Comparative analysis of DNA methylation changes in two rice genotypes under salt stress and subsequent recovery

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Abstract: DNA methylation, which is one of the best understood epigenetic phenomena, plays an important role in plant responses to environmental stimuli. The rice introgression line IL177-103 and its recurrent parent IR64, which show contrasting salt stress tolerance, were used to characterize DNA methylation changes under salt stress and subsequent recovery using methylation-sensitive amplified polymorphism (MSAP) analysis. The introgression line IL177-103 showed significantly improved tolerance to salinity, as represented by higher relative water content, endogenous abscisic acid content, activity of reactive oxygen species scavenging enzymes, and lower Na⁺ concentration in shoots, compared with IR64. The MSAP results showed that less than 10.5% of detected DNA methylation sites were genotype specific, in line with their similar genetic background. Salt-induced DNA methylation changes in both genotypes were mostly detected in roots, and the major portion of the salt-induced DNA demethylation/methylation alterations remained even after recovery, implying their inheritance in the present generation. Furthermore, a few sites with stable DNA methylation differences were identified between salt-tolerant IL177-103 and salt-sensitive IR64, thus providing genotype-specific epigenetic markers. Collectively, these results provide valuable data for further dissection of the molecular mechanisms of salt-stress response and tolerance in rice.

Keywords: DNA methylation; salt tolerance; epigenetic marker; rice

1. Introduction

Salt stress is a severe environmental factor that limits crop productivity and agricultural sustainability in arid and semi-arid regions of the world [1]. Rice is the staple food crop in many parts of the world, and rice plants are sensitive to salt stress at a young seedling stage as well as at the reproductive stage [2]. Developing salt-tolerant rice cultivars by molecular breeding and biotechnology is an efficient approach to reduce the yield loss caused by salt stress and contribute to food security. Rice genotypes vary considerably in salt-stress tolerance. Genetic studies have revealed that a number of quantitative trait loci are associated with salt tolerance in rice [3,4]. However, few studies have investigated the relationship between epigenetics and the molecular mechanisms of salt stress tolerance in rice.

DNA methylation, an important epigenetic mechanism, is involved in the control of all genetic functions, including transcription, post-transcription, DNA repair, gene transposition, and cell differentiation, without an alteration to the DNA sequence [5,6]. DNA methylation in plants is well known to be genotype, species, tissue, and developmental stage specific [7,8]. In rice, about 8% of active genes are methylated within their promoter regions and 31% of expressed genes are methylated in the transcribed regions [9]. More than one-third of all *Arabidopsis* genes experience a degree of methylation within transcription regions and only 5% of genes show methylation within promoter regions; methylation of the latter regions is correlated with differential gene expression in a temporal or spatial manner [10].

Increasing evidence indicates that DNA methylation plays a crucial role in regulating gene expression in plant responses to environmental stresses [11–14]. The global DNA methylation levels of a salinity-tolerant wheat cultivar are reduced by exposure to salinity stress and the alteration of DNA methylation in promoter regions may affect gene expression [15]. Salt, cold, and oxidative stresses induce DNA demethylation in the coding sequence of the *Nicotiana tabacum* glycerophosphodiesterase-like protein gene (*NtGPD*L) in tobacco leaves, and this demethylation is positively correlated with *NtGPD*L gene expression [16]. In rice, the gene body methylation may perform an important role in regulating gene expression in an organ- and genotype-specific manner under salinity stress [17]. Furthermore, whole methylome sequencing of *Arabidopsis* grown in high-salinity soil has revealed that the salt-stressed lineages accumulate ~45% more differentially methylated

cytosines at CG sites than the controls [18].

Recently, it has been shown that plants can remember past environmental stimuli through epigenetic memories and can use these memories to aid responses when these events recur [19]. Ding *et al.* [20] reported that multiple exposures to drought stress enable plants to respond to a new stress by making more rapid adaptive changes to gene expression patterns compared with plants not previously exposed to drought stress. 75% of differentially methylated cytosine positions induced by salinity stress are inherited, although some may be lost in subsequent generations [18]. However, detailed information on the general pattern of DNA methylation linked with the response of rice to salinity and subsequent recovery, salinity-induced epigenetic memories, and their relationship with salinity tolerance in rice remain to be elucidated.

In this study, two rice genotypes, the salinity-tolerant rice introgression line IL177-103 and its salinity-sensitive recurrent parent IR64, were used to characterize the DNA methylation changes under salt stress and subsequent recovery by methylation-sensitive amplified polymorphism (MSAP) analysis. The genotype- and tissue-specific DNA methylation variations induced by salt stress and a portion of these salt-induced DNA methylation/demethylation sites remain stable during recovery. The present findings provide useful epigenetic information for further molecular dissection of salt stress tolerance in rice.

2. Materials and Methods

2.1 Plant Materials and Growth Conditions

Two rice lines, IL177-103 and IR64, were used in this study. IR64 is a salinity-sensitive cultivar and has been widely grown in many Asian countries. IL177-103 is a salinity-tolerant F₇ line derived from a cross between two ST IR64 introgression lines. SSR markers distributed throughout the rice genome were used to analyze genetic differences between the two genotypes. It was established that IL177-103 differed from IR64 in six genomic segments inherited from two donors, Madhukar and Binam (Figure S1).

Rice seeds were surface sterilized in 0.1% NaClO (v/v) and rinsed several times before germination at 37 °C in an incubator. After 3 days, germinated seeds were transferred to a 20-L tank filled with Yoshida nutrient solution. The seedlings were cultured for 12 days after germination. Salinity treatment was applied by addition of

NaCl to achieve a final concentration of 125 mM. After treatment for 4 days, the stressed plants were recovered for 5 days by culture in Yoshida nutrient solution lacking NaCl. The seedlings were grown in a phytotron with 29/22°C (day/night) temperatures and minimum relative humidity of 70%. The pH of the nutrient solution was adjusted daily to 5.5 by adding sulfuric acid. The nutrient solution was refreshed weekly.

2.2 Phenotypic and Physiological Trait Evaluation

After treatment for 4 days, phenotypic and physiological traits of IL177-103 and IR64 were characterized under control and salt-stress conditions. Shoot length (cm) was measured for each plant. The fresh weight of the roots and shoots was measured before oven drying at 70–75°C for 72 h and the total biomass was determined. Relative water content of each plant was calculated as $(FW-DW)/(TW-DW)*100$, where FW, DW, and TW are the fresh weight, dry weight, and total weight of each plant [21]. Sodium and potassium concentrations and the sodium: potassium ratio in shoots were measured in accordance with the method described by Zang *et al.* [22].

Leaf tissue from the well-cultured (control) and salinity-stressed plants were used to analyze the endogenous ABA content and activity of antioxidant enzymes. Endogenous ABA content was measured using the plant abscisic acid (ABA) ELISA Kit (Catalog no. CSB-E09159PI). Activity of the antioxidant enzymes SOD, POD, and CAT were determined following previously reported methods [23].

2.3 Methylation-sensitive Amplification Polymorphism Analysis

Leaf and root tissue from three replicates were collected from the well-cultured (control), salinity-stressed, and recovered plants at the seedling stage. Genomic DNA of both IL177-103 and IR64 samples were isolated using the improved CTAB method. The MSAP analysis was performed as described by Wang *et al.* [8]. Three biological replicates for the control and salinity-stressed samples and two biological replicates for the recovered samples were used for the MSAP analysis.

2.4 Cloning and Characterization of the Differentially Amplified DNA Fragments

Selected differentially amplified fragments were isolated, reamplified, and purified with Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). The reamplified DNA fragments were purified and cloned using the T-vector (Takara, Dalian, China) for sequencing. The sequences obtained were analyzed

against sequences in the National Center for Biotechnology Information databases using the BLAST tool (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

3. Results

3.1 Phenotypical and Physiological Characterization of IL177-103 and IR64 under Salt Stress

Under the non-stressed condition no notable phenotypical difference was observed between the IL177-103 and IR64 genotypes at the seedling stage (Table 1). Growth parameters for the two genotypes were evaluated after salt stress treatment. A significantly negative effect on growth parameters including relative water content, shoot length, and total biomass was observed in both genotypes after stress treatment. These parameters were more strongly inhibited for IR64 compared with those of IL177-103 under the saline condition. The Na⁺ concentration and Na⁺: K⁺ ratio were significantly increased in shoots of both genotypes under salt stress. The increase in these two parameters was more evident in IR64 (Table 1), which indicated that shoots of IL177-103 suffered less severe Na⁺ toxicity.

To investigate physiological changes in response to salt stress for each genotype, endogenous abscisic acid (ABA) content and activity of antioxidant enzymes in the leaf were measured. Compared with the control condition, both genotypes exhibited higher endogenous ABA content and activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) under salinity treatment (Figure 1). The endogenous ABA content and activity of CAT in IL177-103 were significantly higher than those of IR64 (Figure 1A and 2B) after salinity stress for 4 days. However, the activities of POD and SOD did not show significant differences between IL177-103 and IR64 (Figure 1C and 1D). These results indicated that the endogenous ABA content and CAT might play important roles in the salinity tolerance of IL177-103.

3.2 General DNA Methylation Patterns in IL177-103 and IR64 under Different Treatments

Forty-three to 50 primer combinations were used for the MSAP analysis, with which 694–826 clear and reproducible fragments were amplified in leaf or root

samples of IL177-103 and IR64 growing under control, salt stress, and subsequent recovery conditions. Tables 2 and 3 summarize the general cytosine methylation patterns in the leaves and roots of each genotype under the three conditions. Based on the MSAP profiles, the number of methylated (hemi- and fully methylated) DNA bands was determined. A total of 193–236 bands (Type II + Type III + Type IV), accounting for 26.5–28.7% of the total bands, were polymorphic among the samples. The total DNA methylation levels in leaves of both genotypes were similar (Table 2). However, when measured by the number and percentage of total fully-methylated bands in roots under the three conditions, for both genotypes the general pattern was control > stress > recovery (Table 3).

A comparative analysis of the DNA methylation patterns in leaves or roots of both genotypes under each condition was performed. As shown in Supplemental Table S2, the major portion of the detected bands was common to IL177-103 and IR64. A total of 6.79–10.42% of the bands were genotype specific, e.g. 51/47 and 52/48 bands (Type I+II+III) were specifically amplified by MSAP in leaves of IL177-103/IR64 under control and salt stress conditions, respectively. In contrast, 73/61 and 85/70 bands (Type I+II+III) were detected in roots of IL177-103/IR64 under control and salt stress conditions, respectively. A slightly higher number of genotype-specific bands were detected for IL177-103 than for IR64.

3.3 DNA Methylation Changes in IL177-103 and IR64 under Salt Stress and Subsequent Recovery

The DNA methylation alterations for both genotypes under salt stress and subsequent recovery were further comparatively analyzed. Salt stress resulted in only slight changes in DNA methylation in leaves of both genotypes; only four DNA bands were identified as methylated/demethylated under the stress or recovery conditions (Table 4). However, significant changes in DNA methylation were observed in roots of both genotypes under salt stress and subsequent recovery (Table 4). The same number of demethylated DNA bands (22 bands in each genotype; classes **a+b**) was detected in both genotypes under salt stress; these bands were remethylated (class **a**) or remained hypomethylated (class **b**) on recovery. In addition, a few bands (4 and 7 in IL177-103 and IR64, respectively) were methylated under salt stress, but demethylated (class **c**) or remained methylated (class **d**) under subsequent recovery.

Collectively, these results indicated that salt-stress-induced DNA methylation changes were largely root specific, and mostly involved demethylation; in addition, the major portion (classes **b** and **d**) of these DNA methylation changes (demethylation /methylation) were stable in the recovery condition (after the stress was removed).

3.4 Epigenetic Alleles between IL177-103 and IR64

Four MSAP fragments showed stable DNA methylation polymorphism between salinity-tolerant IL177-103 and salinity-sensitive IR64 under the control, stress and recovery conditions (Table 5 and Figure S1). The fragments V1 and V2 were hypomethylated in IR64 under the control, salinity, and recovery conditions, but in IL177-103 both fragments were hypermethylated under the three conditions. The fragments V3 and V4 were hypermethylated in IR64 but hypomethylated in IL177-103. Two possible reasons for this polymorphism are differences at the genetic level (DNA sequences) between IR64 and IL177-103, and differences at the epigenetic level (DNA methylation patterns, i.e., epigenetic alleles). To validate the observed polymorphism, two experiments were performed. First, we sequenced the four fragments, and based on the BLAST results the four fragments were located on chromosomes 2, 3, and 7, respectively (Table 5). However, no differences were observed between IL177-103 and IR64 based on a survey of 385 simple sequence repeat (SSR) markers distributed across the rice genome (Figure S2). Second, we designed sequence-specific PCR primers based on the four fragment sequences and sequenced the PCR products. No differences in DNA sequence between IL177-103 and IR64 were observed within the four regions. Those two results indicated that DNA methylation differences may be responsible for the polymorphisms between IL177-103 and IR64. These stable DNA methylation differences (epigenetic alleles) between cultivars that show different salinity tolerance may be candidate epigenetic markers, which previously have been reported to be potentially linked to phenotypic variations between closely related strains [24].

3.5 BLAST Results and Methylation Patterns of Polymorphic Methylated DNA Fragments

A random set of 19 polymorphic methylated fragments (four from leaf tissue and 15 from root tissue) were cloned and sequenced. The sequences of the cloned bands

showed an average length of 173 bp, ranging from 62 to 602 bp (Table 6). The fragments were distributed widely in the rice genome except on chromosomes 7, 9, 11, and 12, indicating that salinity induced genome-wide alteration in DNA methylation/demethylation.

The methylation patterns of the cloned fragments from leaf tissue are summarized in Table 6. Two fragments (L1 and L2) belonged to class **b** (demethylated during salinity stress, and remained hypomethylated after recovery). The other two fragments (L3 and L4) belonged to class **e** (DNA methylation pattern remained unchanged under salinity stress, but changed after recovery). Based on the BLAST results, the four fragments were homologous to genes encoding a receptor-like protein kinase, mitochondrial-processing peptidase, and Myb-like transcription factor.

The methylation pattern in root tissue induced by salinity was more complicated than that of leaf tissue. Eight fragments belonged to class **b**, two fragments belonged to class **e**, one fragment belonged to class **d** (methylated during salinity stress, and remained methylated after recovery), three fragments belonged to class **a** (demethylated during salinity stress, but remethylated after recovery), and one fragment belonged to class **c** (methylated during salinity stress, but demethylated after recovery). Among the 15 polymorphic methylated DNA fragments analyzed from root tissue, four encoded reverse transcriptase, DNA polymerase, ATPase, and hexokinase. Six fragments were highly homologous to ribosomal protein, RING-H2 finger protein, thaumatin, basic 7S globulin, and histone-like transcription factor. The remaining fragments were homologous to genes encoding DUF domains, transposon protein, hypothetical proteins, and cyclic nucleotide-gated ion channel. These results indicate that the salinity-induced methylation/demethylation bands detected involved genes with a wide range of functions, including those associated with stress responsiveness.

4. Discussion

Epigenetic mechanisms such as DNA methylation play a key role in plant response to environmental stresses by adjusting physiological and developmental mechanisms through differentially regulating genome-wide gene expression [25–27]. Demethylation usually leads to transcriptional activation of certain functionally inactive genes when plants are exposed to abiotic stresses [28]. The present results

showed that salinity induced genome-wide changes in the DNA methylation status of the salinity-tolerant rice introgression line IL177-103 and salinity-sensitive cultivar IR64. In particular, salinity tended to reduce the overall DNA methylation levels in root tissue of both genotypes. These results are consistent with previous reports that showed environmental stimuli, such as drought, salinity, cold, heat, and heavy metals, tend to cause hypomethylation of the genomic DNA or stress-response genes [8,14,16,29–31]. Tissue-specific alterations were observed among the salinity-induced DNA methylation changes in rice. In root tissue, salinity stress dramatically decreased the methylation level, but in leaf tissue only slight changes in methylation level were observed in this study. Tissue-specific gene expression is known to be controlled by DNA methylation [32]. Differences in DNA methylation changes in roots and leaves are indicative of the unique biological functions of rice roots and leaves in response to salinity stress. The possible reason for the tissue-specific DNA methylation changes may be because of the fact that root tissue experiences the stress first, and is in direct contact with the surrounding high-salinity environment. Hypomethylation in roots suggests the preparedness of more stress-responsive genes to respond to salinity stress [17,32]. The present results suggest that tissue-specific DNA methylation changes may be one of the epigenetic mechanisms involved in rice adaptation to salinity stress.

Increasing evidence indicates that epigenetic mechanisms such as DNA methylation are essential for stress memories and adaptation in plants [18,20,33]. For example, heat stress causes epigenomic and physiological changes in *Arabidopsis thaliana*, and these changes may be transmitted to progeny partially reliant on *DCL2* and *DCL3* [34]. Transgenerational epigenetic inheritance may be the key reason for the transgenerational phenotypic changes induced by heat and salt stress in *Arabidopsis thaliana* [35]. In the current study, two major types of salinity-induced methylation changes in root tissue were identified. The first type, including classes **a** and **c**, consisted of demethylation/methylation under salt stress, but reversal to the original status after 5 days recovery. The second type, including classes **b** and **d**, comprised DNA hypomethylation/hypermethylation changes that persisted even after subsequent recovery (Table 4). The second type of salinity-induced DNA methylation changes has the capacity to ‘remember’ the environmental stress and maintain the stress-induced epigenetic states, and to be inherited through mitotic cell divisions in the present generation. However, whether these salt-induced DNA methylation alterations can be transmitted to the next generation needs further study.

Four epigenetic alleles showed stable different DNA methylation states between IL177-103 and IR64, which differ in salinity tolerance. These DNA methylation differences (epigenetic alleles) between the two genotypes may be candidate epigenetic markers, which previously have been reported to be potentially linked to phenotypic variation between closely related strains. Epialleles might cause phenotypic differences between cultivars with a genetically close relationship. Identification of epialleles underlying natural variation will be an important issue in future rice breeding [24,36]. Epigenetic alleles may be involved in diverse phenomena ranging from development to genome evolution and defense against transposons[37]. Zhang *et al.* [38] identified a gain-of-function epiallele (*Epi-df*) of rice *FIE1*; this allele caused a dwarf stature and various floral defects. In plants, commonly encountered natural environmental stresses can accelerate the accumulation and change the profiles of novel inherited variants [18]. During long-term interactions between plants and environmental stresses, salinity-tolerant and -sensitive genotypes may form distinct fixed methylation patterns in some genomic or gene regions. These distinct methylation patterns might be linked to stress tolerances and be inherited in multiple subsequent generations.

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Table legends:

Table 1. Morphological and physiological traits of IR64 and IL177-103 under control and salinity conditions

Table 2. DNA methylation changes in leaves of IL177-103 and IR64 under salt stress and subsequent recovery

Table 3. DNA methylation changes in roots of IL177-103 and IR64 under salt stress and subsequent recovery

Table 4. Summary of DNA methylation pattern changes of IL177-103 and IR64 under salt stress and subsequent recovery

Table 5. BLAST results and DNA methylation patterns of epigenetic alleles between IR64 and IL177-103

Table 6. BLAST results and methylation patterns of a randomly selected polymorphic methylated DNA fragments

Figure legends:

Figure 1. Endogenous ABA content and activity of antioxidant enzymes in seedlings of the two rice genotypes under control (CK) and salinity conditions.

Supplementary Materials

Supplementary materials can be found at

Table S1. Adapter and primer sequences used for MSAP analysis

Table S2. Comparative analysis of different MSAP bands in leaves and roots of IL177-103 and IR64 under control, salt stress, and recovery conditions

Figure S1. Genetic composition of IL177-103 at 6 genomic regions (loci) introgressed and pyramided from two different donors, Madhukar and Binam, in the IR64 genetic background based on SSR markers.

Figure S2. DNA methylation patterns in IL177-103 and IR64 under control, salt stress and recovery conditions

Table 1. Morphological and physiological traits of IR64 and IL177-103 under control and salinity conditions

Treatment	Genotype	Relative water content (%)	Shoot length (cm)	Total biomass (mg/plant)	Shoots Na ⁺ concentration (mmol/g dry weight)	Na ⁺ /K ⁺ ratio in shoots
Control	IR64	83.7±5.1	32.8±0.1	53.34±1.8	0.03±0.01	0.03
	IL177-103	82±3.0	34.5±0.9	53.83±2.5	0.04±0.01	0.03
Salt	IR64	64.5±3.7	18.0±0.8	24.93±1.6	1.31±0.11	1.63
	IL177-103	76.9±2.1	24.8±1.0	33.61±2.2	0.96±0.05	1.25

Table 2. DNA methylation changes in leaves of IL177-103 and IR64 under salt stress and subsequent recovery

Genotype	IL177-103			IR64		
MSAP Band Types ^{a)}	Control	Salinity	Recovery	Control	Salinity	Recovery
I	500	502	500	499	501	499
II	3	3	3	3	3	3
III	193	193	193	190	190	190
IV	2	0	2	2	0	2
Total amplified bands	698	698	698	694	694	694
Total methylated bands	198	196	198	195	193	195
MSAP (%)	28.37	28.08	28.37	28.1	27.81	28.1
Full methylated bands	195	193	195	192	190	192
Full methylation ratio (%)	27.94	27.65	27.94	27.67	27.38	27.67

a) Type II = hemi-methylated bands and types III+IV are full methylated bands. Total methylated bands = II+III+IV

Table 3. DNA methylation changes in roots of IL177-103 and IR64 under salt stress and subsequent recovery

Genotype	IL177-103			IR64		
MSAP Band Types ^{a)}	Control	Stress	Recovery	Control	Stress	Recovery
I	590	601	605	580	588	598
II	5	7	5	5	7	5
III	205	210	206	203	208	203
IV	26	8	10	26	11	8
Total amplified bands	826	826	826	814	814	814
Total methylated bands	236	225	221	234	226	216
MSAP (%)	28.57	27.24	26.76	28.75	27.76	26.54
Full methylated bands	231	218	216	229	219	211
Full methylation ratio (%)	27.97	26.39	26.15	28.13	26.90	25.92

a) Type II = hemi-methylated bands and types III+IV are full methylated bands. Total methylated bands = II+III+IV

Table 4. Summary of DNA methylation pattern changes of IL177-103 and IR64 under salt stress and subsequent recovery

Band class *	Leaves		Roots	
	IL177-103	IR64	IL177-103	IR64
a	2	2	6	5
b	0	0	16	17
c	0	0	2	4
d	0	0	2	3
e	2	2	4	4
f	694	690	795	781
Total	698	694	826	814

* **a:** Demethylated by salinity, but remethylated after recovery; **b:** Demethylated by salinity, and remaining hypomethylated after recovery; **c:** Methylated by salinity, but demethylated after recovery; **d:** Methylated by salinity, and remaining methylated after recovery; **e:** DNA methylation pattern remained unchanged under salinity, but changed after recovery; **h:** DNA methylation pattern was unchanged under all three conditions; **f:** DNA methylation pattern was unchanged under all conditions

Table 5.BLAST results and DNA methylation patterns ofepigenetic alleles between IR64 and IL177-103

Code	Primer combination	Size (bp)	MSAP Fragments				IR64								IL177-103			
			Accession	Physical position (bp)	Nucl.id (%)	Sequence homology	Control		Salinity		Recovery		Control		Salinity		Recovery	
			No./Locus Name				E/H	E/M	E/H	E/M	E/H	E/M	E/H	E/M	E/H	E/M	E/H	E/M
V1	H5/M39	141	NC_008400.2	7106272-7106386	98	Oryza sativa Japonica Group DNA, chr 7	+	+	+	+	+	+	—	—	—	—	—	—
V2	H6/M34	308	LOC_Os03g57660	33717128-33717409	99.44	Peptidase, putative, expressed	+	+	+	+	+	+	—	—	—	—	—	—
V3	H7/M39	215	LOC_Os02g01790	434365-434553	100	Expressed protein	—	—	—	—	—	—	+	+	+	+	+	+
V4	H9/M37	286	LOC_Os02g46510	29247359-29247496	100	Adaptor complexes medium subunit family domain	—	—	—	—	—	—	+	+	+	+	+	+

“**H**”: *Hpa* II; “**M**”: *Msp* I; “**E**”: *Eco*R I; “+”: Present; “—”: Absent

Table 6.BLAST results and methylation patterns of a randomly selected polymorphic methylated DNA fragments

Code	Primer combination	Tissue	MSAP fragments				Control				Salinity				Recovery			
			Size	Accession	Nucl.id(%)	Sequence homology	IL177-103		IR64		IL177-103		IR64		IL177-103		IR64	
			(bp)	No./Locus Name			E/H	E/M	E/H	E/M	E/H	E/M	E/H	E/M	E/H	E/M	E/H	E/M
L1	H3/M311	Leaves	235	LOC_Os02g06600	99	Receptor-like protein kinase 2 precursor	—	—	—	—	+	+	+	+	+	+	+	+
L2	H6/M34	Leaves	116	NC_008397.2	97	Oryza sativa Japonica Group DNA, chr 4	—	—	—	—	+	+	+	+	+	+	+	+
L3	H1/M316	Leaves	602	LOC_Os01g09560	98	Mitochondrial-processing peptidase subunit alpha	+	+	+	+	+	+	+	+	—	—	—	—

L4	H1/M316	Leaves	128	LOC_Os06g40710	77	Myb-like DNA-binding domain containing protein	+	+	+	+	+	+	+	+	+	+	+	+	+
R1	H8/M312	Roots	166	Os05g0126900	86	Reverse transcriptase, RNA-dependent DNA polymerase family protein	—	—	—	—	+	+	+	+	+	+	+	+	+
R2	H1/M34	Roots	62	Os05g0282900	95	Conserved hypothetical protein	—	—	—	—	+	+	+	+	+	+	+	+	+
R3	H1/M35	Roots	67	Os02g0627700	88	Cyclic nucleotide-gated ion channel	—	—	—	—	+	+	+	+	+	+	+	+	+
R4	H7/M35	Roots	243	Os05g0402800	92	ATPase, P-type, K/Mg/Cd/Cu/Zn/Na/Ca/Na/H-transporter	—	—	—	—	+	+	+	+	+	+	+	+	+
R5	H10/M38	Roots	108	Os03g0852300	81	Histone-like transcription factor and archaeal histone	—	—	—	—	+	+	+	+	+	+	+	+	+
R6	H8/M36	Roots	65	Os02g0241500	95	DUF4216; Domain of unknown function	—	—	—	—	+	+	+	+	+	+	+	+	+
R7	H1/M34	Roots	260	LOC_Os10g41310	70	DUF630/DUF632 domains containing protein	—	—	—	—	+	+	+	+	+	+	+	+	+
R8	H6/M310	Roots	185	LOC_Os01g33410	63	Transposon protein	—	—	—	—	+	+	+	+	+	+	+	+	+
R9	H8/M34	Roots	168	Os06g0647100	89	Ribosomal protein L35	—	—	—	—	—	—	—	—	+	+	+	+	+
R10	H9/M313	Roots	131	LOC_Os04g28074	67	Expressed protein	—	—	—	—	—	—	—	—	+	+	+	+	+
R11	H8/M312	Roots	106	LOC_Os08g34550	69	RING-H2 finger protein	+	+	+	+	—	—	—	—	—	—	—	—	—
R12	H10/M38	Roots	195	Os04g0689900	88	Thaumatococcus	—	—	—	—	+	+	+	+	—	—	—	—	—
R13	H5/M33	Roots	130	Os07g0197100	78	Hexokinase	—	—	—	—	+	+	+	+	—	—	—	—	—
R14	H3/M37	Roots	167	LOC_Os05g33400	66	Basic 7S globulin precursor	—	—	—	—	+	+	+	+	—	—	—	—	—
R15	H5/M39	Roots	156	Os10g0124000	92	Ribosomal protein L7Ae	+	+	+	+	—	—	—	—	+	+	+	+	+

“**H**”: *Hpa* II; “**M**”: *Msp* I; “**E**”: *EcoR* I; “+”: Present; “—”: Absent

Figure 1. Endogenous ABA content and activity of antioxidant enzymes in seedlings of the two rice genotypes under control(CK) and salinity conditions. (A) Endogenous ABA content, (B) CAT activity, (C) POD activity, (D) SOD activity. IR64 cultivar (white) and IL177-103 cultivar (black). Values are means of 3 replicates. **p < 0.01; *p < 0.05 versus IR64 (ANOVA, Dunnett’s multiple comparison test).