Journal of Experimental Botany
doi:10.1093/jxb/erz368 Advance Access Publication 15 October 2019

RESEARCH PAPER

The *Saltol* QTL-localized transcription factor OsGATA8 plays an important role in stress tolerance and seed development in Arabidopsis and rice

Kamlesh K Nutan[1], Sneh L Singla-Pareek[2,] and Ashwani Pareek[1,*,]

[1] Stress Physiology and Molecular Biology Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India
[2] Plant Stress Biology, International Centre for Genetic Engineering and Biotechnology, New Delhi 110067, India

* Correspondence: ashwanip@mail.jnu.ac.in

Received 30 April 2019; Editorial decision 30 July 2019; Accepted 6 September 2019

Editor: Om Dhankher, University of Massachusetts, Amherst, USA

Abstract

GATA represents a highly conserved family of transcription factors reported in organisms ranging from fungi to angiosperms. A member of this family, OsGATA8, localized within the *Saltol* QTL in rice, has been reported to be induced by salinity, drought, and ABA. However, its precise role in stress tolerance has not yet been elucidated. Using genetic, molecular, and physiological analyses, in this study we show that OsGATA8 increases seed size and tolerance to abiotic stresses in both Arabidopsis and rice. Transgenic lines of rice were generated with 3-fold overexpression of OsGATA8 compared to the wild-type together with knockdown lines with 2-fold lower expression. The overexpressing lines showed higher biomass accumulation and higher photosynthetic efficiency in seedlings compared to the wild-type and knockdown lines under both normal and salinity-stress conditions. OsGATA8 appeared to be an integrator of diverse cellular processes, including K^+/Na^+ content, photosynthetic efficiency, relative water content, F_v/F_m ratio, and the stability to sub-cellular organelles. It also contributed to maintaining yield under stress, which was ~46% higher in overexpression plants compared with the wild-type. OsGATA8 produced these effects by regulating the expression of critical genes involved in stress tolerance, scavenging of reactive oxygen species, and chlorophyll biosynthesis.

Keywords: Drought, GATA, *Oryza sativa*, RNAi, salinity, *Saltol* QTL, transcription factor, overexpression, tolerance, yield.

Introduction

As sessile organisms, the growth and development of plants is greatly influenced by environmental factors such as salinity, drought, and heat (Vij and Tyagi, 2007; Pareek *et al.*, 2010). Salinity is a major factor affecting plant productivity, as it limits the uptake of water by the roots and is associated with the accumulation of toxic ions, and thus it influences the overall cellular physiology of the plant (Munns and Tester, 2008). To cope with salinity, plants have evolved a diverse set of adaptive strategies, which include accumulation of osmoprotectants, activation of membrane-bound antiporters, and triggering of numerous stress-responsive pathways, which are regulated by the orchestrated action of specific transcription factors (Chinnusamy *et al.*, 2005; Nakashima *et al.*, 2007; Joshi *et al.*, 2016; Nutan *et al.*, 2017). Thus, a large subset of stress-related genes are coordinately and differentially expressed, depending on the dose and duration of the stress imposed (Seki *et al.*, 2002; Zhu, 2002; Kumari *et al.*, 2009a; Das *et al.*, 2015; Singh *et al.*, 2015). The fine regulatory control required to achieve such precise expression patterns is provided by transcription factors (TFs). TFs influence almost every aspect of plant life,

such as growth, development, and responses to abiotic and biotic stresses (Lakra *et al.*, 2013; Joshi *et al.*, 2016).

GATA TFs are a major class of transcriptional regulators that are widely distributed in eukaryotes. They are characterized by the presence of conserved, type-IV zinc-finger motifs (C-X_2-C-X_{17-20}-C-X_2-C) followed by a highly basic region. The first evidence of their existence in plants came with the identification of GATA motifs in the regulatory regions of genes responsive to light and circadian rhythms (Argüello-Astorga and Herrera-Estrella, 1998), including many of those involved in, or related to, photosynthesis, such as the Rubisco small subunit and the chlorophyll *a*/*b* binding protein (Koch, 1996). In the genome of rice (*Oryza sativa*), a total of 28 loci encoding GATA transcription factors have been annotated (Reyes *et al.*, 2004; Gupta *et al.*, 2017). Studies on single GATA-domain-containing proteins have revealed their role in diverse phenomena in plants such as regulation of processes by light, leaf greening, seed germination, and flowering (Richter *et al.*, 2013a, b; Klermund *et al.*, 2016). In Arabidopsis, the paralogous and functionally redundant GATA transcription factors GATA, NITRATE-INDUCIBLE, CARBON METABOLISM INVOLVED (GNC) and GNC-LIKE/CYTOKININ-RESPONSIVE GATA FACTOR1 (GNL/CGA1) have been shown to be involved in the control of flowering time, leaf greening, and cold tolerance (Richter *et al.*, 2013a, 2013b). Functional studies on GNC/GNL mutants and overexpressing plants of Arabidopsis have indicated a role of these proteins downstream of signaling by hormones such as auxin, gibberellin (GA), and cytokinin (CK), and in this way they contribute to processes such as leaf greening, cold tolerance, determination of seed size, reduction/suppression of germination, flowering time, and senescence (Richter *et al.*, 2010, 2013a, 2013b; Behringer and Schwechheimer, 2015). However, the role of these TFs in other abiotic stresses such as salinity and drought have not yet been reported.

Rice shows a lot of genetic diversity, including in the response to salinity; for example, a traditional landrace, Pokkali, has been found to be tolerant, while the commercial variety IR64 has been shown to be sensitive (El-Shabrawi *et al.*, 2010). Morphological and physiological analyses of seedlings under salinity stress have demonstrated that the basis of the higher tolerance in Pokkali is higher chlorophyll and proline contents, increased shoot growth, and reduced membrane injury compared with IR64 (Kumari *et al.*, 2009a). Extensive transcriptome and proteome analyses in these genotypes has revealed differential expression of various categories of stress-related genes and proteins that contributes to the higher tolerance in Pokkali (Karan *et al.*, 2009; Kumari *et al.*, 2009a; Soda *et al.*, 2013; Nutan *et al.*, 2017; Lakra *et al.*, 2018, 2019). The *Saltol* QTL on the short arm of chromosome I in rice was identified from a recombinant inbred line population developed between Pokkali and IR29, and it has been shown to account for 62–80% of phenotypic variation under salinity stress (Bonilla *et al.*, 2002; Thomson *et al.*, 2010). The importance of this QTL in the salinity response in tolerant genotypes is indicated by the identification within *Saltol* of *SKC1*, which encodes a Na^+ selective transporter (Ren *et al.*, 2005), and by the increased abundance of transcripts of other genes involved in alleviation of salinity (Kumari *et al.*, 2009a). Consequently, *Saltol* and its constituent genes have been the focus of research targeting the improvement of salinity tolerance in high-yielding rice genotypes. Functional validation of the *Saltol* QTL has been achieved using traditional marker assisted breeding programs for varietal improvement (Singh *et al.*, 2018), as well as through transgenic technology by ectopically expressing salinity-induced genes localized in *Saltol* such as OsHBP1b and OsIF in tobacco as well as in rice (Soda *et al.*, 2018; Das *et al.*, 2019). Rice genomes modified in this way exhibit improved tolerance towards multiple abiotic stresses, thus reflecting the importance of the constituent genes that make up the QTL. Expression analysis of genes related to signaling (Soda *et al.*, 2013) and to transcription factors (Nutan *et al.*, 2017) that show higher constitutive transcript abundance in the tolerant genotype (Pokkali) and induced expression in the sensitive genotype (IR64) has led to the identification of a novel transcription factor, OsGATA8 and its characterization as playing a role in the response to salinity stress (Gupta *et al.*, 2017). Out of the 28 gene loci (encoding 35 putative GATA transcription factors) that we have found in the rice genome, only GATA8 is localized within the *Saltol* QTL (Gupta *et al.*, 2017), indicating its possible role in salinity tolerance. Additional support for this hypothesis comes from the fact that the GATA transcription factors GLN3 and GAT1 have been shown to be involved in salt tolerance in yeast (*Saccharomyces cerevisiae*; Crespo *et al.*, 2001). Based on the localization of OsGATA8 within the *Saltol* QTL, its differential regulation in rice genotypes with contrasting salinity tolerance, and the involvement of its orthologs in salt tolerance in yeast, we hypothesize that OsGATA8 may be a master regulator of stress responses in rice that determines survival and yield under stress. Although the GATA family of TFs has been a subject of interest for many years, their roles in abiotic stress tolerance have only recently started to become apparent as tools have become available for producing mutants and overexpression lines in plants. In the present study, we examined the function of OsGATA8 in providing tolerance to salinity and drought stress by using transgenic plants of rice and Arabidopsis in which the gene was either overexpressed or knocked down by RNAi. Our results demonstrate that OsGATA8 plays an important role in both species in processes associated with chlorophyll biosynthesis, ion homeostasis, scavenging of reactive oxygen species, grain size, stress tolerance, and yield.

Materials and methods

Plant growth, stress treatments, and transcript abundance analysis for rice

Seeds of rice (*Oryza sativa* cv IR64 and Pokkali) were washed with de-ionized water and soaked in dark for two days after which seeds were allowed to germinate in a hydroponic system filled with half-strength Yoshida medium (Yoshida *et al.*, 1971). The system was kept inside a growth chamber maintained at 28±1 °C, 70% humidity, under a 12/12 h light/dark cycle with illumination of 500–600 μmol m^{-2} s^{-1}. For the salinity stress treatment, 7-d-old seedlings were treated with half-strength Yoshida medium supplemented with 200 mM NaCl; controls were maintained without adding NaCl. Seedlings were harvested after 10, 20,

30 min, and 1, 2, 24, 48, and 72 h of stress as described previously (Kumari *et al.*, 2009a). At each sampling time, 2–3 seedlings within each treatment (control, salinity) were pooled to form a replicate, and RNA was extracted from three replicates per treatment. The RNA was further used for cDNA synthesis. qRT-PCR analysis was performed as described by Soda *et al.* (2013) with three replicates.

Transcript abundance analysis was carried out on leaves of field-grown plants at the tillering stage. Strips of ~2 cm were cut from the youngest fully emerged leaf of 2–3 plants and placed in half-strength Yoshida medium supplemented with 200 mM NaCl. Samples (200 mg FW) were harvested after 15 min, 30 min, 24 h, 48 h, and 72 h and used for RNA extraction. Three replicates were used, and control samples were placed in medium without NaCl.

Transcript abundance analysis was also carried out for mature plants at 60 d and 95 d after anthesis for IR64 and Pokkali, respectively. Samples were taken from three primary tillers from three different plants. Samples of leaves were taken from different parts of the plant: upper (leaf next to the flag leaf), middle (third leaf after the flag leaf), and lower (fifth leaf after the flag leaf). The stems were divided into three portions and samples taken from the upper, middle, and lower parts. The roots and inflorescences were also sampled. Each sample was placed in either half-strength Yoshida medium with 200 mM NaCl for 15 min, 30 min, 24 h, 48 h, and 72 h or in medium without NaCl (control) and used for RNA gel blotting analysis as described previously (Kumari *et al.*, 2009a).

Subcellular localization of OsGATA8–GFP fusion protein

The coding sequence of *OsGATA8* was amplified using the primer pair *OsGATA8-SL* (Supplementary Table S1 at *JXB* online), which gave the desired amplicon without the stop codon and the required restriction site at both ends for cloning in the expression vector. The PCR product was sub-cloned into the pCAMBIA1304 vector to generate pCAMBIA-*OsGATA8-GFP* containing an *OsGATA8-GFP* fusion construct under the control of the cauliflower mosaic virus (CaMV) 35S promoter. The construct was used for transformation of epidermal cells of onion (*Allium cepa*) by biolistic bombardment, as previously described (Sharan *et al.*, 2017). The transformed cells were incubated in Murashige and Skoog (MS) medium at 28 °C for 36–48 h and then observed using a FluoView FV1000 laser-scanning confocal microscope (Olympus).

Transactivation assays

In order to identify the activation domain in the transcription factor, the pGBD-C1 vector containing a GAL4 DNA-binding domain (James *et al.*, 1996) was used. Yeast one-hybrid assays were performed using the AH109 strain, which was transformed with the pGBD-C1-OsGATA8 construct. Yeast transformants were selected on single drop-out medium (SD/−Trp) for 3–5 d at 30 °C. Further, they were transferred onto double drop-out medium [SD/−Trp/−His +5 mM 3-amino-1,2,4-triazole (3-AT)] to check the activation of His reporter gene, and the transformed colonies were subsequently streaked onto triple drop-out medium (SD/−Trp/−His/−Ade) supplemented with 5 mM 3-AT. Yeast cells carrying the empty vector (pGBD-C1, containing only the GAL4 DNA-binding domain) were used as the negative control. For the positive control, OsRR26, a response regulator protein from rice that is known to exhibit auto-activation (Sharan *et al.*, 2017), was cloned into the pGBD-C1 vector.

Arabidopsis transformation, stress treatments, and phenotyping at the seedling and reproductive stages

The *OsGATA8* gene cloned in the plant expression vector (pCAMBIA1304-*OsGATA8*) was transformed into *Agrobacterium* (GV3101) and transformation of Arabidopsis was performed using the floral dip method (Clough and Bent, 1998). For abiotic stress-tolerance assays on plates, wild-type and 35S-*OsGATA8* seeds (T3) were germinated on MS medium. At 5 d after germination, seedlings from each line were carefully transferred to new MS media supplemented with different concentrations of NaCl, mannitol, ABA, or PEG. After 10 d of growth on the treatment media, root length, number of lateral roots, and the fresh weight of the seedlings were measured. For drought tolerance, 35S-OsGATA8 and wild-type (WT) seeds were germinated on MS medium, and 5-d-old seedlings were transferred to pots (10×10×8 cm) containing a mixture of vermiculite and humus (1:1). The plants were maintained in a growth chamber for 5 weeks (22 °C, 70% humidity, 12/12 h light/dark cycle, 120–150 μmol m^{-2} s^{-1}) before stress treatments were applied. For the drought treatment, water was withheld from plants for 20 d and they were then rewatered for next 3 d. For the salinity stress treatment, water was withheld for 5 d and the plants were then well irrigated for 20 d with 150 mM of NaCl solution, which was poured into a tray in which the pots were standing. Measurement of plant survival was based on the total number of seedlings surviving at the end of the stress period (for salinity) or after the 3-d recovery period (for drought). In each case, control plants were maintained in a well-watered state. For each subsequent experiment, three biological replicates were used. The following measurements were taken at the end of the 20-d treatment periods for salinity and drought stress. Chlorophyll content was determined in the cauline leaves following the method described by Lakra *et al.* (2015). *In situ* accumulation of $O_2^{\bullet-}$ and H_2O_2 were assayed in the fourth fully expanded leaf from the base of the plant, using histochemical staining by nitroblue tetrazolium (NBT) and 3, 3'-diaminobenzidine (DAB), respectively (Lakra *et al.*, 2015; Das *et al.*, 2019). The stained leaves were bleached in 95% ethanol, and after complete removal of chlorophyll, they were imaged and densiometric analysis was carried out using the ImageJ software (Schneider *et al.*, 2012). At the end of the experiment, the length and width of seeds were measured using images taken under a microscope (Nikon SMZ1500) and using the Nikon NIS-Elements software.

Rice transformation, stress treatments, and phenotyping at the seedling and reproductive stages

The gene cloned in the plant expression vector pCAMBIA1304-*OsGATA8* and RNAi vector pFGC1008-*OsGATA8* were transformed into *Agrobacterium* (LBA4404). Transformation and regeneration were performed in the IR64 rice callus according to Sahoo *et al.* (2011). After 4 d of salinity treatment at 200 mM NaCl in the hydroponics system, seedlings were harvested for physiological analyses. Control seedlings without NaCl treatment were also harvested. The parameters measured were fresh weight, shoot length, number of lateral roots per seedling, root length, number of leaves per seedling, relative water content, electrolyte leakage, proline content, and K^+/Na^+ in shoots. The ultrastructure of chloroplast was examined in leaves after 2 d of salinity stress using an electron microscope (JEOL) in the Advanced Instrument Research Facility, Jawaharlal Nehru University according to the protocol described by Das *et al.* (2019).

To evaluate the tolerance of plants at the reproductive stage, T2 seeds of the WT, overexpressing (OE), and knockdown (KD) lines were sown in soil in pots (15 cm diameter). Five pots each of WT, OE7, OE24, KD41, and KD81 were used and salinity stress at 200 mM NaCl was imposed after 60 d of growth. Photosynthetic parameters (F_v/F_m, net photosynthetic rate, stomatal conductance, and transpiration rate) were recorded after 15 d of treatment in the second leaf from top of the plant using a LI-COR 6400-40 IRGA with default settings. The relative water content was measured for the same leaf. For this purpose, the fresh weight (WF) of the leaf was determined immediately after harvesting from the plant and then its rehydrated weight (WR) was measured after floating the leaf in deionized water at 4 °C overnight. The leaves were then dried at 80 °C for 24 h and the dry weight (WD) was obtained. RWC (%) was calculated as $[(WF-WD)/(WR-WD)]\times100$. The Na^+ and K^+ contents were measured in the fifth leaf from the top of the plant using a flame photometer (Corning EEL, UK), as described previously (Kumar *et al.*, 2009). After 45 d of salinity treatment, the saline solution was removed and the plants were then rewatered for 5 d to allow the completion of their life cycle, and morphological and yield-related parameters were scored as described previously (Tripathi *et al.*, 2016).

Expression analysis of target genes in transgenic rice plants

For gene expression analysis, shoot tissues were collected from 10-d-old seedlings of the WT, OE, and KD lines that had been subjected to either control conditions or salinity treatment with 200 mM NaCl for 24 h. Total RNA was extracted from three replicates per treatment, with each replicate formed of 2–3 seedlings, cDNA was prepared, and transcript analysis was carried out using the protocol described by Soda *et al.* (2013). Gene expression data was normalized using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) taking *actin* gene as the internal control. We focused on genes involved in ROS scavenging, stress-responsive genes encoding transcription factors, and three genes encoding PROTOCHLOROPHYLLIDE OXIDOREDUCTASE (*POR A*, *B*, *C*), which is involved in a critical step of chlorophyll biosynthesis, namely the conversion of protochlorophyllide to chlorophyllide *a* (Thomas, 1997). The primers used are listed in Supplementary Table S1.

Statistical analysis

The data were analysed using one-way ANOVA followed by Tukey's *post hoc* test in SigmaPlot 12.0 (Systat Software Inc.).

Results

OsGATA8 is localized in the nucleus in rice

OsGATA8 is a member of the conserved GATA family of TFs in rice and has a single GATA domain (Reyes *et al.*, 2004). To investigate the function of OsGATA8, we isolated *OsGATA8* (LOC_Os01g24070) from the commercial rice cultivar IR64. The full-length coding sequence (CDS) of *OsGATA8* consisted of 396 bp and its corresponding protein sequence was predicted to have 131 amino acids (NCBI accession: KU377163) (Fig. 1A). *In silico* analysis of the sequence of OsGATA8 showed the presence of a GATA_Zn_Finger_1 domain of 26 amino acids (aa), from 21 to 46 aa, which is the characteristic feature of GATA proteins. Use of cNLS Mapper (http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi) confirmed the presence of a nuclear localization signal (NLS) of 11 aa (47 to 57 aa) adjacent to the GATA domain. The deduced amino acid sequence of OsGATA8 showed a maximum identity (86%) with BdGATA23-like from *Brachypodium distachyon*, followed by 74% identity with ObGATA23-like from wild rice (*Oryza brachyantha*), 72% identity with SiGATA23-like from *Setaria italica*, and 60% identity with ZmGATA22 from *Zea mays*. AtGATA23 of Arabidopsis showed a minimum identity of 38.33%. A phylogenetic tree based on the full-length sequences of these related GATA domain-containing proteins indicated that OsGATA8, ObGATA23-like, and BdGATA23-like were closely related (Fig. 1B), while the other proteins formed a separate clade of different origin.

Since OsGATA8 is a known TF, we wanted to examine its subcellular localization. For this purpose, *OsGATA8* was fused 'in-frame' with the GFP reporter gene under the control of the CaMV 35S promoter and GFP was visualized in onion epidermal cells. The results clearly confirmed the presence of the protein in the nucleus (Fig. 1C). To confirm the role of the NLS in targeting to the nucleus, we cloned *OsGATA8* in two halves, one encoding the NLS sequence together with the N-terminal part of the protein (OsGATA8-N) and the other having the rest of the sequence without NLS (OsGATA8-C). The localization of the OsGATA8-N fragment in the nucleus is shown in Fig. 1D; in the absence of the NLS sequence the protein was only present in the cytoplasm (Fig. 1E). These results confirmed OsGATA8 to be localized in the nucleus.

To determine whether OsGATA8 alone could act as a transcriptional activator, yeast one-hybrid assays were performed. The full-length *OsGATA8* cDNA was fused to the DNA-binding domain of GAL4 (pGBD-C1-OsGATA8) and transformed into yeast strain AH109 (Supplementary Fig. S1A). The results showed that the yeast cells carrying only the empty vector could not grow on the selective media while the ones carrying either OsRR26 or the OsGATA8::GAL4 could activate the downstream reporter genes and could grow on SD/−Trp /−His/−Ade media supplemented with 5 mM 3-AT (Supplementary Fig. S1B, C). It was therefore concluded that OsGATA8 has autoactivation activity and is capable of initiating transcription in yeast when fused with GAL4BD.

Transcript abundance of OsGATA8 is influenced by salinity and development stage in rice

We have previously reported that *OsGATA8* is a salinity-inducible gene and is differentially regulated in seedlings of rice genotypes with contrasting tolerances to salinity (Nutan *et al.*, 2017). High expression of *OsGATA8* has also been observed during early (germination, seedling, and tillering) and late stages (milking and dough) of growth, as indicated by available microarray data (https://www.genevestigator.com/gv/) (Supplementary Fig. S2A). We conducted our own observations through RNA blotting analysis. Constitutive expression of *OsGATA8* in response to salinity was detected at the seedling stage with a bi-phasic pattern of expression, which showed an initial peak of expression after 30 min of stress and a second peak at ~24 h of stress (Supplementary Fig. S2B). Analysis at the tillering stage using leaves of 40-d-old plants again showed a high abundance of *OsGATA8* transcripts at 30 min and 24 h of stress (Supplementary Fig. S2C).

To assess the tissue specificity of expression of *OsGATA8*, we performed blotting analysis using RNA isolated from various tissues of mature plants (Supplementary Fig. S2D). Negligible amounts of transcript were found in the inflorescence under control conditions but there was slight induction after 48 h of salinity stress and this continued to 72 h. In the leaves, high constitutive expression was seen, and inducibility by salinity stress was observed for short durations but showed a decline by the final time-point after 72 h of stress. Lower leaves and the lower culm also showed high abundance of *OsGATA8* transcripts under control condition. The roots showed a completely different pattern, with negligible constitutive expression but strong salinity-induced expression by 24 h, which was maintained through to 72 h. The role of OsGATA8 in salinity stress in rice is therefore tissue-specific and varies according to the stage of plant development.

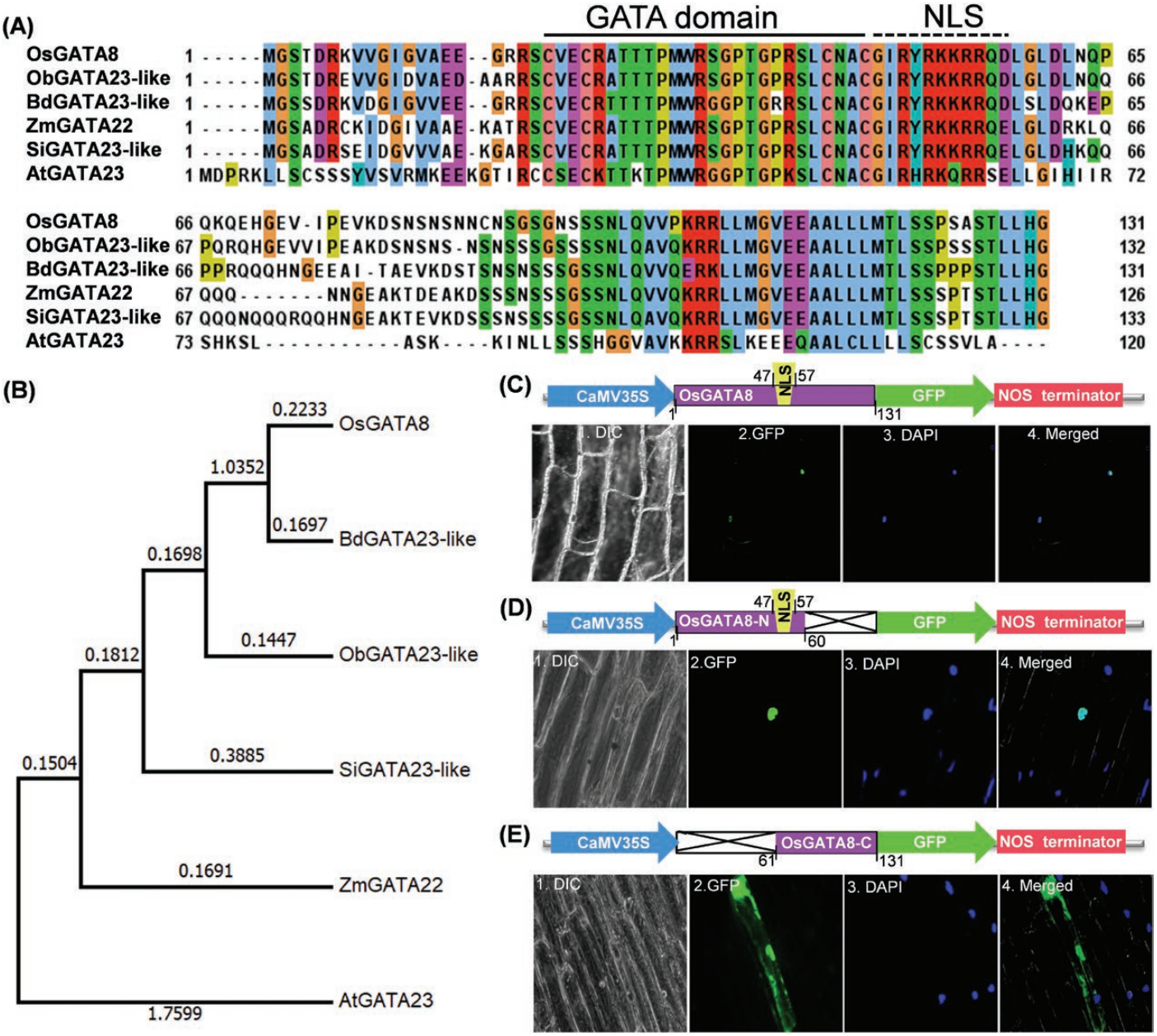

Fig. 1. Characteristic features of the OsGATA8 protein confirm it to be an authentic member of the GATA family. (A) Multiple sequence alignment of the deduced amino acid sequences of full-length proteins containing the GATA domain (GATA_Zn_Finger_1) from *Oryza sativa* (OsGATA8), *Oryza brachyantha* (ObGATA23-like), *Brachypodium distachyon* (BdGATA23-like), *Zea mays* (ZmGATA22), *Setaria italica* (SiGATA23-like), and *Arabidopsis thaliana* (AtGATA23). The alignment was carried out using Clustal Omega multiple sequence alignment tool with default settings (https://www.ebi.ac.uk/Tools/msa/clustalo/). The GATA domain and the nuclear localization signal (NLS) are indicated at the top. The GATA domain is positioned according to InterPro tools (https://www.ebi.ac.uk/interpro/) and the NLS is positioned according to cNLS Mapper (http://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi). (B) Phylogenetic tree of the GATA domain-containing proteins shown in (A). The tree was constructed using MEGA (https://www.megasoftware.net/) and the branch lengths are shown. (C–E) Subcellular localization of OsGATA8 with and without the NLS using green fluorescent protein (GFP) constructs transformed into onion epidermal cells and visualized using confocal microscopy. (C) Full-length OsGATA8, (D) OsGATA8-N terminal with NLS, and (E) OsGATA8-C terminal without NLS. Schematic diagrams above the images show the construct used. The deleted portion of the GATA protein (either N-terminal or C-terminal) is shown as a white box with a cross. In each case, image 1 shows the cells as observed under bright light (differential interference contrast, DIC), image 2 shows the GFP expression, image 3 shows the peel stained with diamidino-2-phenylindole (DAPI), and image 4 shows the merged image of GFP and DAPI.

OsGATA8 *contributes to tolerance to multiple abiotic stresses in Arabidopsis at the seedling stage*

To determine the function of salinity-inducible *OsGATA8*, the corresponding full-length cDNA was isolated from the rice cultivar IR64, cloned into the pCAMBIA1304 vector (Supplementary Fig. S3A), and transformed into Arabidopsis. The integration of the gene and its copy number were confirmed by PCR (Supplementary Fig. S3B) and Southern blotting (Fig. S3C), respectively. In the Southern analysis, no band corresponding to the rice *OsGATA8* CDS probe was detected in WT Arabidopsis while the putative transgenic lines showed

the signal, thus indicating the unique identity of the probe corresponding to OsGATA8. Three representative single-copy transgenic lines (L1, L3, and L4; Fig. S3D) were selected and multiplied to obtain T3 homozygous lines (35S-*OsGATA8*).

To examine the role of OsGATA8 in tolerance to various abiotic stresses, detailed phenotyping analyses were performed. The 35S-*OsGATA8* Arabidopsis and WT plants were grown for 5 d under control conditions and were then transferred to various stress conditions. Under control conditions (MS medium alone), the WT and the overexpression lines showed negligible differences in their root length, number of lateral roots, fresh weight of seedlings, and leaf greening (Fig. 2A). In contrast, significant differences were observed in response to treatment with PEG, mannitol, NaCl, and ABA. Root length and the number of lateral roots of the transgenic seedlings were found to be increased relative to the WT after 10 d of growth on medium containing PEG as an osmoticum (Fig. 2A–D). To determine further whether OsGATA8 was involved in general osmotic stress, 5-d-old seedlings grown on control medium were transferred to medium with 200 mM or 300 mM mannitol. After 10 d of treatment, it was found that the growth of WT seedlings was clearly inhibited compared to the 35S-OsGATA8 plants (Fig. 2A, E–G).

Leaf greening, root growth, and fresh weight of the WT were strongly inhibited at the seedling stage when plants were treated with 125 mM NaCl (Fig. 2A, H–J). At 180 mM NaCl, the growth of the WT was severely inhibited and chlorophyll bleaching of seedlings was observed, whereas OsGATA8 transgenic seedlings were not quite as severely affected. To examine the responsiveness to ABA, 5-d-old seedlings were treated with either 2 μM or 14 μM of ABA. At 2 μM ABA, the transgenic plants had longer roots, a greater number of lateral roots, and higher fresh weight than the WT. At 14 μM ABA, severe retardation of growth was observed in both the WT and the transgenic lines. However, 35S-*OsGATA8* seedlings had a greater number of lateral roots and higher fresh weight than the WT (Fig. 2A, K–M). Taken together, the transgenic plants ectopically expressing OsGATA8 exhibited better growth

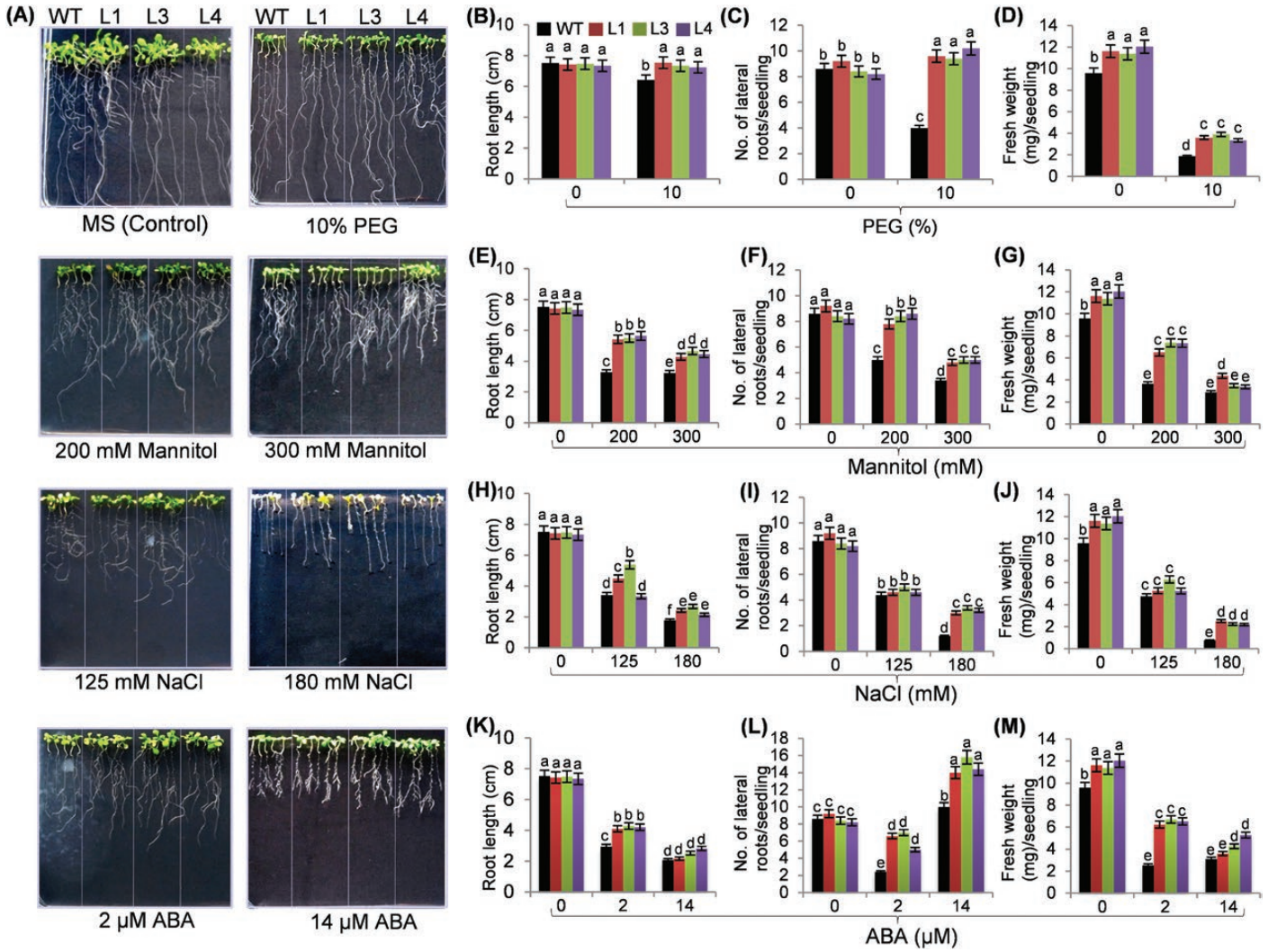

Fig. 2. OsGATA8 promotes tolerance to multiple abiotic stresses in Arabidopsis seedlings. (A) Effects on seedling growth of drought (PEG), salinity (NaCl), osmotic stress (mannitol), and ABA. WT, wild-type; L1, L3, and L4 are 35S-*OsGATA8* overexpressing transgenic lines; MS, Murashige and Skoog medium only. (B–M) Root length, number of lateral roots per seedling, and fresh weight per seedling under (B–D) drought, (E–G) osmotic stress, (H–J) salinity stress, and (K–M) ABA. Data are means (±SE) of three biological replicates. Different letters indicate significant differences between means as determined using one-way ANOVA followed by Tukey's *post hoc* test ($P<0.05$).

under the different stress conditions as compared to the WT plants.

Leaf greening and ROS homeostasis is promoted by OsGATA8 under salinity and drought stress in Arabidopsis

35S-*OsGATA8* and WT Arabidopsis plants were grown in soil for 5 weeks and then watered with 150 mM NaCl. After 20 d of treatment, signs of salinity stress were very clear on WT plants but the 35S-*OsGATA8* plants were much less affected (Fig. 3A). 35S-*OsGATA8* plants were found to have ~60% higher chlorophyll content then the WT under stress (Fig. 3B). The survival of the transgenic plants on saline soil was much higher (70–80%) than that of the WT (~30%) (Fig. 3C).

To assess performance under drought stress, another set of 35S-*OsGATA8* and WT plants were grown in soil for 5 weeks and then water was withheld for 20 d. The plants were then rewatered for 3 d (Fig. 3D). Before rewatering, wilting in the WT was more prominent than in the transgenic plants. Under stress conditions, the transgenic plants had 2-fold higher chlorophyll content than the WT (Fig. 3E). After rewatering, ~80% of the transgenic plants recovered, whereas only ~20% of WT plants recovered (Fig. 3F).

After the 20-d periods of salinity and dehydration stress, leaves of WT and transgenic plants were excised and stained with DAB (Fig. 3G, H) or NBT (Fig. 3I, J). Under control conditions, the patterns of DAB and NBT staining between the WT and transgenic lines were very similar. However, under salinity and drought stress, leaves of WT plants showed denser

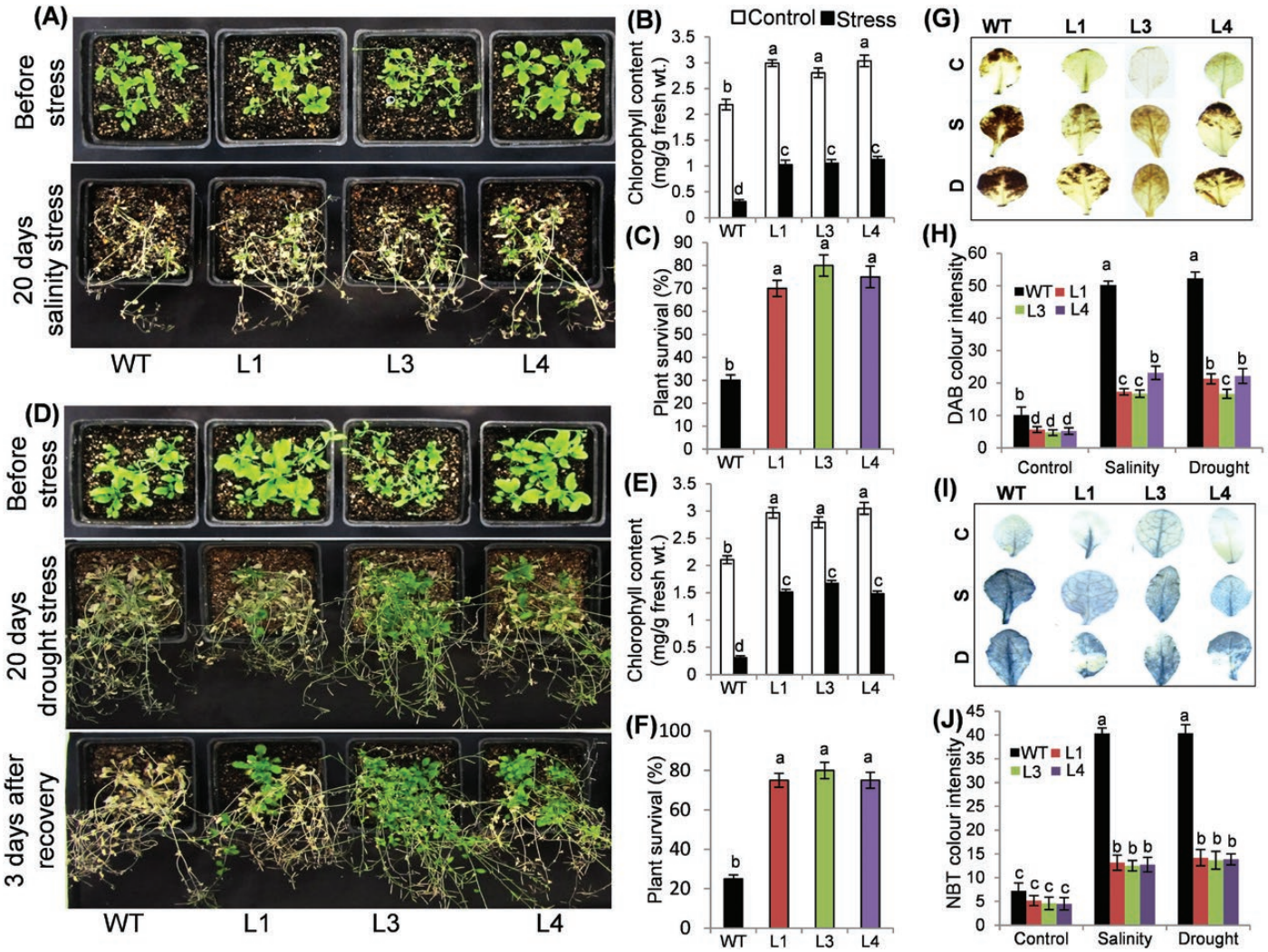

Fig. 3. Overexpression of *OsGATA8* in Arabidopsis increases tolerance towards salinity and drought at the reproductive stage. (A–C) A solution of NaCl (150 mM) was applied to 5-week-old Arabidopsis plants of the wild-type (WT) along with three 35S-*OsGATA8* overexpressing transgenic lines (L1, L3, L4) and measurements were taken after 20 d. (A) Morphology, (B) chlorophyll content, and (C) percentage plant survival. (D–F) Drought stress was imposed on 5-week-old plants by withholding watering for 20 d. The plants were then rewatered for 3 d, at which time survival was assessed. Other measurements were taken at the end of the drought period. (D) Morphology, (E) chlorophyll content, and (F) percentage plant survival. (G) Representative images of leaves showing accumulation of H_2O_2 as revealed by staining with diaminobenzidine (DAB) in response to the salinity (S) and drought (D) stress treatments (C, control), and (H) quantification of colour intensity in these leaves. (I) Representative images of leaves showing accumulation of $O_2^{\bullet-}$ as revealed by staining with nitroblue tetrazolium (NBT) in response to the salinity and drought stress treatments, and (J) quantification of colour intensity in these leaves. Data are means (±SE) of three biological replicates. Different letters indicate significant differences between means as determined using one-way ANOVA followed by Tukey's *post hoc* test (P<0.05).

staining than the transgenic lines (Fig. 3G–J). The staining patterns under salinity were similar to those under dehydration, indicating that the transgenic lines accumulated less H_2O_2 and $O_2^{\bullet-}$ than the WT under both these stresses. The seed length of the transgenic plants was found to be significantly greater than that of the WT (~494 μm versus ~445 μm, respectively), but no significant differences were observed in seed width (Supplementary Fig. S4B). The thousand-seed weight of the transgenic lines was significantly higher than that of the WT (~19.8 mg versus ~17.5 mg, respectively).

Under normal growth conditions, OsGATA8 is determinant of biomass and leaf greening in rice in both seedlings and the mature plant

Agrobacterium containing pCAMBIA1304-*OsGATA8* was used to transform rice IR64 to produce overexpressing (OE) plants. Stable integration and copy number of the *OsGATA8* transgene was confirmed by PCR and Southern blot analysis, respectively (Supplementary Fig. S5). Similarly, *Agrobacterium* containing pFGC1008-*OsGATA8* was used to produce RNAi-based knockdown (KD) plants (Supplementary Fig. S6). qRT-PCR analysis demonstrated that the KD lines (KD41 and KD81) showed 1.8-fold down-regulation of *OsGATA8* transcripts whilst the OE lines (OE7 and OE24) showed 3-fold up-regulation (Supplementary Fig. S6D). We used T2 homozygous lines of these transgenic genotypes.

Under control growth conditions, 20-d-old seedlings of the OE lines showed vigorous growth with more and longer roots compared to the KD lines and the WT (Supplementary Fig. S7). The fresh weight of the WT and OE seedlings were significantly higher than the KD lines (Supplementary Fig. S7C), and similar results were found for other growth parameters (Supplementary Fig. S7D–G). Following transfer to a greenhouse, mature plants showed similar patterns, with growth of the OE lines being greater that the WT and KD lines (Supplementary Fig. 7H). Chlorophyll content in mature plants was also significantly higher in OE lines as compared to the WT and KD lines (Supplementary Fig. S7I).

OsGATA8 *is essential for salinity tolerance in rice seedlings*

After 4 d of salinity stress, the *OsGATA8*-OE lines retained more chlorophyll than the WT and KD lines (Fig. 4A). In contrast to the fully expanded leaves seen in the OE lines, leaf rolling was evident in the WT and KD lines after just a day of stress. After 4 d of salinity, OE lines were able to survive, but the WT and KD lines showed severe stress-induced senescence. After 4 d of stress, the fresh weight of WT seedlings was reduced by ~57% compared to the controls whereas the OE seedlings showed only ~41% reduction (Fig. 4B). The KD seedlings showed reduction in fresh weight by over 66%. The relative tolerance of the OE lines and sensitivity of the KD lines compared to the WT was reflected in the results from other parameters (Fig. 4C–H). For example, the OE lines showed no significant reductions in relative water content under salinity stress compared with control conditions whereas the WT showed a reduction of 17% and the KD lines showed reductions of 43% (Fig. 4C). Similarly, electrolyte leakage in the OE lines under salinity stress was lower as compared to the WT and KD lines (Fig. 4D). However, the proline content and the K^+/Na^+ ratio in the OE lines were significantly higher under salinity stress than in the WT and KD lines (Fig. 4E, H).

OsGATA8 is an essential integrator of photosynthetic efficiency, and seed size and yield under salinity stress in rice

To obtain a simple assessment of salinity tolerance in mature rice plants, leaf-strip senescence assays were performed (Tripathi *et al.*, 2016) in which 2-cm strips were incubated either in water or in 200 mM NaCl solution. After 5 d of treatment, higher chlorophyll bleaching and leaf rolling were observed in the *OsGATA8*-KD lines as compared to the WT and OE lines (Supplementary Fig. S8A). Chlorophyll bleaching was greater in the WT than in the OE lines but was less than in the KD lines (Supplementary Fig. S8B). This is an important indicator for salinity tolerance, and hence demonstrated a positive role of OsGATA8 TF in salinity stress tolerance. After, 2 d of salinity stress, a higher degree of damage to the thylakoid membranes in the chloroplasts of the WT seedlings was observed compared to the OE lines. This damage was even more severe in the chloroplasts of the KD plants (Supplementary Fig. S8C).

We also performed the yield analysis of transgenic rice under salinity stress as previously described (Tripathi *et al.*, 2016). Plants were cultivated in soil in pots and salinity stress (200 mM NaCl) was imposed after 60 d of growth. After 15 d of treatment the OE plants showed mild morphological effects of salinity toxicity (Fig. 5A). Under salinity stress, the shoot K^+/Na^+ ratio, RWC, F_v/F_m, net photosynthesis, stomatal conductance, and transpiration rate were all significantly higher in the OE lines and lower in the KD lines as compared to the WT (Supplementary Fig. S9). After 45 d of treatment, leaves of the WT and KD lines were yellowish and showed senescence while those of the OE lines still appeared light-green, indicating their higher tolerance to salinity stress (Fig. 5A). The plants were then recovered by normal watering until they had completed their life cycle. After 5 d of recovery, the OE lines had revived and turned green but the WT and KD lines failed to do so. Under control conditions, the OE lines produced larger grains than the WT, whilst the KD lines produced smaller grains (Fig. 5B). The grain length of the OE lines was significantly greater (~20%) than that of the WT, while the KD lines produced grains that were significantly shorter (~16%) than the WT (Fig. 5C). No significant differences were observed in the width of the grains (Fig. 5D). Under control conditions, the OE lines had a higher number of panicles per plant (~10%), more filled grains per panicle (~18%), higher 1000-grain weight, and a higher total yield (~10%) as compared to the WT (Fig. 5E, Table 1). Under salinity stress, reductions in the yield parameters were observed in all the genotypes, but the total yield penalty in the OE lines was less (~29%) compared to the WT (~53%) and the KD lines (~87%) (Fig. 5F).

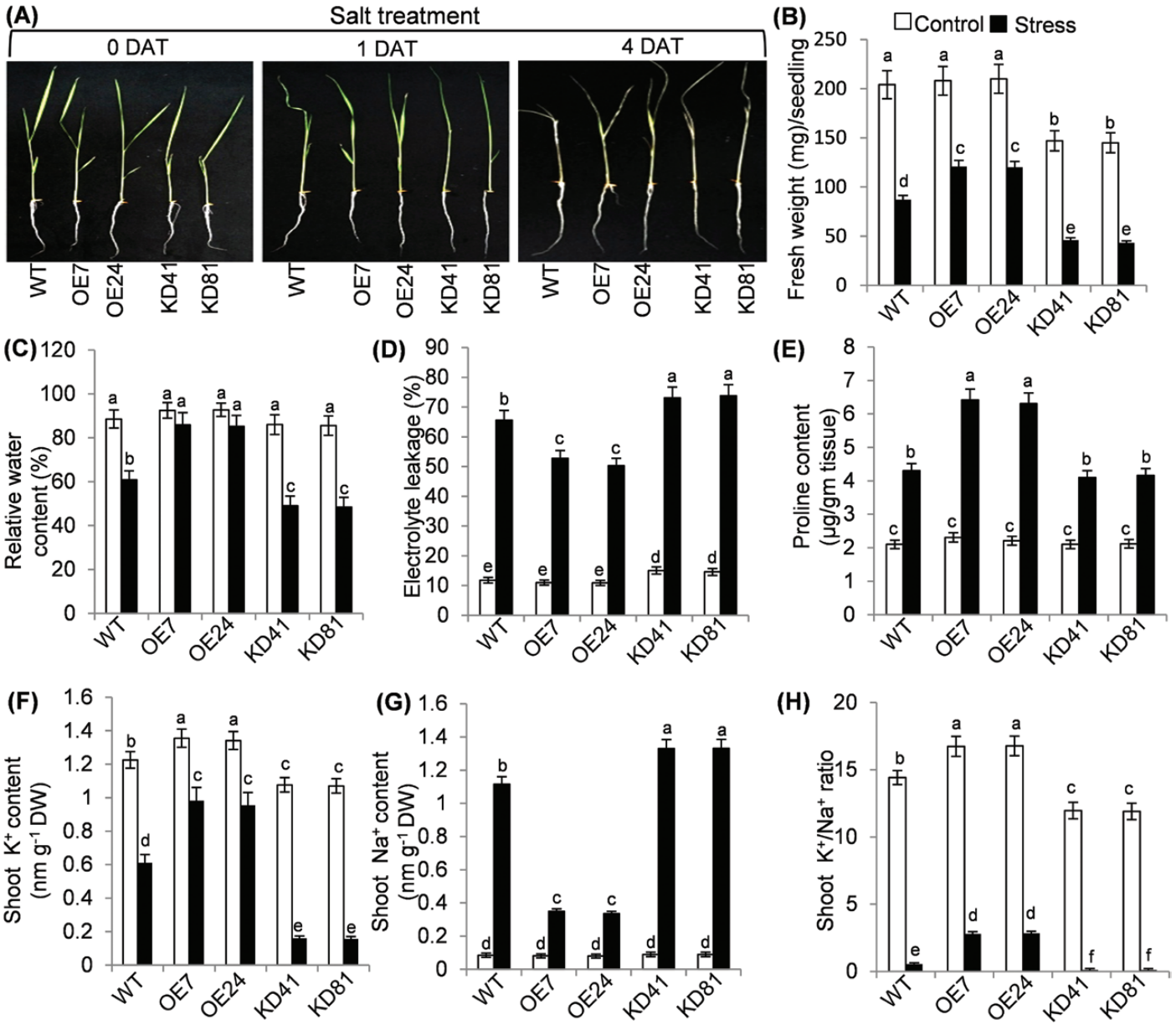

Fig. 4. OsGATA8 is important for salinity tolerance in rice seedlings via maintaining ion homeostasis and restricting membrane damage. 7-d old Wild-type (WT) plants, *OsGATA8*-overexpressing lines (OE7, OE24), and *OsGATA8*-knockdown lines (KD41, KD81) were treated with 200 mM NaCl solution. (A) Representative images of seedlings at 0 d of treatment (DAT), 1 DAT, and 4 DAT. (B–H) Physiological measurements taken at 4 DAT: (B) fresh weight per seedling, (C) relative water content, (D) electrolyte leakage, (E) proline content, (F) K^+ content, (G) Na^+ content, and (H) K^+/Na^+ ratio. Data are means (±SE) of three biological replicates. Different letters indicate significant differences between means as determined using one-way ANOVA followed by Tukey's *post hoc* test ($P<0.05$).

OsGATA8 operates through regulating the expression of downstream genes involved in leaf greening, ROS scavenging, and stress tolerance

To investigate the possible molecular functions of OsGATA8 in rice, we examined the expression dynamics of different categories of genes in 10-d-old plants (WT, OE and KD lines) that were treated with 200 mM NaCl for 24 h. We focused on genes involved in ROS scavenging, stress-responsive genes encoding transcription factors, and three genes encoding PROTOCHLOROPHYLLIDE OXIDOREDUCTASE (POR A, B, C). Genes encoding ROS-scavenging enzymes such as SOD and CAT showed 2–2.5-fold higher constitutive expression in the OE lines as compared to the WT under control conditions (Fig. 6A). Under salinity stress, a significant increase in expression of *SOD* was observed in both the WT and OE lines. The response of *APX* was different, with a 4-fold salinity-induced increase in expression being observed in the OE lines only.

We also studied the expression of key transcription factors that have been reported to be induced under environmental stresses. Under control conditions, *OsDREB1A* and *OsNAC6* showed 3-fold and 1.7-fold higher expression, respectively, in the OE lines compared to the WT (Fig. 6B); under salinity stress, the differences in expression increased to ~6-fold and ~2.5-fold for *OsDREB1A* and *OsNAC6*, respectively.

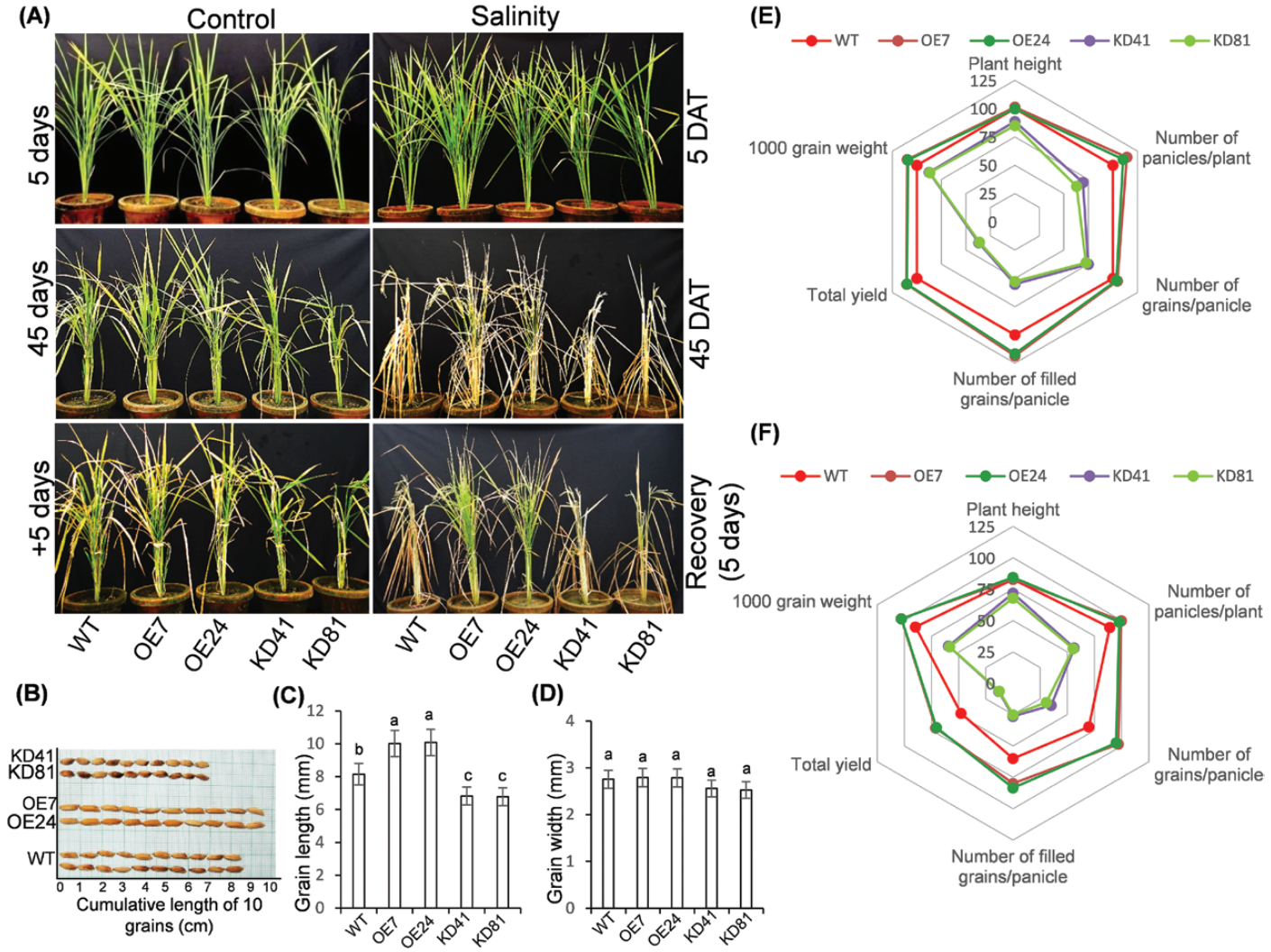

Fig. 5. Overexpression of *OsGATA8* promotes stress tolerance and influence grain size and yield in rice. Wild-type (WT) plants, *OsGATA8*-overexpressing lines (OE7, OE24), and *OsGATA8*-knockdown lines (KD41, KD81) were grown in a greenhouse for 60 d and then treated with 200 mM NaCl for 45 d, followed by 5 d of rewatering (recovery). (A) Images of representative plants after 5 d and 45 d of salinity treatment (DAT), and after the 5-d recovery period. (B–D) Grain size of the WT, OE, and KD lines grown under control conditions: (B) images showing the cumulative length of 10 grains, (C) individual grain length, and (D) grain width. Data are means (±SE) of three biological replicates. Different letters indicate significant differences between means as determined using one-way ANOVA followed by Tukey's *post hoc* test ($P<0.05$). (E, F) Web diagrams showing the yield components from the WT, OE, and KD lines under (E) control conditions and (F) salinity stress, plotted by taking the WT under control conditions as 100%.

OsDREB1A was induced by salinity in both the WT and the OE lines. In comparison to control conditions, expression of *OsbZIP23* was significantly up-regulated under salinity stress in all the genotypes; however, no differences in expression between the genotypes under salinity stress were observed, suggesting that OsbZIP23 may not be involved in the differences in salinity tolerance in these plants.

Transcript abundance for *POR A* and *POR B* was ~3.5 and ~4.2-fold higher, respectively, in the OE lines as compared to the WT under control conditions (Fig. 6C). Transcripts of *POR C* were also relatively higher in the OE lines but to a lesser extent, and the levels in general were much lower than those of *POR A* and *POR B*. Under salinity stress, the transcript levels for all these genes were decreased relative to the controls, but they were always higher in the OE lines.

The expression of all the genes in the different categories was greatly affected in the KD lines, with lower expression than in the WT under control conditions and generally no induction under salinity stress (the one exception being *OsbZIP23*). Indeed, the *POR* genes were down-regulated under stress. Overall, the results indicated the possible roles of OsGATA8 in the regulation of genes encoding ROS-scavenging enzymes, transcription factors, and chlorophyll-biosynthesis enzymes involved in stress alleviation.

Discussion

Although the roles of GATA proteins in plant development and carbon/nitrogen metabolism are well established, their involvement in responses associated with tolerance towards abiotic stresses have not yet been determined (Gupta *et al.*, 2017). In the present study, we aimed to characterize one of the members of the GATA family of proteins in rice, namely OsGATA8. OsGATA8 has a single GATA domain with a strongly predicted

Table 1. *Yield-related parameters for rice wild-type (WT) plants,* OsGATA8*-overexpression (OE) lines, and* OsGATA8*-knockdown (KD) lines under control conditions or subjected to salinity stress*

	Plant height (cm)	Number of panicles/plant	Number of grains/panicle	Number of filled grains/panicle	Total yield*	1000 grain weight (g)
Control						
WT	79.2±0.92	18±2.1	115±1.9	80±3.0	1630±12.2	31±0.78
OE7	80.2±0.57	20±1.8	120±2.1	95±3.5	1801±10.2	34±0.55
OE24	79.1±0.65	20±1.5	119±2.3	93±4.1	1795±15.5	34±1.8
KD41	70.2±0.41	12±0.9	86±1.8	44±1.6	600±10.5	27±0.86
KD81	67.3±0.61	11±1.1	83±1.6	42±1.5	590±8.2	27±0.80
Salinity stress						
WT	65±1.6	16±0.82	80±2.4	48±1.7	780±11.8	28±0.55
OE7	65.7±1.2	18±0.79	111±3.2	64±2.4	1160±12.4	32±1.1
OE24	66.9±1.3	17±0.66	109±4.2	67±2.1	1150±6.3	32±0.92
KD41	56.8±0.81	10±0.45	40±1.6	21±0.88	213±4.2	18±0.74
KD81	53.7±0.9	10±0.5	35±1.2	20±0.5	205±4.5	18±0.62

Plants were grown in soil in pots for 60 d, then subjected to 200 mM NaCl for 45 d, after which they were well-watered until they had completed their life cycle. Control plants were well-watered throughout.
* Total yield = total number of filled grains per plant.

NLS sequence adjacent to it (GATA_Zn_Finger_1). OsGATA8 showed significant homology with various orthologous proteins from diverse plant species, indicating its evolutionary conservation within plants (Fig. 1). The multiple sequence alignment showed good homology (86%) with orthologous members of the family from wild rice. OsGATA8 also showed 38% homology with Arabidopsis AtGATA23, a member of the well-studied B-GATA class of transcription factors (TFs) that play an important role in plant development (Behringer *et al.*, 2014; Behringer and Schwechheimer, 2015). Previous studies in rice and Arabidopsis have classified OsGATA8 as a GATA-like zinc finger domain-containing B-GATA class protein (Reyes *et al.*, 2004; Gupta *et al.*, 2017).

Examination of its subcellular localization clearly showed OsGATA8 to be present in the nucleus, as targeted by the NLS (Fig. 1). Yeast one-hybrid assays further confirmed the probable role of OsGATA8 in the transcription process (Supplementary Fig. S1). The binding of GATA proteins on specific *cis-acting* elements on DNA and hence their functions as TFs has been clearly demonstrated (Ko and Engel, 1993; Lowry and Atchley, 2000; Crespo *et al.*, 2001; Doubeikovskaia *et al.*, 2001; Richter *et al.*, 2013b). Taken together, these findings clearly establish OsGATA8 to be a functional TF.

Plants modulate their responses to changes in their physical and biological environment through complex gene networks that operate in a cell- and tissue-specific manner (Pareek *et al.*, 2010). The regulation of gene expression in response to environmental stresses has been well studied (Walia *et al.*, 2007; Kumari *et al.*, 2009b). Our previous analysis of *OsGATA8* showed that it has differential expression in shoots of seedlings of contrasting rice cultivars under salinity stress (Nutan *et al.*, 2017). We have also reported induced transcript abundance of OsGATA8 together with various other members of the GATA family in rice seedlings in response to salinity, ABA, and drought (Gupta *et al.*, 2017). In our current study, we found that expression of OsGATA8 has consistently been reported in rice leaves at both the tillering and milking stages and expression is further induced in response to salinity stress (Supplementary Fig. S2). Consistent with our results that showed reduced expression in the inflorescence, culm, and roots at the milking stage under normal (no stress) conditions, Nishi *et al.* (2000) found that a ZIM (zinc-finger protein expressed in inflorescence meristem) gene in Arabidopsis encoding a GATA-type zinc-finger protein with a C-X2-C-X20-C-X2-C motif also showed differential patterns of expression at the reproductive stage. Further, ZIM expression was observed in shoot apices and in the roots at the vegetative stage (Shikata *et al.*, 2004). In our study, the lower culm and lower leaves of mature rice plants showed high expression of *OsGATA8* under non-stress conditions (as compared to the upper parts of the plant), which may indicate a role for these tissues in acting as sinks for toxic substances during development, the accumulation of which thus induces the expression (Supplementary Fig. S2D). Different environmental stress signaling cascades in plants form a complex network with convergent and divergent signaling circuits (Zhu, 2002; Shinozaki *et al.*, 2003; Wani *et al.*, 2018). The complex pattern of expression of *OsGATA8* in these tissues is a result of this complexity of regulatory circuits.

In Arabidopsis, GATA family proteins have been shown to be involved in chlorophyll biosynthesis (Jeong *et al.*, 2003; Bi *et al.*, 2005). We examined the effect of ectopic expression of OsGATA8 in Arabidopsis under the control of the 35S promoter. Expression of OsGATA8 in Arabidopsis resulted in a phenotype with higher chlorophyll content and reduced water loss under salinity and drought stress, indicating its role in abiotic stress tolerance (Figs 2, 3). Our results were also consistent with a previous report that GATA TFs promote increased seed size (Supplementary Fig. S4; Behringer and Schwechheimer, 2015). Thus, our study gives a clear indication of the conserved functions of members of this TF family across different genera.

In agreement with the response seen in transgenic Arabidopsis and rice with overexpression (OE) of *OsGATA8* also exhibited increased seedling weight (Fig. 4), higher chlorophyll content and increased photosynthetic efficiency

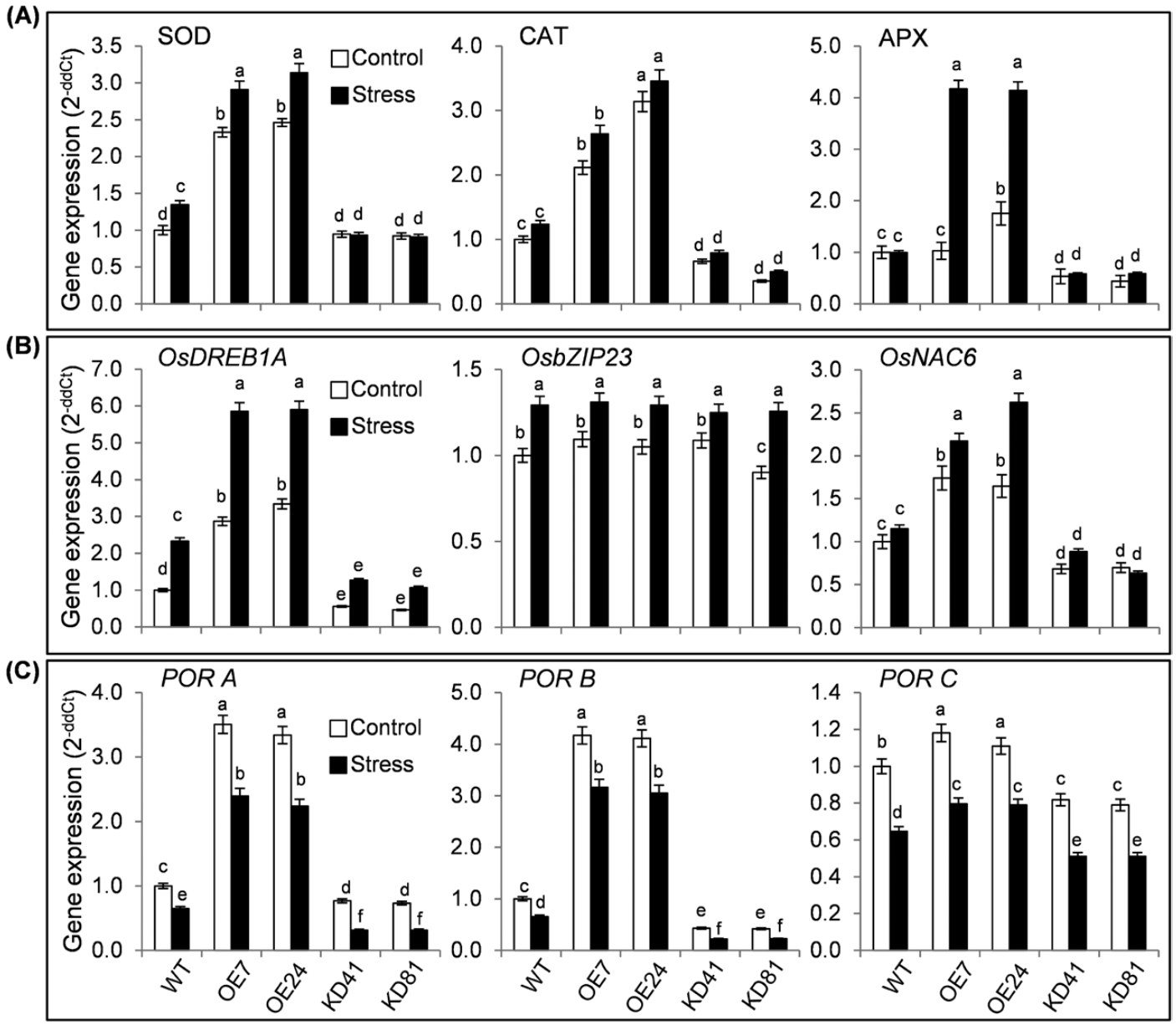

Fig. 6. OsGATA8 positively regulates the expression of downstream genes that have roles in leaf greening, ROS-scavenging, and stress tolerance. Wild-type (WT) plants, *OsGATA8*-overexpressing lines (OE7, OE24), and *OsGATA8*-knockdown lines (KD41, KD81) were grown for 10 d and then treated with 200 mM NaCl for 24 h. Total RNA was then extracted, cDNA was synthesized, and qRT-PCR was carried out. Gene expression was normalized using *actin* as the internal control and the value for the WT under control conditions was set as 1. (A) ROS-scavenging genes, *SOD*, *CAT*, and *APX*; (B) stress-responsive genes, *OsDREB1A*, *OsbZIP23*, and *OsNAC6*, and (C) and chlorophyll biosynthesis genes, *POR A*, *POR B*, and *POR C*. Data are means (±SE) of three biological replicates. Different letters indicate significant differences between means as determined using one-way ANOVA followed by Tukey's *post hoc* test ($P<0.05$). *SOD*, superoxide dismutase; *CAT*, catalase; *APX*, ascorbate peroxidase; *OsDREB1A*, rice dehydration responsive element binding factor 1A; *OsbZIP23*, rice basic leucine zipper 23; *OsNAC6*, rice NAM, ATAF, and CUC (NAC) 6; *POR A–C*, protochlorophyllide oxidoreductase A–C.

(Supplementary Figs S8,S9) compared to the WT or knockdown (KD) plants under salinity stress. Using Arabidopsis plants overexpressing GNC and GNL (B-GATA members) together with their single- and double-mutants, Richter *et al.* (2010, 2013b) confirmed a role of GATA TFs in increased accumulation of chlorophyll in the hypocotyl region, and also found a positive effect on leaf numbers. Similar observations have also been reported in transgenic rice overexpressing *HIGHER YIELD RICE* (*HYR*) (Ambavaram *et al.*, 2014). In comparison to the WT, rice seedlings overexpressing OsGATA8 showed an improved phenotype under salinity stress in terms of higher biomass, and increase in RWC, electrolyte leakage, proline content, and K^+/Na^+ ratio (Fig. 4), all indicating the higher tolerance of OE plants to salinity stress. Overexpression of rice *OsbZIP23* (Xiang *et al.*, 2008), *OsRAN2* (Zang *et al.*, 2010), *NAC1* (Hu *et al.*, 2006), *AP37* (Oh *et al.*, 2009), and *HYR* (Ambavaram *et al.*, 2014), and tomato *TSRF1* (Quan *et al.*, 2010) have also been shown to increase tolerance to abiotic stresses in rice. Leaf strip assays showed that the OE lines had reduced chlorophyll bleaching under salinity stress

(Supplementary Fig. S8A, B). When subjected to salinity stress at the reproductive stage, the OE lines showed improved yield parameters compared to WT plants, including increase in grain length and number of filled grains per panicle, which resulted in a total yield that was ~46% higher than in the WT. The KD lines showed an even lower yield than the WT. Our results are in agreement with a previous study that showed increased seed size in transgenic Arabidopsis overexpressing GNC and GNL (Behringer and Schwechheimer, 2015). Under drought stress at the reproductive stage, a similar yield gain has been seen in transgenic rice overexpressing *HYR* and *AP37* (Oh *et al.*, 2009; Ambavaram *et al.*, 2014). We observed a minimal effect of salinity stress on the ultrastructure of chloroplasts in the leaves of OE plants (Supplementary Fig. S8C), which was in agreement with the relatively high photosynthetic efficiency in OE plants at the reproductive stage (Supplementary Fig. S9). Drought-tolerant transgenic rice overexpressing *HYR* shows minimal thylakoid separation under stress, which contributes to higher tolerance in comparison to the WT (Ambavaram *et al.*, 2014). The role of members of LLM domain-containing B-GATA TF, GNC, and GNL in chloroplast development, chlorophyll accumulation, and stomatal development are well correlated with our results (Richter *et al.*, 2010, 2013a, 2013b; Hudson *et al.*, 2013; Ranftl *et al.*, 2016). The B-GATA members GNC and GNL acting in coordination with the MADS box TF SOC1 have been reported to be involved in processes associated with greening and cold tolerance (Richter *et al.*, 2013a). The expression of *POR C* in the OE lines was different to that of *POR A* and *POR B* under both control and salinity stress conditions (Fig. 6). This may be due to the fact that these chlorophyll biosynthesis genes are regulated by two different hormones and together with various light-signaling components (Liu *et al.*, 2017). Cytokinin controls the expression of *POR A* and *POR B* through EIN3/EIL1, a master TF (Chao *et al.*, 1997; Guo and Ecker, 2004), while *POR C* is expressed under the regulation of the DELLA protein in the GA signaling pathway (Cheminant *et al.*, 2011). We found higher transcript abundance in the OE plants compared to the WT and KD plants for the POR genes, TF genes, and ROS-scavenging genes under both control and salinity stress conditions (Fig. 6). Higher expression of POR genes has also been found in Arabidopsis plants overexpressing GNC and GNL (Richter *et al.*, 2010). Higher accumulation of various stress-related and photosynthesis genes have also been reported in transgenic rice under drought stress (Oh *et al.*, 2009; Ambavaram *et al.*, 2014). Based on these observations and the available literature, we propose a model to describe the functioning of OsGATA8 in promoting tolerance to stresses (Fig. 7). Expression of *OsGATA8* is probably induced by various stresses such as salinity and drought through an ABA-mediated pathway. It also appears to be induced by various other hormones such as GA, auxin, and CK (Behringer and Schwechheimer, 2015). Our data clearly show that OsGATA8 regulates the expression of various downstream genes involved in leaf greening, ion homeostasis, chlorophyll biosynthesis, stress tolerance, and ROS-scavenging enzymes. Taken together, OsGATA8 appears to be a master regulator of stress response in plants interacting directly or indirectly with diverse cellular mechanisms that contribute to stress tolerance.

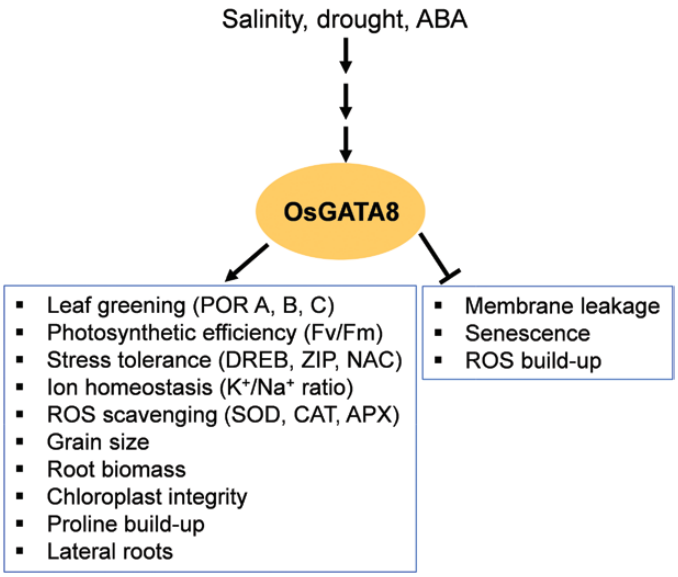

Fig. 7. Model depicting the regulatory control of OsGATA8 in plant growth and development under abiotic stresses. The multiple arrows indicate multi-step processes, the single arrow indicates positive regulation, and the blocked line indicates negative regulation. Various environmental stresses such as salinity and drought together with ABA lead to increased expression of *OsGATA8*, which integrates biomass production, leaf greening, ROS-scavenging, and ion homeostasis resulting in improved tolerance to the stress.

Since salinity and drought stress are considered to be the two major threats to agriculture in the near future, the results of our present study have the potential to help improve crop productivity in affected areas. *OsGATA8* appears to be a highly suitable candidate gene for studies targeting the improvement of tolerance to multiple stresses in crops plants, which is a topic of great importance in the context of climate change. Field-level evaluation of new plant types is required to assess their suitability for environments where combinations of stresses will determine their performance and yield. Testing of more 'multi-stress responsive genes' through functional genomics is urgently needed to ensure global food security in the years to come.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. Yeast one-hybrid assay for *OsGATA8*.

Fig. S2. *OsGATA8* expression is salinity-responsive and tissue-specific.

Fig. S3. Molecular confirmation of transgenic Arabidopsis ectopically expressing *OsGATA8*.

Fig. S4. OsGATA8 positively influences the seed size in transgenic Arabidopsis.

Fig. S5. Molecular confirmation of rice lines overexpressing *OsGATA8*.

Fig. S6. Molecular confirmation of rice lines with knockdown of *OsGATA8*.

Fig. S7. OsGATA8 increases biomass, greening, and net photosynthesis in rice under control conditions.

Fig. S8. OsGATA8 promotes leaf greening and maintains chloroplast structure under salinity stress in rice.

Fig. S9. OsGATA8 increases leaf greening and photosynthetic rate under salinity stress in mature rice plants.

Table S1. List of primers used in this study.

Acknowledgements

The laboratory of AP is financially supported by funds received from the Department of Biotechnology, Government of India, the Indo-US Science & Technology Forum (IUSSTF), and the Indo-US Advanced Bioenergy Consortium (IUABC).

References

Ambavaram MM, Basu S, Krishnan A, Ramegowda V, Batlang U, Rahman L, Baisakh N, Pereira A. 2014. Coordinated regulation of photosynthesis in rice increases yield and tolerance to environmental stress. Nature Communications **5**, 5302.

Argüello-Astorga G, Herrera-Estrella L. 1998. Evolution of light-regulated plant promoters. Annual Review of Plant Physiology and Plant Molecular Biology **49**, 525–555.

Behringer C, Bastakis E, Ranftl QL, Mayer KF, Schwechheimer C. 2014. Functional diversification within the family of B-GATA transcription factors through the leucine-leucine-methionine domain. Plant Physiology **166**, 293–305.

Behringer C, Schwechheimer C. 2015. B-GATA transcription factors – insights into their structure, regulation, and role in plant development. Frontiers in Plant Science **6**, 90.

Bi YM, Zhang Y, Signorelli T, Zhao R, Zhu T, Rothstein S. 2005. Genetic analysis of Arabidopsis GATA transcription factor gene family reveals a nitrate-inducible member important for chlorophyll synthesis and glucose sensitivity. The Plant Journal **44**, 680–692.

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. The Philippine Agricultural Scientist **85**, 68–76.

Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR. 1997. Activation of the ethylene gas response pathway in Arabidopsis by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. Cell **89**, 1133–1144.

Cheminant S, Wild M, Bouvier F, Pelletier S, Renou JP, Erhardt M, Hayes S, Terry MJ, Genschik P, Achard P. 2011. DELLAs regulate chlorophyll and carotenoid biosynthesis to prevent photooxidative damage during seedling deetiolation in Arabidopsis. The Plant Cell **23**, 1849–1860.

Chinnusamy V, Jagendorf A, Zhu J-K. 2005. Understanding and improving salt tolerance in plants. Crop Science **45**, 437.

Clough SJ, Bent AF. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. The Plant Journal **16**, 735–743.

Crespo JL, Daicho K, Ushimaru T, Hall MN. 2001. The GATA transcription factors GLN3 and GAT1 link TOR to salt stress in *Saccharomyces cerevisiae*. The Journal of Biological Chemistry **276**, 34441–34444.

Das P, Lakra N, Nutan KK, Singla-Pareek SL, Pareek A. 2019. A unique bZIP transcription factor imparting multiple stress tolerance in rice. Rice **12**, 58.

Das P, Nutan KK, Singla-Pareek SL, Pareek A. 2015. Understanding salinity responses and adopting 'omics-based' approaches to generate salinity tolerant cultivars of rice. Frontiers in Plant Science **6**, 712.

Doubeikovskaia Z, Aries A, Jeannesson P, Morle F, Doubeikovski A. 2001. Purification of human recombinant GATA-1 from bacteria: implication for protein–protein interaction studies. Protein Expression and Purification **23**, 426–431.

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**, 85–96.

Guo H, Ecker JR. 2004. The ethylene signaling pathway: new insights. Current Opinion in Plant Biology **7**, 40–49.

Gupta P, Nutan KK, Singla-Pareek SL, Pareek A. 2017. Abiotic stresses cause differential regulation of alternative splice forms of GATA transcription factor in rice. Frontiers in Plant Science **8**, 1944.

Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L. 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proceedings of the National Academy of Sciences, USA **103**, 12987–12992.

Hudson D, Guevara DR, Hand AJ, Xu Z, Hao L, Chen X, Zhu T, Bi YM, Rothstein SJ. 2013. Rice cytokinin GATA transcription Factor1 regulates chloroplast development and plant architecture. Plant Physiology **162**, 132–144.

James P, Halladay J, Craig EA. 1996. Genomic libraries and a host strain designed for highly efficient two-hybrid selection in yeast. Genetics **144**, 1425–1436.

Jeong MJ, Jeong MJ, Shih MC. 2003. Interaction of a GATA factor with *cis*-acting elements involved in light regulation of nuclear genes encoding chloroplast glyceraldehyde-3-phosphate dehydrogenase in Arabidopsis. Biochemical and Biophysical Research Communications **300**, 555–562.

Joshi R, Wani SH, Singh B, Bohra A, Dar ZA, Lone AA, Pareek A, Singla-Pareek SL. 2016. Transcription factors and plants response to drought stress: current understanding and future directions. Frontiers in Plant Science **7**, 1029.

Karan R, Singla-Pareek SL, Pareek A. 2009. Histidine kinase and response regulator genes as they relate to salinity tolerance in rice. Functional & Integrative Genomics **9**, 411–417.

Klermund C, Ranftl QL, Diener J, Bastakis E, Richter R, Schwechheimer C. 2016. LLM-domain B-GATA transcription factors promote stomatal development downstream of light signaling pathways in *Arabidopsis thaliana* hypocotyls. The Plant Cell **28**, 646–660.

Ko LJ, Engel JD. 1993. DNA-binding specificities of the GATA transcription factor family. Molecular and Cellular Biology **13**, 4011–4022.

Koch KE. 1996. Carbohydrate-modulated gene expression in plants. Annual Review of Plant Physiology and Plant Molecular Biology **47**, 509–540.

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. Journal of Plant Physiology **166**, 507–520.

Kumari S, Sabharwal VP, Kushwaha HR, Sopory SK, Singla-Pareek SL, Pareek A. 2009a. Transcriptome map for seedling stage specific salinity stress response indicates a specific set of genes as candidate for saline tolerance in *Oryza sativa* L. Functional & Integrative Genomics **9**, 109–123.

Kumari S, Singh P, Singla-Pareek SL, Pareek A. 2009b. Heterologous expression of a salinity and developmentally regulated rice cyclophilin gene (*OsCyp2*) in *E. coli* and *S. cerevisiae* confers tolerance towards multiple abiotic stresses. Molecular Biotechnology **42**, 195–204.

Lakra N, Kaur C, Anwar K, Singla-Pareek SL, Pareek A. 2018. Proteomics of contrasting rice genotypes: identification of potential targets for raising crops for saline environment. Plant, Cell & Environment **41**, 947–969.

Lakra N, Kaur C, Singla-Pareek SL, Pareek A. 2019. Mapping the 'early salinity response' triggered proteome adaptation in contrasting rice genotypes using iTRAQ approach. Rice **12**, 3.

Lakra N, Nutan KK, Das P, Anwar K, Singla-Pareek SL, Pareek A. 2015. 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. Journal of Plant Physiology **176**, 36–46.

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. Plant Stress **7**, 84–99.

Liu X, Li Y, Zhong S. 2017. Interplay between light and plant hormones in the control of Arabidopsis seedling chlorophyll biosynthesis. Frontiers in Plant Science **8**, 1433.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C^T}$ method. Methods **25**, 402–408.

Lowry JA, Atchley WR. 2000. Molecular evolution of the GATA family of transcription factors: conservation within the DNA-binding domain. Journal of Molecular Evolution **50**, 103–115.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annual Review of Plant Biology **59**, 651–681.

Nakashima K, Tran LS, Van Nguyen D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K. 2007. Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. The Plant Journal **51**, 617–630.

Nishii A, Takemura M, Fujita H, Shikata M, Yokota A, Kohchi T. 2000. Characterization of a novel gene encoding a putative single zinc-finger protein, ZIM, expressed during the reproductive phase in *Arabidopsis thaliana*. Bioscience, Biotechnology, and Biochemistry **64**, 1402–1409.

Nutan KK, Kushwaha HR, Singla-Pareek SL, Pareek A. 2017. Transcription dynamics of *Saltol* QTL localized genes encoding transcription factors, reveals their differential regulation in contrasting genotypes of rice. Functional & Integrative Genomics **17**, 69–83.

Oh SJ, Kim YS, Kwon CW, Park HK, Jeong JS, Kim JK. 2009. Overexpression of the transcription factor AP37 in rice improves grain yield under drought conditions. Plant Physiology **150**, 1368–1379.

Pareek A, Sopory SK, Bonhert H, Govindjee. 2010. Abiotic stress adaptation in plants. Dordrecht: Springer.

Quan R, Hu S, Zhang Z, Zhang H, Zhang Z, Huang R. 2010. Overexpression of an ERF transcription factor TSRF1 improves rice drought tolerance. Plant Biotechnology Journal **8**, 476–488.

Ranftl QL, Bastakis E, Klermund C, Schwechheimer C. 2016. LLM-domain containing B-GATA factors control different aspects of cytokinin-regulated development in *Arabidopsis thaliana*. Plant Physiology **170**, 1849–1860.

Ren ZH, Gao JP, Li LG, Cai XL, Huang W, Chao DY, Zhu MZ, Wang ZY, Luan S, Lin HX. 2005. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nature Genetics **37**, 1141–1146.

Reyes JC, Muro-Pastor MI, Florencio FJ. 2004. The GATA family of transcription factors in Arabidopsis and rice. Plant Physiology **134**, 1718–1732.

Richter R, Bastakis E, Schwechheimer C. 2013a. 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 Physiology **162**, 1992–2004.

Richter R, Behringer C, Müller IK, Schwechheimer C. 2010. The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. Genes & Development **24**, 2093–2104.

Richter R, Behringer C, Zourelidou M, Schwechheimer C. 2013b. Convergence of auxin and gibberellin signaling on the regulation of the GATA transcription factors GNC and GNL in *Arabidopsis thaliana*. Proceedings of the National Academy of Sciences, USA **110**, 13192–13197.

Sahoo KK, Tripathi AK, Pareek A, Sopory SK, Singla-Pareek SL. 2011. An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars. Plant Methods **7**, 49.

Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods **9**, 671–675.

Seki M, Narusaka M, Ishida J, *et al.* 2002. Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. The Plant Journal **31**, 279–292.

Sharan A, Soni P, Nongpiur RC, Singla-Pareek SL, Pareek A. 2017. Mapping the 'Two-component system' network in rice. Scientific Reports **7**, 9287.

Shikata M, Matsuda Y, Ando K, Nishii A, Takemura M, Yokota A, Kohchi T. 2004. Characterization of Arabidopsis ZIM, a member of a novel plant-specific GATA factor gene family. Journal of Experimental Botany **55**, 631–639.

Shinozaki K, Yamaguchi-Shinozaki K, Seki M. 2003. Regulatory network of gene expression in the drought and cold stress responses. Current Opinion in Plant Biology **6**, 410–417.

Singh AK, Kumar R, Tripathi AK, Gupta BK, Pareek A, Singla-Pareek SL. 2015. Genome-wide investigation and expression analysis of sodium/calcium exchanger gene family in rice and Arabidopsis. Rice **8**, 54.

Singh VK, Singh BD, Kumar A, Maurya S, Krishnan SG, Vinod KK, Singh MP, Ellur RK, Bhowmick PK, Singh AK. 2018. Marker-assisted introgression of *Saltol* QTL enhances seedling stage salt tolerance in the rice variety "Pusa Basmati 1". International Journal of Genomics **2018**, 8319879.

Soda N, Gupta BK, Anwar K, Sharan A, Govindjee, Singla-Pareek SL, Pareek A. 2018. Rice intermediate filament, OsIF, stabilizes photosynthetic machinery and yield under salinity and heat stress. Scientific Reports **8**, 4072.

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. Functional & Integrative Genomics **13**, 351–365.

Soda N, Sharan A, Gupta BK, Singla-Pareek SL, Pareek A. 2016. Evidence for nuclear interaction of a cytoskeleton protein (OsIFL) with metallothionein and its role in salinity stress tolerance. Scientific Reports **6**, 34762.

Thomas H. 1997. Chlorophyll: a symptom and a regulator of plastid development. New Phytologist **136**, 163–181.

Thomson MJ, De Ocampo M, Egdane J, *et al.* 2010. Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. Rice **3**, 148–160.

Tripathi AK, Pareek A, Singla-Pareek SL. 2016. A NAP-family histone chaperone functions in abiotic stress response and adaptation. Plant Physiology **171**, 2854–2868.

Vij S, Tyagi AK. 2007. Emerging trends in the functional genomics of the abiotic stress response in crop plants. Plant Biotechnology Journal **5**, 361–380.

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 Molecular Biology **63**, 609–623.

Wani SH, Tripathi P, Zaid A, Challa GS, Kumar A, Kumar V, Upadhyay J, Joshi R, Bhatt M. 2018. Transcriptional regulation of osmotic stress tolerance in wheat (*Triticum aestivum* L.). Plant Molecular Biology **97**, 469–487.

Xiang Y, Tang N, Du H, Ye H, Xiong L. 2008. Characterization of OsbZIP23 as a key player of the basic leucine zipper transcription factor family for conferring abscisic acid sensitivity and salinity and drought tolerance in rice. Plant Physiology **148**, 1938–1952.

Yoshida S, Forno DA, Cock JH. 1971. Laboratory manual for physiological studies of rice. Los Baños, Philippines: IRRI.

Zang A, Xu X, Neill S, Cai W. 2010. Overexpression of *OsRAN2* in rice and Arabidopsis renders transgenic plants hypersensitive to salinity and osmotic stress. Journal of Experimental Botany **61**, 777–789.

Zhu JK. 2002. Salt and drought stress signal transduction in plants. Annual Review of Plant Biology **53**, 247–273.