact, functional genomics studies targeting expression of key members of stress responsive machinery (identified from these studies) have been proven to be showing improved

salinity tolerance when expressed ectopically (Kumar et al. 2012b; Singh et al. 2012; Lakra et al. 2014; Joshi et al. 2016a; Soda et al. 2016). Soda et al. (2013) carried out the *in silico* analysis of genes localized in the *Saltol* QTL (9.3 to 16.4 Mb) and classified them on the basis of their possible functions, assigned using MIPS functional categories. Transcription factors (TFs) are considered as the master regulator of the gene expression. These TFs bind specifically to the *cis*-acting elements in the promoter, thus regulating the gene expression either by inducing (activators) or repressing (repressor) the activity of the RNA polymerase (Riechmann et al. 2000). It is also reported that Pit-1 TF may function as activator or repressor depending on the *cis*-acting elements where it binds on the gene promoter (Latchman 2001). Various studies have shown that the induction of genes encoding TF under various abiotic stresses and overexpression of these genes lead to enhanced tolerance in transgenic plants (Lakra et al. 2013; Joshi et al. 2016b). In the present analysis, we have identified the various transcription factors localized within the *Saltol* QTL (henceforth referred as ‘SalTFs’). We have analysed relative expression of 14 of these SalTFs genes in contrasting genotypes—Pokkali and IR64, under control and salinity stress conditions. We have further made an attempt to correlate the expression of these SalTFs with the single-nucleotide polymorphisms (SNPs) present in the coding as well as promoter region of these genes. This study will open up new avenues for understanding the molecular basis of salinity tolerance in crop plants.

Materials and methods

In silico analysis of SalTFs

With the annotation of the genes present in the *Saltol* QTL region of chromosome I (Soda et al. 2013), the gene sequences of the corresponding loci were obtained from TIGR database (<http://rice.plantbiology.msu.edu/>). Using FunCat/MIPS databases (<http://mips.gsf.de/proj/funecatDB>), functional annotation was performed for genes localized in *Saltol* QTL region. Out of total 783 genes present in *Saltol* QTL region, 14 genes were predicted to be encoding transcription factors, which were selected for further analysis (Table 1). Predicted gene structure of these SalTFs was obtained from TIGR database.

Plant material and stress treatments

Seeds of rice (IR64 and Pokkali) were thoroughly washed with de-ionized water and germinated on hydroponic system filled with one half strength Yoshida medium as nutrient supplier. Hydroponic system was kept in plant growth chamber at 28 ± 1 °C temperature, 70 % humidity, 60,000 lx light intensity and 12 h light/12 h dark cycle. Seedlings were grown for 7 days

Table 1 List of ‘transcription factors’ localized within *Saltol* QTL

S. no	Gene name	Abbreviated gene name	Locus	Orientation	Genomic DNA (bp)	CDS (bp)	Protein (AA)
1	<i>BSD domain containing protein</i>	<i>OsBSD</i>	LOC_Os01g16670	Sense	1948	1614	537
2	<i>HBP1b transcription factor</i>	<i>OsHBP1b</i>	LOC_Os01g17260	Sense	7116	990	329
3	<i>MYB2 transcription factor</i>	<i>OsMYB2</i>	LOC_Os01g18240	Sense	2450	1287	428
4	<i>Basic helix-loop-helix dimer</i>	<i>OsHLH</i>	LOC_Os01g18290	Sense	3417	1914	637
5	<i>OsMADS88-MADS-box TF with M-gamma type Box</i>	<i>OsMADS88</i>	LOC_Os01g18420	Sense	723	723	240
6	<i>OsMADS89-MADS-box TF with M-gamma type box</i>	<i>OsMADS89</i>	LOC_Os01g18440	Sense	921	921	306
7	<i>Squamosa promoters binding (SPB) like protein 12</i>	<i>OsSPL12</i>	LOC_Os01g18850	Sense	6641	2589	862
8	<i>MYB78 like transcription factor</i>	<i>OsMYB78L</i>	LOC_Os01g19330	Sense	1098	882	293
9	<i>MYB-like transcription factor</i>	<i>OsMYBL</i>	LOC_Os01g19970	Sense	6357	954	317
10	<i>Transcription initiation factor IID</i>	<i>OsTFIID</i>	LOC_Os01g23630	Sense	3130	552	183
11	<i>OsMADS93-MADS-box TF with M-beta type box</i>	<i>OsMADS93</i>	LOC_Os01g23760	Sense	1224	1224	407
12	<i>OsMADS94-MADS-box TF with M-beta type box</i>	<i>OsMADS94</i>	LOC_Os01g23770	Sense	1295	972	323
13	<i>GATA8 transcription factor</i>	<i>OsGATA8</i>	LOC_Os01g24070	Sense	2716	396	131
14	<i>Histone like transcription factor and Archaeal histone</i>	<i>OsHL</i>	LOC_Os01g24460	Sense	1424	1335	444

in this hydroponic setup with continuous air bubbling and renewal of nutrient media after every 2 days interval. For salinity stress, 7-day-old seedlings were exposed to 200 mM NaCl for 30 min, 24 h and 48 h in the hydroponic system, as reported earlier (Soda et al. 2013). Shoots of the seedlings were harvested, frozen in liquid nitrogen and stored in -80°C for further use. Seedlings grown in normal media (without NaCl) served as control.

Total RNA isolation and cDNA synthesis

Total RNA was isolated from the control and stress samples by TRIzol Reagent (Life Technologies, Rockville, MD, USA). PolyA⁺ RNA enrichment was carried out using streptavidin-linked paramagnetic beads (Roche GMBH, Mannheim, Germany) and biotin-labelled oligo d(T)₂₀ primer (Operon Biotechnologies, Cologne, Germany). From the mRNA, first-strand complementary DNA (cDNA) synthesis was carried out using first strand cDNA synthesis kit (Fermentas Life Sciences, Burlington, ON, Canada). Procedure for total RNA isolation to cDNA synthesis was followed as described by Soda et al. (2013).

Quantitative real-time PCR analysis

To ensure high specificity for the primer binding, 3' UTR region of the genes was selected for the purpose of primer designing (Primer Express 3.0 software, Applied Biosystems,

California, USA) using default parameters (Table 2). The uniqueness of each primer pair to amplify selected gene was confirmed by BLASTn using the KOME and NCBI databases. The PCR mixture contained 5 μl of cDNA (20 times diluted), 12.5 μl of 2 \times SYBR Green PCR Master Mix (Applied Biosystems, California, USA) and 10 nM of each gene-specific primer in a final volume of 25 μl . The real-time PCR was performed employing StepOne™ Real-Time PCR System having StepOne™ software Version 2.0 (Applied Biosystems, California, USA). All the PCRs were performed under the following conditions: 10 min at 95 $^{\circ}\text{C}$ and 40 cycles of 15 s at 95 $^{\circ}\text{C}$, 1 min at 62 $^{\circ}\text{C}$ and 30 s at 72 $^{\circ}\text{C}$ in 96-well optical reaction plates. The specificity of the amplification was tested by dissociation curve analysis and agarose gel electrophoresis. The expression of each gene in different RNA samples was normalized with the expression of rice actin gene (*OsAct*) used as the internal control. The transcript level for each candidate gene in different samples was calculated relative to its expression in IR64 control seedlings using $\Delta\Delta\text{CT}$ method (Soda et al. 2013). Two biological and three technical replicates were taken for each sample for data analysis.

Expression analysis of SalTFs in rice plants at various developmental stages of growth

Expression patterns of SalTFs in rice at various stages of development, such as germination, seedling, tillering, stem elongation, booting, heading, flowering, milking and dough

Table 2 List of primers used in qRT-PCR and their sequences

Locus	Gene name	Abbreviated gene name	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
LOC_Os01g16670	<i>BSD domain containing protein</i>	<i>OsBSD</i>	GGATTTCACAGTGCTGGTT	CAAACAAACAACCCCATTGA
LOC_Os01g17260	<i>HBP1b transcription factor</i>	<i>OsHBP1b</i>	CCCGTGGGTATTGACATCT	CCAATGCAACTGAATGGTCA
LOC_Os01g18240	<i>MYB2 transcription factor</i>	<i>OsMYB2</i>	CAAGGATTTGCAAAGGATGTC	TTCATGTGGTGCTCTGTCC
LOC_Os01g18290	<i>Basic helix-loop-helix dimer</i>	<i>OsbHLH</i>	CTTCAGCCCAAACAAAAGC	TGTTTCAGCCCCATCTCTCT
LOC_Os01g18420	<i>OsMADS88-MADS-box TF with M-gamma type Box</i>	<i>OsMADS88</i>	TGGAAGGGAACAAGAGGATG	GCCAACACCAGTATTGCCTT
LOC_Os01g18440	<i>OsMADS89-MADS-box TF with M-gamma type box</i>	<i>OsMADS89</i>	CCATGCCGCTGTCTTCTATG	GCCATGGTTGATGAAGTGGT
LOC_Os01g18850	<i>Squamosa promoters binding (SPB) like protein 12</i>	<i>OsSPL12</i>	AATTCCATTGGACAGGCATC	GCTCTGCTCACGGTAAAACC
LOC_Os01g19330	<i>MYB78 like transcription factor</i>	<i>OsMYB78L</i>	AGACACCGGAGACAACACTGCT	CAGAAATTATCAGTACGGCTGC
LOC_Os01g19970	<i>MYB-like transcription factor</i>	<i>OsMYBL</i>	ACGTGTTTGGGTTTACCAT	GCATCAACCTCTCTACCCCA
LOC_Os01g23630	Transcription initiation factor IID	<i>OsTFIID</i>	TTCTCGTCAGTCTCTCGT	GAGAAAGGCTTGGCAAGATG
LOC_Os01g23760	<i>OsMADS93-MADS-box TF with M-beta type box</i>	<i>OsMADS93</i>	GGATTCCAGCTCGGTGACA	TGATGAAGCTCCCCAATTC
LOC_Os01g23770	<i>OsMADS94-MADS-box TF with M-beta type box</i>	<i>OsMADS94</i>	TACGGGATCGGAAACCAGAT	CCGACTGGGTATTGCATTG
LOC_Os01g24070	<i>GATA8 transcription factor</i>	<i>OsGATA8</i>	CATCTGCATCCACATTGCTA	CCCTCCCCTATCTCATCCTG
LOC_Os01g24460	<i>Histone like transcription factor and Archaeal histone</i>	<i>OsHL</i>	ACGACGGTGTTTTGCACAG	TTGGCATTGTACTACTTGGG
Actin	<i>Actin</i>	<i>OsAct</i>	CAGCCACACTGTCCCCATCTA	AGCAAGGTCGAGACGAAGGA

were extracted from the publicly available microarray data (<https://www.genevestigator.com/gv/>). Locus ID mentioned in Table 1 was used to analyse and illustrate the expression of SalTFs.

Analysis of *Tos17* Mutant population

The *Tos17*-tagged lines (<https://tos.nias.affrc.go.jp/>) were searched using the TIGR sequence of all the SalTFs. The search was made using the default search criteria. The information thus obtained has been presented as Table 3.

Analysis of protein–protein interaction

To study the interaction of these SalTFs with other rice proteins, we have analysed the protein–protein interaction using rice protein–protein interaction data obtained from TIGR (<http://rice.plantbiology.msu.edu/>).

Analysis of SNPs and InDels

The analysis of SNPs in IR64 and Pokkali was performed using the Rice SNP discovery database (<http://www.nipgr.res.in/mjain.html>) which holds the resequenced data for the rice cultivars (Jain et al. 2014). The SNPs and InDels were extracted from the database using in-house PERL programmes.

The representative figure showing various SNPs and InDels in *Salto1* genes was made using the R statistical package.

Results

SalTFs are structurally very diverse in rice

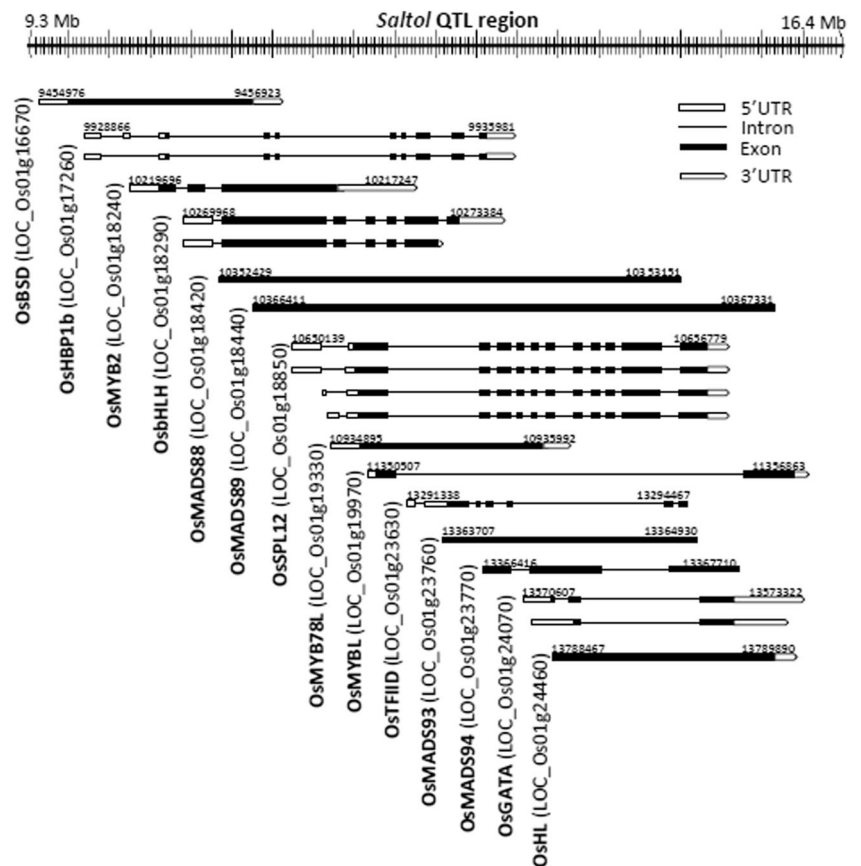
Out of 14 SalTFs analysed in this study, *in silico* analysis of gene structure revealed six members to be intron-less. These members are *OsBSD*, *OsMADS88*, *OsMADS89*, *OsMYB78L*, *OsMADS93* and *OsHL* (full names of these proteins are mentioned in Table 1). On the other hand, genes having exons were predicted to have introns ranging from two to 11, having variable lengths (Fig. 1). *OsMYBL* was predicted to possess the longest single intron (5403 bp) with two small flanking exons. Among the various SalTFs analysed *in silico*, *OsSPL12* transcribed into maximum of four alternate spliced transcripts and three other SalTFs *viz* *OsHBP1b*, *OsHLH* and *OsGATA8* could have two such splice variants. Rest of the members were not predicted to encode for any alternative splicing product (Fig. 1). Except the MADS-box TF family (4 genes), all other genes possess both 5' and 3' UTR. *OsHL* transcription factor was an exception where only 3' UTR was predicted.

Table 3 List of rice *Tos17* mutant for transcription factors encoded by genes present in *Saltol* QTL

Locus	Gene	e-value	Mutant line	Mutant phenotype
LOC_Os01g16670	<i>OsBSD</i>	NA	NA	NA
LOC_Os01g17260	<i>OsHBP1b</i>	5e-33	H0281	Vivipary, relatively high yield, Dwarf
LOC_Os01g18240	<i>OsMYB2</i>	2e-11	NG1332	Lethal
LOC_Os01g18290	<i>OsHLH</i>	NA	NA	NA
LOC_Os01g18420	<i>OsMADS88</i>	NA	NA	NA
LOC_Os01g18440	<i>OsMADS89</i>	7e-04	NF6845	Sterile (50 %), Dwarf and dense panicle
LOC_Os01g18850	<i>OsSPL12</i>	1e-36	ND0060	Lethal, Late germination, less fertile, Vivipary
LOC_Os01g19330	<i>OsMYB78L</i>	NA	NA	NA
LOC_Os01g19970	<i>OsMYBL</i>	4e-70	NF3709	Fertility 75 %
LOC_Os01g23630	<i>OsTFIID</i>	NA	NA	NA
LOC_Os01g23760	<i>OsMADS93</i>	NA	NA	NA
LOC_Os01g23770	<i>OsMADS94</i>	1e-05	NF6845	Sterile (fertility 50 %), Dwarf dense panicle
LOC_Os01g24070	<i>OsGATA8</i>	9e-91	NC2533	Low fertility, comparatively low yield
LOC_Os01g24460	<i>OsHL</i>	NA	NA	NA

NA mutant not available

Fig. 1 Schematic representation of *Saltol* QTL region showing localization of SalTFs. The diagram is constructed on the basis of information available in TIGR database. The upper scale shows the physical location of the *Saltol* QTL and the gene transcripts with their alternate splice forms are arranged by their locus ID in ascending order. CDS coordinates (5'-3') are mentioned above the gene structure. 5' UTR, 3' UTR, intron and exon are also shown for each gene



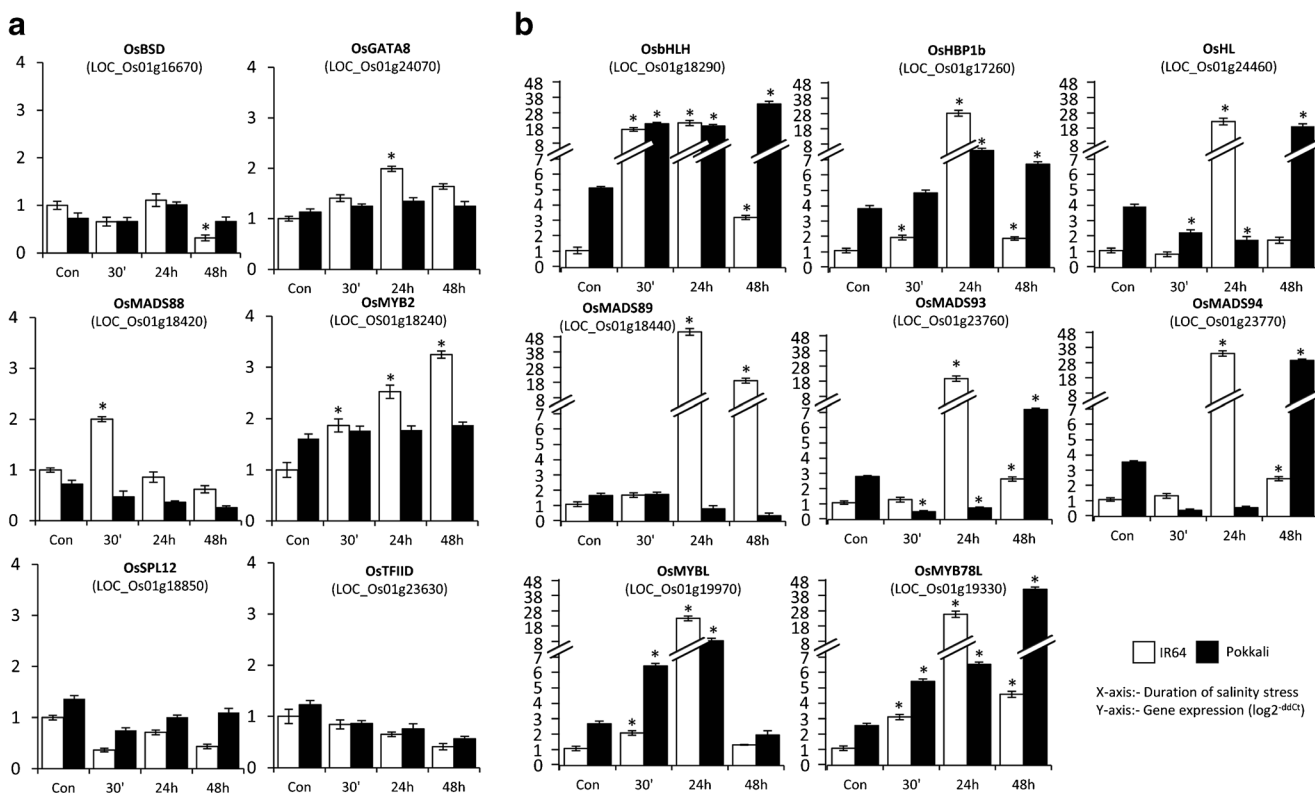


Fig. 2 Bar graphs showing qRT-PCR result of SalTFs in contrasting cultivars of rice. **(a)** The genes showing low level of alterations in their expression under various durations of salinity stress. **(b)** The genes showing high level of alterations in their expression under various durations of salinity stress. Seven-day-old seedlings of IR64 (salt sensitive) and Pokkali (salt tolerant) were subjected to 200 mM NaCl stress for different time intervals. Bar graphs were plotted between stress duration (x-axis) and $\log 2^{-ddCt}$ value in number (y-axis). The values represented are the mean of

two biological and three technical replicates; standard error is shown on bar. Gene expression data was normalized with the plant reference gene ‘actin’ for rice as an internal control. Relative expression of genes was plotted against the expression of IR64 control. Con control, 30’ 30-min salt stress, 24 h 24-h salt stress, 48 h 48-h salt stress. Results are represented as mean \pm standard error (SE). For statistical significance, Student’s *t* test was performed and asterisk above the graph means significant differences from their respective control (Con) at $P \leq 0.05$

Salinity-induced expression patterns of SalTFs in contrasting cultivars of rice showed them to be differentially regulated and fine-tuned with respect to a given genotype

qRT-PCR-based transcript abundance analysis was carried out in contrasting rice genotypes, where the salt sensitive cultivar of rice was IR64, while the salt tolerant landrace was Pokkali (Fig. 2). Various studies have revealed distinct group of genes showing either altered expression after quick exposure to stress (early responsive genes) or group of genes showing up/downregulation after only a long exposure (late responsive genes) to stress in contrasting genotypes of rice under salinity (Kumari et al. 2009), chilling (Zhang et al. 2012) and drought stress (Baldoni et al. 2016). Hence, in the present study, to get a snapshot of transcript abundance for SalTFs, analysis was carried out after early (30 min) and late (24 h and 48 h) duration of stress. On the basis of the amplitude of expression (fold change of transcript abundance), these SalTFs could be divided into two major categories represented as Fig. 2a, b. In the first category, the change in the expression of genes was to the

tune of 2–4-folds only, while the second category comprised of members which showed change in expression up to 40–50-folds. Among the first category, the representative members are OsBSD, OsMADS88, OsSPL12, OsGATA8, OsMYB2 and OsTFIID, while the rest of the members comprise the second category.

On relative basis, constitutive expression of majority of the SalTFs was found to be higher in Pokkali as compared to IR64. Exception to this observation were the two members—OsBSD and OsMADS88, where no difference or slightly lower abundance of transcript was noticed in the former genotype (Fig. 2a). Maximum difference in constitutive expression among the contrasting genotypes was reported for OsbHLH, where the tolerant genotype exhibited 5 times more transcripts than the sensitive genotype.

In response to very short period of salinity stress (30 min only, where only osmotic shock prevail), majority of the SalTFs showed alteration in their transcript abundance. Members belonging to the first category did not show conspicuous change in their expression, except the two members viz. OsMADS88 and OsMYB2. Interestingly, OsMADS88

showed induction only in the sensitive genotypes IR64 after 30 min of salinity and then its expression goes down below the constitutive expression level in response to extended durations of salinity stress (24 and 48 h). However, in Pokkali, expression of this gene decreases upon salinity stress. In OsMYB2, after 30 min of salinity, transcript abundance increases up to ~2-fold in IR64 and it continues to increase further. Though, constitutive expression of this gene was higher in Pokkali, but no induction in transcript was observed in response to salinity stress (30 min, 24 h and 48 h). Among the members of the second category, all showed upregulation of transcript in response to 30 min of salinity, except the members of OsHL and MADS family (OsMADS89, OsMADS93 and OsMADS94). OsbHLH showed significant upregulation of its transcript under these conditions, where up to 18-folds increase in abundance was reported. Rest of the members of this category also showed upregulation of the transcript ranging from 2 to 6-folds.

After 24 h of stress, when the plant probably senses actual ionic stress, the expression of SalTFs showed a whole reprogramming where a majority of the genes from second category showed upregulation in response to salinity stress in both the genotypes with a few exception to this pattern.

For example, OsMADS89 showed no change, and OsHL, OsMADS93 and OsMADS94 showed decrease in the expression in the tolerant genotype Pokkali with a significant upregulation (upto 48-folds) in the sensitive genotype IR64. The transcript abundance for all the SalTFs was reported to be higher in IR64 as compared to Pokkali under these conditions. Further increase in stress duration up to 48 h, when the sodium toxicity build up in the cells and causes disturbance in the ion homeostasis, these SalTFs showed even higher transcript abundance in Pokkali as compared to IR64. Interestingly, members such as OsHBP1b, OsHL, OsMADS93, OsMADS94 and OsMYB78L showed significantly high abundance of transcript up to 30–40-folds in Pokkali with their corresponding transcripts showing only 2–4-folds in IR64. In contrast, the transcripts for OsMADS89 showed a strikingly different pattern of accumulation where the tolerant genotype showed hardly any change in abundance while the sensitive genotype IR64 maintained even 20-fold transcripts after 48 h of salinity stress.

The unique pattern of transcript accumulation for various SalTFs observed here clearly confirm the salinity response to be highly complex in rice seedlings and tuned ‘finely’ in a given genotype. Based on the dynamics of the transcript

Fig. 3 Grouping of SalTFs on the basis of their transcript abundance at particular stress duration in contrasting cultivars of rice. **(a)** Group I includes late (24 h stress) inducible genes in IR64 **(b)** group II includes genes upregulated at 30' in IR64 **(c)** Group III includes genes downregulated at 30' in IR64. Salt stress duration is mentioned on the x-axis and $\log 2^{-ddCt}$ value is on the y-axis. Con control, 30' 30-min salt stress, 24 h 24-h salt stress, 48 h 48-h salt stress

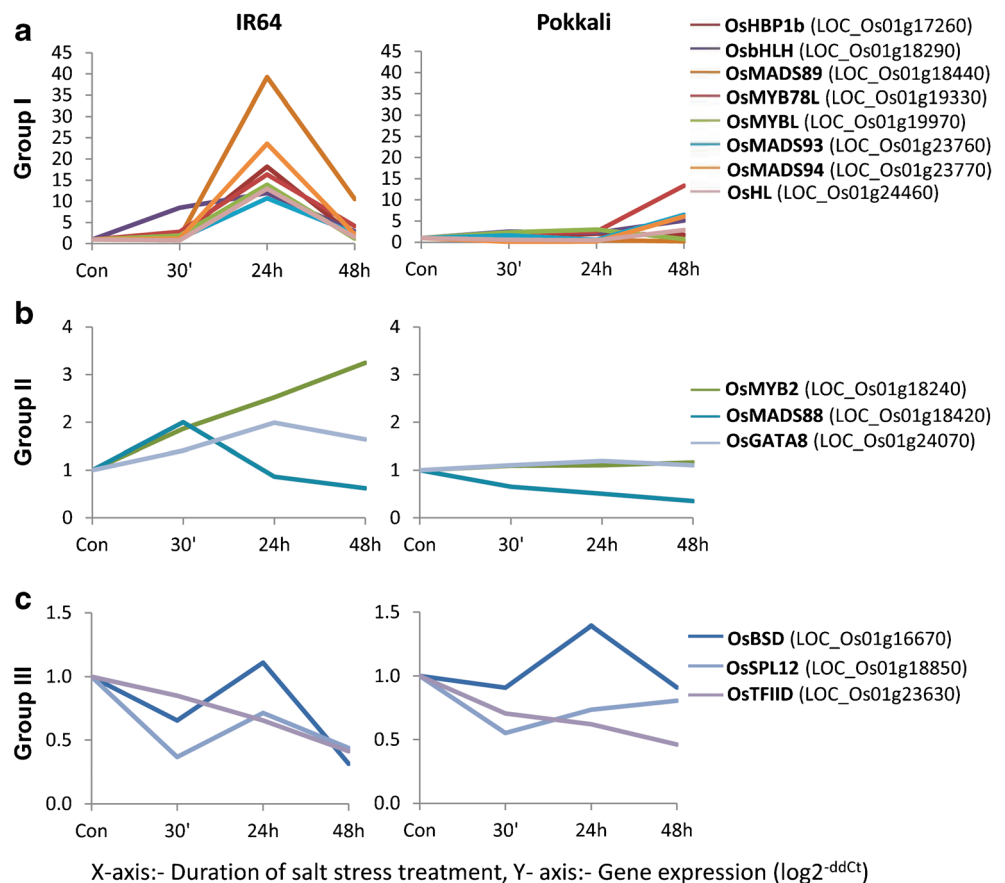
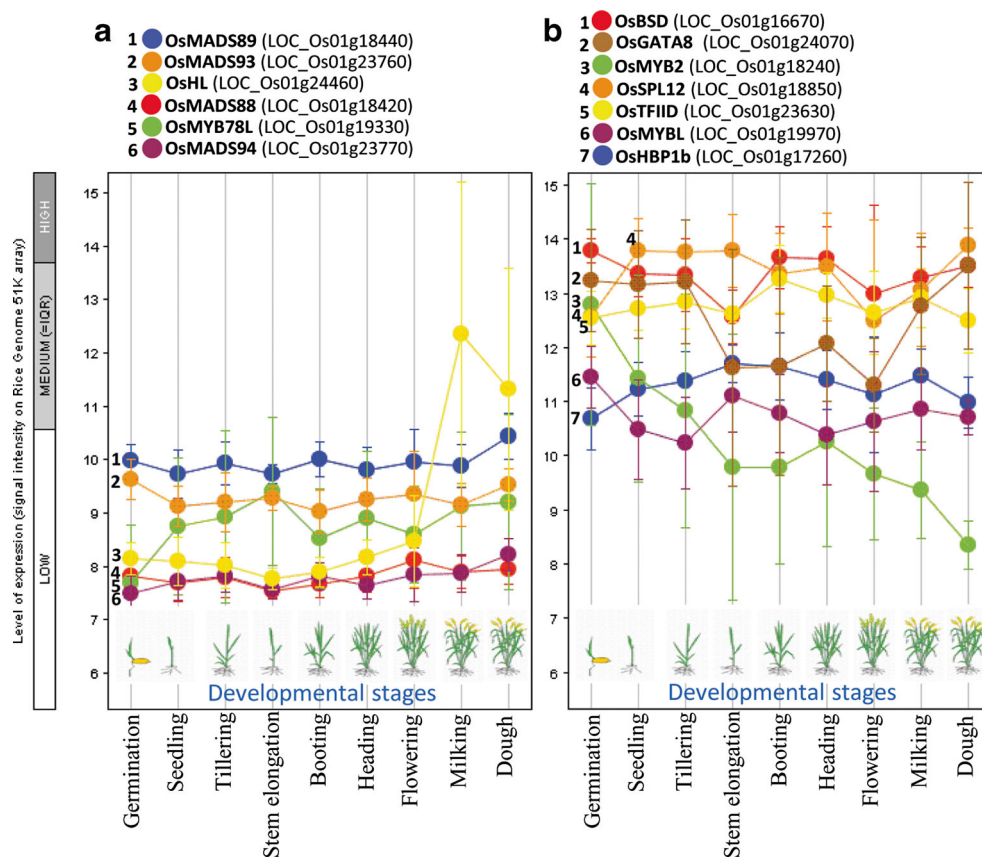


Fig. 4 Development regulated expression of SalTFs genes in rice, based on microarray data (<https://www.genevestigator.com/gv/>). The stage of development of plant is also shown diagrammatically below the figure. **(a)** Genes showing low transcript abundance during various stages of life cycle. **(b)** Genes showing high transcript abundance during the various stages of life cycle. The stages of rice shown from *left to right* are germination, seedling, tillering, stem elongation, booting, heading, flowering, milk and dough



accumulation, these genes could be further classified into three groups as shown in Fig. 3. Members of Group I showed highest transcript abundance at 24 h of salinity stress in IR64, after which they exhibited a sharp decline. However, the same set of genes showed little or no change in expression up to 24 h of stress in Pokkali, after which they showed induction. Second group of members were those which showed upregulation after 30 min of stress in the sensitive genotype IR64 but did not show any change in tolerant genotype Pokkali. Third group of genes were those where initial decline (after 30 min) in transcript abundance was reported in both the genotypes with further increase after 24 h followed by a sharp decline in transcript abundance at 48 h of stress.

Expression of SalTFs is further fine-tuned with respect to the developmental stage of the rice plant

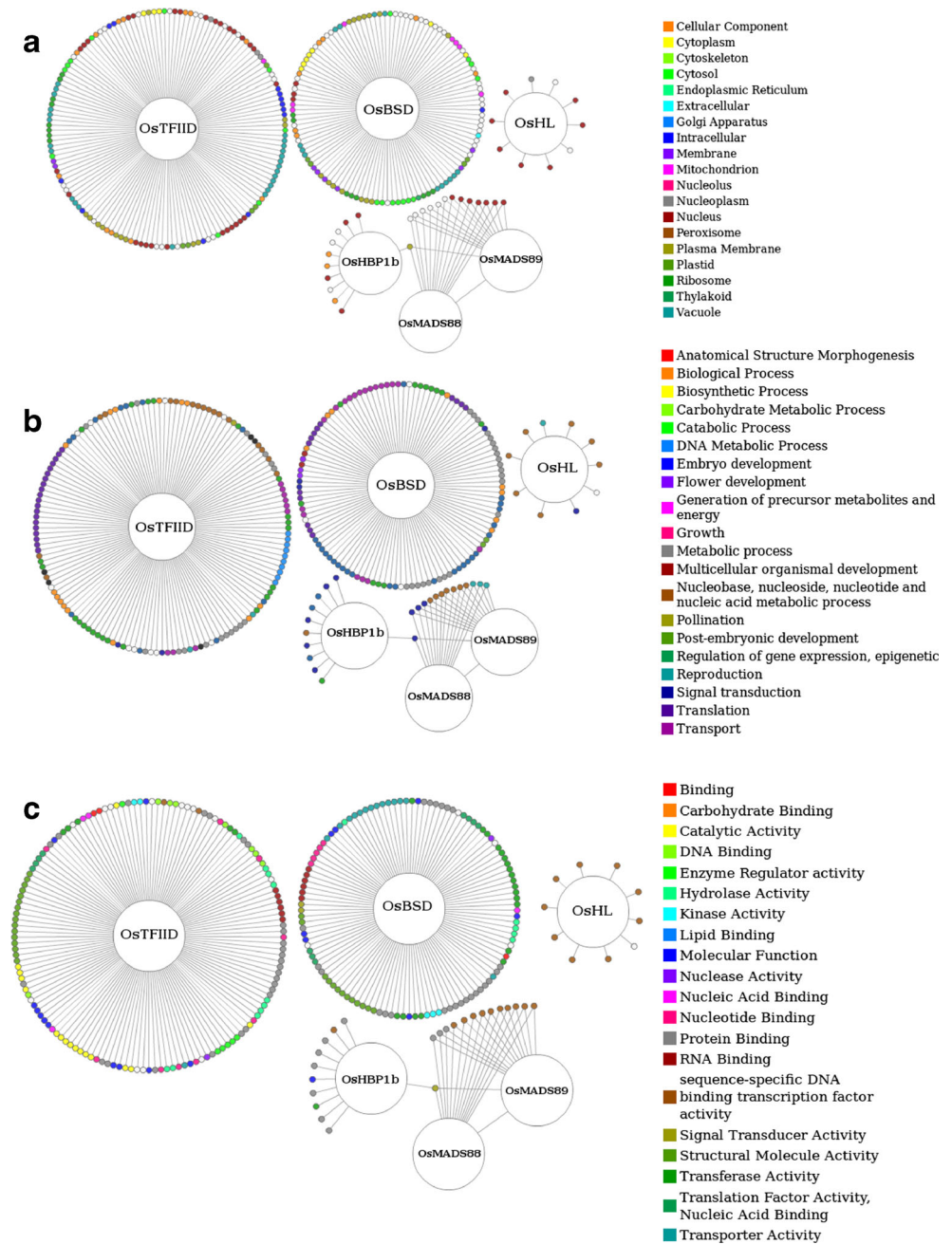
In order to dissect out the role of SalTF encoding genes at various growth stages in rice, we have used the expression datasets publicly available in the genevestigator database (<https://www.genevestigator.com/gv/>). This database gives a reliable normalized expression data about rice genes. Using this tool, we could analyse the expression of SalTFs in various tissues of rice from distinct developmental stages viz.

germination, seedling, tillering, stem elongation, booting, heading, flowering, milk and dough stage. Based on their expression patterns, two clear groups of SalTFs could be easily seen. One group is where the expression was low (Fig. 4a), while the other group showed medium to very high transcript abundance (Fig. 4b). The first group comprised of six members viz. *OsMADS88*, *OsMADS89*, *OsMYB78L*, *OsMADS93*, *OsMADS94* and *OsHL* which showed relatively low transcript abundance at almost all the growth stages of rice, except for *OsHL* which showed higher expression at milk and dough stage of rice (Fig. 4a). On the other hand, seven SalTFs, namely *OsBSD*, *OsHBP1b*, *OsMYB2*, *OsSPL12*, *OsMYBL*, *OsTFIID* and *OsGATA8* were found to have relatively higher expression, except for *OsMYB2*, which showed low expression at milk, and dough stage of rice constituted the second group of SalTFs (Fig. 4b). The expression data for *OsbHLH* was not found in the genevestigator database.

Tos17 mutant analysis for SalTFs relates them with their contribution in plant growth and development

Mutant libraries can assist in developing an understanding of the role of specific genes in an organism. In rice, mutant libraries have been developed using endogenous retrotransposon

Fig. 5 Interaction of SalTFs with other proteins as predicted based on protein–protein interaction data obtained from TIGR (<http://rice.plantbiology.msu.edu/>). The interacting proteins were classified on the basis of their Gene Ontology (GO) classification, namely (a) component, (b) process and (c) function. The figure is made using open source Cytoscape software used for visualizing complex networks



Tos17 tags, which essentially integrate itself into the genic regions, as its hotspots for integration are distributed throughout the genome (Miyao et al. 2003). We have performed a search in the *Tos17* retrotransposon insertion mutants available from Rice *Tos17* Insertion Mutant Database (<https://tos.nias.affrc.go.jp/>). Among the 14 SalTFs genes analysed in the present study, seven SalTFs, namely *OsHBP1b*, *OsMYB2*, *OsMADS89*, *OsSPL12*, *OsMYBL*, *OsMADS94* and *OsGATA8* were found in the database. These mutants for SalTFs showed the phenotypic character of sterility, low fertility and vivipary

(Table 3), indicating their critical role(s) in survival and reproductive success of the plant.

Protein interaction analysis of SalTFs indicated them to be involved in diverse functions in plant system

TFs are well known as the master regulators of expression of various genes at the level of transcription. TFs interact with diverse set of genes and proteins, which directly or indirectly play major role in determining the response of plants towards stress. In

order to hypothesize the role of these SalTFs, we have analysed their interaction with the other proteins using rice protein–protein interaction data. Further, for understanding the impact of interaction of SalTFs with other proteins, we have classified the interacting proteins on the basis of their Gene Ontology (GO) classification, namely compartment, process and function. In case of compartments, that is, the localization of proteins interacting with SalTFs, maximum number (131) were found to be in the various cellular component followed by various intracellular proteins (105). Other members were found to be compartmentalized in the cytoskeleton (82), extracellular, nucleoplasm (63) and plasma membrane (61) (Fig. 5a). In terms of process involving proteins having interaction with SalTFs, maximum numbers of interacting proteins were found to be associated with growth (332) and reproduction (113). Also, some proteins were found to be associated with the process of metabolite generation and energy (71) along with the other metabolic processes (65) (Fig. 5b). The proteins, which have been found to be interacting with the SalTFs, were observed to be involved in various binding functions (108), such as DNA binding, other nucleotide binding and various other interacting functions. Some proteins (total 38) were also found to be involved in the catalytic functions (Fig. 5c). This analysis clearly suggests diverse roles for these SalTFs in a plant system.

SalTFs exhibit SNPs and InDels in both upstream promoter as well as in genic regions

In order to decipher the SNPs in the SalTFs, we relied on the whole genome single-nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) identified in the rice genotypes having varied response for salinity stress. In the current analysis, we have analysed the SNPs and InDels in three rice genotypes viz IR64 and Pokkali with reference to the Nipponbare rice genome. The SNPs found in the *Saltol* QTLs were classified into the upstream promoter or the genic regions (Table S1). The genes such as *OsBSD*, *OsHBP1b*, *OsMADS89*, *OsMADS93* and *OsHL* were observed to carry 5–7 SNPs in their upstream promoter region (Table S1). The maximum number of SNPs in the upstream promoter region was 12 and nine in *OsTFIID* and *OsSPL12*, respectively. The minimum number of SNPs in the upstream promoter region, i.e. only one, was found in *OsGATA8* and *OsMADS94* gene. No SNPs were observed in the upstream region of the two SalTFs, namely *OsMYBL* and *OsMYB78L*. Interestingly, *OsMYBL* showed SNPs only in the genic region and not in the upstream promoter region. Further, in the genic region, maximum number of SNPs were observed in *OsMYBL* and *OsHBP1b*, 21 and 17, respectively. Interestingly, *OsBSD*, *OsMADS89*, *OsMADS93* and *OsMADS94* were found to have SNPs only in the upstream promoter region. The SNPs found in the upstream promoter and genic regions have been

depicted in the *Saltol* QTL region of the chromosome I of rice (Figure S1).

We have also searched for the presence of Insertion/Deletion (InDels) in SalTFs. Among all the SalTFs, *OsBSD*, *OsHBP1b*, *OsMADS89*, *OsSPL12*, *OsMYBL*, *OsTFIID*, *OsMADS93*, *OsGATA8* and *OsHL* showed the presence of InDels in their intergenic and upstream promoter regions (Table S2).

Discussion

Response to salinity stress exhibited by a plant has been proven to be highly complex and integrated and comprised of numerous genes operating at tandem. Over the years, a number of attempts have been made to unveil this complex response architecture using comparative genomics approach (Karan et al. 2009; Kumari et al. 2009; Walia et al. 2009; Mangelsen et al. 2011). In rice, *Saltol* QTL is localized on the chromosome I contributing to more than 60 % of the phenotypic variation in salinity tolerance in rice (Bonilla et al. 2002). The well-accepted significance of the *Saltol* QTL has taken a new dimension by the analysis of signalling related genes localized within *Saltol* QTL in the contrasting genotypes of rice, which has resulted in identification of a suit of new genes defining salinity stress tolerance at the seedling stage (Soda et al. 2013). The analysis presented in the current paper can be considered as an addition to the dimension, as we have analysed the various transcription factors (total 14) present in the *Saltol* QTL region of the chromosome I of rice. These transcription factors were observed to be the part of various well characterized families of TFs, namely MYB, MADS, TFIID, HLH and HL. We have analysed the gene structure of SalTFs in the current report, which showed variations in their gene structure, with a few members such as *OsHBP1b* having a relatively large intron and a very short stretch of exon. On the other hand, *OsMADS88*, *OsMADS89*, *OsMYB78L*, *OsMADS93* and *OsHL* were predicted to have no introns. The genomic structure of various genes can be correlated with respect to their evolutionary origin, while it has been documented that gene structure within the same family may differ. This has been shown through analysis for various gene families, such as MADS (Arora et al. 2007), MYB (Katiyar et al. 2012) and WRKY (Jiang et al. 2013).

qRT-PCR analysis of SalTFs in contrasting genotypes showed that salt tolerant landrace Pokkali maintains high constitutive transcript levels of most of the SalTFs. Exceptions to this observation could be found in genes encoding *OsBSD* and *OsMADS88* TF, in which constitutive expression in IR64 was higher, as compared to Pokkali. Similar studies suggested the basis of stress tolerance (observed in the tolerant genotype of plant) to be its well preparedness to handle stress by keeping its transcripts at a level higher than that of sensitive

one (Walia et al. 2007; Karan et al. 2009; Kumari et al. 2009; Soda et al. 2013).

On the basis of expression, these SalTFs could be categorized into two broad categories—one has the genes which showed minimal alterations in response to salinity stress in either of the genotypes studied here, while the other one comprises genes with high amplitude of changes in their expression in response to salinity stress (Fig. 2a, b). In a similar work, salinity responsive genome-wide expression analysis lead to categorisation of genes based on their differential expression patterns in contrasting cultivars of rice (Walia et al. 2005, 2007). Various groups of genes, including transcription factors such as, MADS-box protein, Myb-like DNA-binding domain have been found to be upregulated in sensitive genotype (Walia et al. 2005). Similarly, differential expression of genes (≥ 2 -fold) was recorded in these cultivars for transporters in response to 50 mM NaCl for 12 days thus leading to the identification of genes involved in ion homeostasis in rice (Senadheera et al. 2009). Further, on the basis of expression dynamics, SalTFs were reclassified into three groups. Group I genes showed higher expression after 24 h and decline after 48 h of stress in IR64 and all the SalTFs from category two falls in this group. However, the transcript for these genes was found to be induced progressively even after long durations of stress in the tolerant genotype of rice. Groups II and III showed increased and decreased expression, respectively, after 30 min of salinity stress in IR64. With this observation, we propose that SalTFs belonging to Group I might be involved in salinity stress responses and Groups II and III in other processes such as plant growth and development. Our study is supported by various previous observations where expression analysis carried out in roots of salt sensitive cultivar (IR29) and tolerant genotype (FL478, Pokkali and IR63731) has been reported to result in categorisation of genes based on their differential expression patterns (Cotsaftis et al. 2011). Similarly, comparative transcriptomic profiling of leaf tissue of rice carried out during late duration of salinity stress (24 h) reported upregulation of many genes encoding transcription factors (do Amaral et al. 2016).

We also investigated the microarray-based expression patterns of these SalTFs at various developmental stages of rice. In this analysis again, we could clearly see two broad groups of genes. SalTFs from Group I (shortlisted based on salinity responsive transcript analysis; Fig. 2b), i.e. *OsMADS89*, *OsMYB78L*, *OsMADS93*, *OsMADS94* and *OsHL* (*bHLH* could not be found in this database), were surprisingly found to be ‘low expressing’ at all the developmental stages of rice plant ranging from germination to dough stages (Fig. 4a). *OsHBP1b* and *OsMYBL* of Group I were found to be ‘low medium expressing’ category of expression at developmental stages (Fig. 4b). This observation clearly establishes that the molecular circuitry responsible for controlling the salinity-induced expression of these genes is totally different from the

one controlling their developmental expression. Members of Groups II and III fall in ‘medium to high expressing’ category of expression at developmental stages except *OsMADS88*.

Presence of MADS and MYB family members of SalTFs in Group I and Group II suggests their role in abiotic stress responses and in plant growth and development. Various members of MADS family have been shown to have diverse role(s) in plant growth and development (Pelaz et al. 2000; Causier et al. 2002) and abiotic stress tolerance (Fujita et al. 2004; Tran et al. 2004; Mane et al. 2007). The SalTFs belonging to the MADS family showed very high expression in response to stress, suggesting thereby their crucial role in salt stress response. Genome-wide analysis of rice (Arora et al. 2007) and *Brachypodium* (Wei et al. 2014) reported the induced expression of some of the MADS-box members under abiotic stress along with their role in floral organ development. Our results are also corroborated with the recent work of Aglawe et al. (2012) showing that some members of MADS family, in sorghum shoots, were upregulated and some were downregulated under drought stress, thus suggesting their role in floral development as well as in abiotic stress. Also, the members of TFs belonging to the MYB family showed high expression in response to salinity stress. Earlier, the role of MYB gene family has been associated with the plant development, secondary metabolism, hormone signal transduction and abiotic stress tolerance (Allan et al. 2008; Cominelli et al. 2009). In our analysis, *OsMYB2* (a R2R3-MYB) expression in IR64 increased after 30 min of salinity stress (about 2-folds) and unlike the other SalTFs, it showed continuous induction up to 48 h of salinity stress (about 3.5-folds). Continued increase in transcript abundance in salt sensitive genotype indicates their important role in response towards salinity stress. Several R2R3-MYB genes were found to be involved in regulating responses to environmental stresses such as salt, drought and cold (Agarwal et al. 2006; Yanhui et al. 2006). Overexpression of a *OsMYB2* (R2R3-MYB gene) in rice has shown an increased tolerance towards multiple abiotic stress (Yang et al. 2012). Abe et al. (2003) has shown bHLH and MYB related TF of Arabidopsis as a transcriptional activator in ABA signalling and its overexpression in Arabidopsis leads to increased tolerance to drought. Our data also match with the similar analysis in contrasting cultivars of rice under salinity stress showing induction of some of the MYB family member genes in sensitive and some in tolerant cultivar (Walia et al. 2005; Jiang et al. 2013).

Our analysis showed high accumulation of transcripts of Group I members viz *OsbHLH*, *OsHBP1b* and *OsHL* under salinity stress at 24 h in IR64, while Pokkali maintained higher transcript even at 48 h of salinity stress, suggesting their major role in salt stress response. Several studies in rice have identified genes from bHLH family, playing distinct roles in stress response, for example *OsbHLH1* in cold response (Wang et al. 2003). Another member, *OrbHLH2* from wild rice (*Oryza*

rufipogon), has been shown to be providing higher salt and osmotic stress tolerance in Arabidopsis, when overexpressed (Zhou et al. 2009). Recently, expression analysis in contrasting cultivars of rice has shown differential expression pattern for genes encoding bHLH and C2H2 TFs (Shankar et al. 2016). Expression data presented in this paper gets a direct support from a recent work from our group where overexpression of OsHBP1b from rice in tobacco has been shown to be correlated with higher tolerance to salinity and oxidative stress (Lakra et al. 2014).

We have observed higher gene expression in members of Groups II and III, viz *OsMYB2*, *OsSPL12*, *OsTFIID*, *OsGATA8* and *OsBSD* at all the developmental stages of rice indicating their exigent role in plant growth. Our data is corroborated with various reports indicating possible role of OsMYB2 in rice (Yanhui et al. 2006), *Squamosa promoters binding (SPB)-like protein* in rice and Arabidopsis (Shao et al. 1999) and Arabidopsis (Shikata et al. 2009), GATA domain containing protein in rice and Arabidopsis (Wang et al., 2009; Richter et al. 2013) and BSD domain containing protein in banana (Ba et al. 2014) in plant growth and development.

The analysis for BSD protein in plants is limited. However, AtBSD1 of Arabidopsis has been known to be expressed in nucleus and having transcriptional activation domain and is expressed in all the tissues but not induced by abiotic stresses (Park et al. 2009). Our analysis suggests very little response of *OsBSD* gene during the salt stress. Similar results were obtained for the OsGATA TF, which has been known for its role in shoot and flower development in Arabidopsis and rice (Zhao et al. 2004; Wang et al. 2009; Hudson et al. 2013). The expression analysis using qRT-PCR showed that IR64 undergoes a sort of complete reprogramming with significant alterations in its transcriptome towards salinity stress, while the response machinery in Pokkali remains relatively stable with its response, suggesting thereby the well preparedness of the latter for handling the exposure to salinity stress.

Analysis of rice *Tos17* mutants of SalTFs genes reveal their important role in plant survival and yield under normal conditions. These *Tos17* mutants either lost their fertility or were unable to complete their life cycle. Further protein–protein interaction analysis of SalTFs corroborated with our expression analysis, which showed that SalTFs from Group II viz OsTFIID and OsBSD interacts with maximum number of proteins present in different cell components, involved in various cellular processes and proteins with different function (Fig. 5). However, SalTFs from Group I which is highly expressed under salinity stress viz OsHL and OsHBP1b interacts with few proteins showing their specific role, i.e. salinity stress response. Protein–protein interaction analysis also showed the interaction of different SalTFs from Group I and Group II with common proteins indicating cross talk among them.

A recent study targeting discovery of the DNA polymorphism in IR64 and Pokkali leads to the identification of the

salinity responsive genes which showed differential expression and harbour several SNPs or large-effect InDels in their promoter or genic region (Jain et al. 2014). We have analysed SNPs and InDels in upstream and genic regions of SalTFs genes, which identified variable numbers of SNPs and/or InDels in contrasting genotypes viz. IR64 and Pokkali. Kim et al. (2009) reported the presence of single feature polymorphism (SFP) which include SNPs and InDels in many genes located within *Saltol* QTL region of IR29 (sensitive) and Pokkali (tolerant) genotypes. Promoters which have TFs binding sites play important role in transcription, and presence of SNPs which can lead to changes in *cis*-acting elements in contrasting cultivars may be one of the reasons for their differential expression. Recently, expression analysis in contrasting rice genotypes viz. IR64 and Pokkali showed differential expression of 507 genes under salinity stress among which a large number of genes encoding members of bHLH and C2H2 TFs were observed (Shankar et al. 2016). Taken together, our studies open up a new opportunity to dissect out the molecular basis of differential regulation of these genes in response to salinity stress and plant growth and development.

Conclusions

The analysis presented in the paper reveals several unique features of seedlings of rice in relation to their response towards salinity stress. Expression of genes encoding ‘transcription factors’ localized within *Saltol* QTL (SalTFs) plays a major role, directly or indirectly, in salinity stress response. Apart from salinity, higher transcript abundance of some of the SalTFs at various developmental stages suggests their role in plant growth and development. Protein interaction analysis of SalTFs highlighted the interaction of SalTFs with other proteins, thus revealing that the members might be playing a crucial role in salinity stress response. High expression of selected transcription factors indicates their importance in the response machinery of rice plant. SNPs and InDels are known to play a major role in the expression of the genes, as SNPs in the promoter region might interfere with the binding of TFs required for the expression of the gene. Thus, the current study has opened up a new vista to dissect out the molecular basis of differential regulation of various genes in response to salinity stress.

Acknowledgments This work was financially supported by the Department of Science and Technology (Indo-Sri Lanka collaborative project). KKN thanks the Department of Biotechnology, India, for providing DBT-JRF fellowship during his PhD programme. HRK acknowledges the financial assistance received as DST-INSPIRE Faculty award.

References

- Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Arabidopsis AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell* 15:63–78
- Agarwal M, Hao Y, Kapoor A, Dong CH, Fujii H, Zheng X, Zhu JK (2006) A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. *J Biol Chem* 281:37636–37645
- Aglawe SB, Fakrudin B, Patole CB, Bhairappanavar SB, Koti RV, Krishnaraj PU (2012) Quantitative RT-PCR analysis of 20 transcription factor genes of MADS, ARF, HAP2, MBF and HB families in moisture stressed shoot and root tissues of sorghum. *Physiol Mol Biol Plants* 18(4):287–300
- Akpınar BA, Avsar B, Lucas SJ, Budak H (2012) Plant abiotic stress signaling. *Plant Signal Behav* 7(11):1450–1455
- Akpınar BA, Lucas SJ, Budak H (2013) Genomics approaches for crop improvement against abiotic stress. *Sci World J* 2013:361921
- Allan AC, Hellens RP, Laing WA (2008) MYB transcription factors that colour our fruit. *Trends Plant Sci* 13:99–102
- Arora R, Agarwal P, Ray S, Singh AK, Singh VP, Tyagi AK, Kapoor S (2007) MADS-box gene family in rice, genome-wide identification, organization and expression profiling during reproductive development and stress. *BMC Genomics* 8:242
- Ba LJ, Shan W, Xiao YY, Chen JY, Lu WJ, Kuang JF (2014) A ripening-induced transcription factor MaBSD1 interacts with promoters of MaEXPI/2 from banana fruit. *Plant Cell Rep* 33(11):1913–1920
- Baldoni E, Bagnaresi P, Locatelli F, Mattana M, Genga A (2016) Comparative leaf and root transcriptomic analysis of two rice japonica cultivars reveals major differences in the root early response to osmotic stress. *Rice* 9(1):25
- Bohnert HJ, Gong Q, Li P, Ma S (2006) Unraveling abiotic stress tolerance mechanisms—getting genomic going. *Curr Opin Plant Biol* 9: 180–188
- Bonilla P, Dvorak J, Mackill D, Deal K, Gregorio G (2002) RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *Philippine J Agric Sci* 85:68–76
- Budak H, Kantar M, Kurtoglu KY (2013) Drought tolerance in modern and wild wheat. *Sci World J* 2013:548246
- Causier B, Kieffer M, Davies B (2002) MADS-box genes reach maturity. *Science* 296:275–276
- Chinnusamy V, Jagendorf A, Zhu JK (2005) Understanding and improving salt tolerance in plants. *Crop Sci* 45:437–448
- Cominelli E, Tonelli C (2009) A new role for plant R2R3-MYB transcription factors in cell cycle regulation. *Cell Res* 19:1231–1232
- Cotsaftis O, Plett D, Johnson AA, Walia H, Wilson C, Ismail AM, Close TJ, Tester M, Baumann U (2011) Root-specific transcript profiling of contrasting rice genotypes in response to salinity stress. *Mol Plant* 4(1):25–41
- do Amaral MN, Arge LW, Benitez LC, Danielowski R, Silveira SF, Farias Dda R, de Oliveira AC, da Maia LC, Braga EJ (2016) Comparative transcriptomics of rice plants under cold, iron, and salt stresses. *Funct Integr Genomics* 16(5):567–579
- El-Shabrawi H, Kumar B, Kaul T, Reddy MK, Singla-Pareek SL, Sopory SK (2010) Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. *Protoplasma* 245(1–4):85–96
- Fan M, Huang Y, Zhong Y, Kong Q, Xie J, Niu M, Xu Y, Bie Z (2014) Comparative transcriptome profiling of potassium starvation responsiveness in two contrasting watermelon genotypes. *Planta* 239:397–410
- Fujita M, Fujita Y, Maruyama K, Seki M, Hiratsu K, Ohme-Takagi M, Tran LSP, Yamaguchi-Shinozaki K, Shinozaki K (2004) A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. *Plant J* 39:863–876
- Gao R, Duan K, Guo G, Du Chen Z, Li L, He T, Lu R, Huang J (2013) Comparative transcriptional profiling of two contrasting barley genotypes under salinity stress during the seedling stage. *Int J Genomics* 3D:972852
- Gelli M, Duo Y, Konda AR, Zhang C, Holding D, Dweikat I (2014) Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. *BMC Genomics* 15:179
- Ghomi K, Rabiei B, Sabouri H, Sabouri A (2013) Mapping QTLs for traits related to salinity tolerance at seedling stage of rice (*Oryza sativa* L.). *OMICS* 17:242–251
- Gregorio GB, Senadhira D, Mendoza RD, Manigbas NL, Roxas JP, Guerta CQ (2002) Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crops Res* 76:91–101
- Gu XY, Mei MT, Yan XL, Zheng SL, Lu YG (2000) Preliminary detection of quantitative trait loci for salt tolerance in rice. *Chin J Rice Sci* 14:65–70
- Hudson D, Guevara DR, Hand AJ, Xu Z, Hao L, Chen X, Zhu T, Bi YN, Rothstein SJ (2013) Rice cytokinin GATA transcription factor1 regulates chloroplast development and plant architecture. *Plant Physiol* 162:132–144
- Islam MR, Salam MA, Hassan L, Collard BCY, Singh RK, Gregorio GB (2011) QTL mapping for salinity tolerance at seedling stage in rice. *Emir J Food Agric* 23:137–146
- Jain M, Moharana KC, Shankar R, Kumari R, Garg R (2014) Genome-wide discovery of DNA polymorphisms in rice cultivars with contrasting drought and salinity stress response and their functional relevance. *Plant Biotechnol J* 12:253–264
- Jiang SY, Ma A, Ramamoorthy R, Ramachandran S (2013) Genome-wide survey on genomic variation, expression divergence, and evolution in two contrasting rice genotypes under high salinity stress. *Genome Biol Evol* 5(11):2032–2050
- Joshi R, Karan R, Singla-Pareek SL, Pareek A (2016a) Ectopic expression of Pokkali phosphoglycerate kinase-2 (OsPGK2-P) improves yield in tobacco plants under salinity stress. *Plant Cell Rep* 35(1):27–41
- Joshi R, Wani SH, Singh B, Bohra A, Dar ZA, Lone AA, Pareek A, Singla-Pareek SL (2016b) Transcription factors and plants response to drought stress: current understanding and future directions. *Front Plant Sci* 7:1029
- Kantar M, Lucas SJ, Budak H (2011) Drought stress: molecular genetics and genomics approaches. In: Kader JC, Delseny M (eds) *Advances in Botanical Research*, vol 57. Academic Press, Burlington, pp 445–493
- Karan R, Singla-Pareek SL, Pareek A (2009) Histidine kinase and response regulator genes as they relate to salinity tolerance in rice. *Funct Integr Genomics* 9:411–417
- Katiyar A, Smita S, Lenka K, Rajwanshi R, Chinnusamy V, Bansal KC (2012) Genome-wide classification and expression analysis of MYB transcription factor families in rice and Arabidopsis. *BMC Genomics* 10:544
- Kim SH, Bhat PR, Cui X, Walia H, Xu J, Wanamaker S, Ismail AM, Wilson C, Close TJ (2009) Detection and validation of single feature polymorphisms using RNA expression data from a rice genome array. *BMC Plant Biol* 9:65
- Kumar G, Purty RS, Sharma MP, Singla-Pareek SL, Pareek A (2009) Physiological responses among Brassica species under salinity stress show strong correlation with transcript abundance for SOS pathway-related genes. *J Plant Physiol* 166:507–520
- Kumar R, Mustafiz A, Sahoo KK, Sharma V, Samanta S, Sopory SK, Pareek A, Singla-Pareek SL (2012a) Functional screening of cDNA library from a salt tolerant rice genotype Pokkali identifies mannose-1-phosphate guanyl transferase gene (OsMPG1) as a key member of salinity stress response. *Plant Mol Biol* 79:555–568

- Kumar G, Kushwaha HR, Panjabi-Sabharwal V, Kumari S, Joshi R, Karan R, Mittal S, Singla-Pareek SL, Pareek A (2012b) Clustered metallothionein genes are co-regulated in rice and ectopic expression of OsMT1e-P confers multiple abiotic stress tolerance in tobacco via ROS scavenging. *BMC Plant Biol* 12:107
- Kumari S, Panjabi-Sabharwal V, Kushwaha HR, Sopory SK, Singla-Pareek SL, Pareek A (2009) Transcriptome map for seedling stage specific salinity stress response indicates a specific set of genes as candidate for saline tolerance in *Oryza sativa* L. *Funct Integr Genomics* 9:109–123
- Lafitte HR, Yongsheng G, Yan S, Li ZK (2007) Whole plant responses, key processes, and adaptation to drought stress: the case of rice. *J Exp Bot* 58:169–175
- Lakra N, Nutan KK, Singla-Pareek SL, Pareek A (2013) Modulating the expression of transcription factors, an attractive strategy for raising abiotic stress tolerant plants. In: *Stress-Mediated Signaling in Plants II*, *Plant Stress* 7, Special Issue 1, (Pandey, GK, ed), pp. 7:84–99 Japan: Global Science Books
- Lakra N, Nutan KK, Das P, Anwar K, Singla-Pareek SL, Pareek A (2014) A nuclear-localized histone-gene binding protein from rice (OsHBP1b) functions in salinity and drought stress tolerance by maintaining chlorophyll content and improving the antioxidant machinery. *J Plant Physiol* 176:36–46
- Latchman DS (2001) Transcription factors: bound to activate or repress. *Trends Biochem Sci* 26(4):211–213
- Lin HX, Zhu MZ, Yano M, Gao JP, Liang ZW, Su WA, Hu XH, Ren ZH, Chao DY (2004) QTLs for Na⁺ and K⁺ uptake of the shoots and roots controlling rice salt tolerance. *Theor Appl Genet* 108:253–260
- Lutts S, Kinet J-M, Bouharmont J (1995) Changes in plant responses to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *J Exp Bot* 46:1843–1852
- Mane SP, Vasquez-Robinet C, Sioson AA, Heath LS, Grene R (2007) Early PLD α -mediated events in response to progressive drought stress in Arabidopsis: a transcriptome analysis. *J Exp Bot* 58(2): 241–252
- Mangelsen E, Kilian J, Harter K, Jansson C, Wanke D, Sundberg E (2011) Transcriptome analysis of high-temperature stress in developing barley caryopses, early stress responses and effects on storage compound biosynthesis. *Mol Plant* 4:97–115
- Miyao A, Tanaka K, Murata K, Sawaki H, Takeda S, Abe K, Shinozuka Y, Onosato K, Hirochika H (2003) Target site specificity of the *Tos17* retrotransposon shows a preference for insertion within genes and against insertion in retrotransposon-rich regions of the genome. *Plant Cell* 15(8):1771–1780
- Negrão S, Courtois B, Ahmadi N, Abreu I, Saibo N, Oliveira MM (2011) Recent updates on salinity stress in rice, from physiological to molecular responses. *Crit Rev Plant Sci* 30:329–377
- Nongpiur R, Soni P, Singla-Pareek SL, Pareek A (2012) Histidine kinases in plants, cross talk between hormone and stress responses. *Plant Signal Behav* 7:1230–1237
- Nongpiur RC, Singla-Pareek SL, Pareek A (2016) Genomics approaches for improving salinity stress tolerance in crop plants. *Curr Genomics* 17(4):343–357
- Pareek A, Sopory SK, Bohnert H, Govindjee (2010) *Abiotic Stress Adaptation in Plants: physiological, molecular and genomic foundation*. Springer, New York
- Park J, Kim MJ, Jung SJ, Suh MC (2009) Identification of a novel transcription factor, AtBSD1, containing a BSD domain in *Arabidopsis thaliana*. *J Plant Biol* 52:141–146
- Pelaz S, Ditta GS, Baumann E, Wisman E, Yanofsky MF (2000) B and C floral organ identity functions require SEPALLATA MADS-box genes. *Nature* 405:200–203
- Prasad SR, Bagali PG, Shailaja H, Shashidhar HE, Hittalmani S (2000) Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). *Curr Sci* 78:162–164
- Richter R, Bastakis E, Schwechheimer C (2013) Cross-repressive interactions between SOC1 and the GATAs GNC and GNL/CGA1 in the control of greening, cold tolerance, and flowering time in Arabidopsis. *Plant Physiol* 162:1992–2004
- Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G (2000) Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290(5499):2105–2110
- Sabouri H, Rezai AM, Moumeni A, Kavousi A, Katouzi M, Sabouri A (2009) QTLs mapping of physiological traits related to salt tolerance in young rice seedlings. *Biol Plant* 53:657–662
- Sahi C, Singh A, Blumwald E, Grover A (2006) Beyond osmolytes and transporters, novel plant salt stress tolerance-related genes from transcriptional profiling data. *Physiol Plant* 127:1–9
- Senadheera P, Singh RK, Maathuis FJ (2009) Differentially expressed membrane transporters in rice roots may contribute to cultivar dependent salt tolerance. *J Exp Bot* 60(9):2553–2563
- Shankar R, Bhattacharjee A, Jain M (2016) Transcriptome analysis in different rice cultivars provides novel insights into desiccation and salinity stress responses. *Sci Rep* 6:23719
- Shao CX, Takenda Y, Hatano S, Matusuoka M, Hiranol HY (1999) Rice gene encoding the SBP domain protein, which is a new type of transcription factor controlling plant development. *Rice Genet Newsl* 16:114
- Sharma R, Mishra M, Gupta B, Parsania C, Singla-Pareek SL, Pareek A (2015) De novo assembly and characterization of stress transcriptome in a salinity-tolerant variety CS52 of *Brassica juncea*. *PLoS One* 105:e0126783
- Shikata M, Koyama T, Mitsuda N, Ohme-Takagi M (2009) Arabidopsis SBP-box genes *SPL10*, *SPL11* and *SPL2* control morphological change in association with shoot maturation in the reproductive phase. *Plant Cell Physiol* 50:2133–2145
- Singh AK, Kumar R, Pareek A, Sopory SK, Singla-Pareek SL (2012) Overexpression of rice CBS domain containing protein improves salinity, oxidative and heavy metal resistance in transgenic tobacco. *Mol Biotechnol* 52:205–216
- Soda N, Kushwaha HR, Soni P, Singla-Pareek SL, Pareek A (2013) A suite of new genes defining salinity stress tolerance in seedlings of contrasting rice genotypes. *Funct Integr Genomics* 13:351–365
- Soda N, Sharan A, Gupta B, Singla-Pareek SL, Pareek A (2016) Evidence for nuclear interaction of a cytoskeleton protein (OsIFL) with metallothionein and its role in salinity stress tolerance. *Sci Rep* 6:34762
- Sreenivasulu N, Sopory SK, Kavi Kishor PB (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. *Gene* 388:1–13
- Thomson MJ, Ocampo MD, Egdane J, Rahman MA, Sajise AG, Adorada DL, Tumimbang-Raiz E, Blumwald E, Seraj ZI, Singh RK, Gregorio GB, Ismail AM (2010) Characterizing the Saltol quantitative trait locus for salinity tolerance in rice. *Rice* 3:148–160
- Tran LSP, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Isolation and functional analysis of Arabidopsis stress inducible NAC transcription factors that bind to a drought responsive cis-element in the early responsive to dehydration stress 1 promoter. *Plant Cell* 16:2481–2498
- Walia H, Wilson C, Condamine P, Liu X, Ismail AM, Zeng L, Wanamaker SI, Mandal J, Xu J, Cui X, Close TJ (2005) Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. *Plant Physiol* 139:822–835
- Walia H, Wilson C, Zeng L, Ismail AM, Condamine P, Close TJ (2007) Genome-wide transcriptional analysis of salinity stressed japonica and indica rice genotypes during panicle initiation stage. *Plant Mol Biol* 63:609–623

- Walia H, Wilson C, Ismail AM, Close TJ, Cui X (2009) Comparing genomic expression patterns across plant species reveals highly diverged transcriptional dynamics in response to salt stress. *BMC Genomics* 10:398
- Wang YJ, Zhang ZG, He XJ, Zhou HL, Wen YX, Dai JX, Zhang JS, Chen SY (2003) A rice transcription factor OsbHLH1 is involved in cold stress response. *Theor Appl Genet* 107:1402–1409
- Wang L, Yin H, Qian Q, Yang J, Huang C, Hu X, Luo D (2009) NECK LEAF 1, a GATA type transcription factor, modulates organogenesis by regulating the expression of multiple regulatory genes during reproductive development in rice. *Cell Res* 19(5):598–611
- Wei B, Zhang RZ, Guo JJ, Liu DM, Li AL, Fan RC, Mao L, Zhang XQ (2014) Genome-wide analysis of the MADS-box Gene Family in *Brachypodium distachyon*. *PLoS One* 9(1):e84781
- Witzel K, Matros A, Strickert M, Kaspar S, Peukert M, Mühling KH, Börner A, Mock HP (2014) Salinity stress in roots of contrasting barley genotypes reveals time-distinct and genotype-specific patterns for defined proteins. *Mol Plant* 7:336–355
- Yang A, Dai X, Zhang WH (2012) A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice. *J Exp Bot* 63(7):2541–2556
- Yanhui C, Xiaoyuan Y, Kun H, Meihua L, Jigang L, Zhaofeng G, Zhiqiang L, Yunfei Z, Xiaoxiao W, Xiaoming Q, Yunping S, Li Z, Xiaohui D, Jingchu L, Xing-Wang D, Zhangliang C, Hongya G, Li-Jia Q (2006) The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. *Plant Mol Biol* 60:107–124
- Yeo AR, Yeo ME, Flowers SA, Flowers TJ (1990) Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *Theor Appl Genet* 79:377–384
- Zhang T, Zhao X, Wang W, Pan Y, Huang L, Liu X, Zong Y, Zhu L, Yang D, Fu B (2012) Comparative transcriptome profiling of chilling stress responsiveness in two contrasting rice genotypes. *PLoS One* 7(8):e43274
- Zhao Y, Medrano L, Ohashi K, Fletcher JC, Yu H, Sakai H, Meyerowitz EM (2004) HANABATARANU is a GATA transcription factor that regulates apical meristem and flower development in Arabidopsis. *Plant Cell* 16:2586–2600
- Zhou J, Li F, Wang JL, Ma Y, Chong K, Xu YY (2009) Basic helix-loop-helix transcription factor from wild rice (OrbHLH2) improves tolerance to salt- and osmotic stress in Arabidopsis. *J Plant Physiol* 166(12):1296–1306