

Article

# Assessing Salinity Tolerance in Rice Mutants by Phenotypic Evaluation Alongside Simple Sequence Repeat Analysis

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**Abstract:** Salinity stress is one of the most severe constraints limiting rice production worldwide. Thus, the development of salt-tolerant rice promises to deal with increasing food demand due to climate change effects. This study investigated the salinity tolerance of mutant rice by evaluating phenotype and genotype, using forty-two simple sequence repeat (SSR) markers linked to the salinity tolerance *Saltol* quantitative trait locus (QTL) in ten cultivars and mutant lines. Results of phenotypic screening showed that the mutant line SKLo/BC15TB and cultivar BC15TB performed salt tolerance, while the mutant line Bao Thai/DT 84 and cultivar DT84DB were sensitive to salt stress. The markers RM 493, RM 562, RM 10748, RM 518, RM 237, and RM 20224 were the most polymorphic in salinity tolerance. Among them, RM 237, RM 10748, and RM 224 showed the highest polymorphism information (PIC = 0.58). This study reveals that the three markers are profitable for classification of salinity tolerance in both cultivar and mutant rice. The mutant line SKLo/BC15TB and cultivar BC15TB were found to be promising candidates for diversity analysis of salt-tolerant rice. Findings of this study are useful for developing new salinity-tolerant rice cultivars towards climate change.

**Keywords:** salinity stress; mutant rice; morphological; SSR markers; genotype; phenotype

## 1. Introduction

Rice is one of the most important crops grown and consumed globally. It ranks the third in agricultural production and provides daily meals for more than half of the world's population. However, ongoing climate change adversely threatens rice production. Both biotic and abiotic stresses

cause a significant yield loss in large rice-growing areas, including high salinity, drought, heat, and cold. Among them, salinity stress is the main hazardous factor of rice productivity [1].

Rice is highly sensitive to salty conditions, especially at the seedling stage. The inhibition in seedling is the initial step that leads to other consequences. The high concentration of toxic ions  $\text{Na}^+$ , induced by salinity, is the main cause of various physiological damages and inhibitory processes of plants. On the other hand, in a physiological view, it disturbs the uptake of potassium, which plays an important role in preserving membrane potential, enzyme activities, and cell turgor. A high  $\text{Na}^+/\text{K}^+$  ratio increases the osmotic gradient that causes cellular dehydration.  $\text{Na}^+$  also affects the activity of enzymes or proteins after entering the cytosol [1]. Besides this, high levels of salt stress are inversely correlated with photosynthesis and photoinhibition, as inducing abscisic acid synthesis leads to stomatal closure and reducing leaf size. All of the factors mentioned above directly influence rice seedling growth, leaf formation, and panicle emergence [2]. Accordingly, salinity reduces rice productivity by a decline in panicle length, number of spikelets per panicle, and number of grains [3]. Additionally, caloric and nutritional values are also changed [4].

Tremendous efforts have been conducted to improve salinity tolerance and to ensure a sustainable rice production. In which the study on rice mutants as new elite materials is an impressive approach, since the high efficiency of mutagenesis has been widely documented and reflected in the enhancement of more than 3000 mutant varieties, including 828 rice cultivars, according to Food and Agriculture Organization/International Atomic Energy Agency Mutant Varieties Database [5]. Basically, mutation can be created by spontaneous mutagenesis or induced mutagenesis (chemical mutagenesis, UV radiation mutagenesis, and ionizing radiation) [6]. Induced mutagenesis has been extensively used for the genetic improvement of all organisms, including microbes, animals, and plants. It is reported that mutation induction is useful for generating potent rice lines. Furthermore, it performs cost- and time-effective strategies in the enhancement of expected characteristics of crops [7,8]. Along with physical mutagens, the chemical mutagens, such as ethyl-nitroso urea (MNU), have been utilized frequently for breeding purposes. MNU is an alkylating agent, which can covalently attach an alkyl group to a biomolecule under physiological conditions (aqueous solution, 37 °C, pH 7.4) [9]. In rice, it is demonstrated to obtain high-frequency mutation and is effective for genetic approach [10].

To accelerate the development of rice cultivation, enormous DNA markers related to *Saltol* quantitative trait loci (QTL) have been developed. Among them, simple sequence repeat (SSR) markers are broadly applied because of their dominances compared to other markers. With 1 to 10 nucleotides, SSRs (or microsatellites) have been used for genotyping plants over the past 20 years. They are abundant, distributed throughout the genome, and highly polymorphic. As multi-allele genetic markers, SSRs can also be replicated and transferred among relative species [11]. SSR markers are valuable in breeding new rice cultivars to obtain elite important yields and quality attributing traits, as well as resistance to disease and environmental stresses [12]. In previous studies, SSR markers performed as a useful indicator to identify salinity tolerance of rice [13–34].

In recent decades, many advanced techniques have been made to enhance tolerant rice varieties which can adapt to environmental stresses such as submergence, drought, chill, high temperature, low pH, salinity, etc. Nevertheless, it is not easy to overcome the challenges of rice salt-tolerant enhancement, because of its complex mechanism. Although phenotype is the eventual expression of molecular compositions, it is adversely influenced by environmental impacts through various physiological and biological processes. Therefore, a combination of genetic and phenotypic investigation was a prior technique for the selection of the most promising genotype.

To date, the screening of salinity tolerance of rice mutants using both morphological analysis and SSR markers is only carried out sporadically, as mentioned above. This study was therefore conducted to evaluate salinity-tolerant abilities of mutant rice lines, and search for potent SSR markers for breeding rice cultivars tolerant to salinity. Although SSR markers have been applied in many earlier researches, especially in marker-assisted selection, in this study, we conducted the genetic analysis in new rice mutants, which were created by “respiratory mutation”. It is the first time that their salt tolerances

were assessed. Additionally, our previous research pointed out that rice mutants expressed better panicle and grain characters than their parents, such as panicle number per plant, full grain per plant, grain weight, and grain yield per ha [33]. Additionally, greater performances of protein, amylose, and lipid contents were also found in these mutants when compared with those of parents [33]. Therefore, findings of this study are useful for the development of target rice varieties, which are not only of high yield and quality, but also resistant to abiotic stresses, to cope with climate change.

## 2. Materials and Methods

### 2.1. Plant Materials

Rice samples were provided by Khai Xuan International Co. Ltd. and Agricultural Genetics Institute (Hanoi, Vietnam). Mutant rice was created by chemical mutation using N-Nitroso-N-methylurea (MNU) [35]. Rice seeds of origin cultivars were soaked in 150 mM MNU for 3 h before drying, and kept in hermetic conditions before storage at 4 °C. The first mutated generation ( $M_1$ ) was self-pollinated to obtain the second ( $M_2$ ) generation.  $M_2$  was used for further works.

In this research, ten rice materials, including mutant lines and parental generations, were used to evaluate their salinity tolerance. Their origins and information are provided in Table 1.

**Table 1.** The origins and classifications of rice cultivars and mutant lines.

Samples	Origins	Classifications
B1	Bao Thai	Cultivar
B2	DT84	Cultivar
B3	Bao Thai/DT84	Mutant
B4	DT84DB	Mutant
B5	SKLo	Cultivar
B6	SKLo/BC15	Mutant
B7	BC15	Cultivar
B8	Khang dan/Wild rice	Mutant
B9	Bao Thai DB	Mutant
B10	BC15/TBR1	Mutant

Initially, rice seeds were soaked in 0.1% NaOCl for 30 min. After washing several times in distilled water, the seeds were immersed in water at 30 °C for 3 days for germination. The germinated seeds then were grown in a floating tray (2 seeds per hole) in plant growth chamber (28 °C day; 25 °C night; 12 h light; 12 h dark). Culture solution was supplied with Yoshida's nutrient [10]. The solution was salinized at the seedling stage (10 days after sowing) by NaCl powder at four levels of electrical conductivity (EC): 0 dS m<sup>-1</sup>, 4 dS m<sup>-1</sup>, 8 dS m<sup>-1</sup>, and 12 dS m<sup>-1</sup>, which describe the levels of salinity that damage crop growth (0–4 dS m<sup>-1</sup>: slight salinity, 4–8 dS m<sup>-1</sup>: moderate salinity, 8–12 dS m<sup>-1</sup>: severe salinity (United State Department of Agriculture). The time for saline treatment was 21 days, a sufficient period to reveal differences in growth and physiological traits. Trays without NaCl were considered as control (0 dS m<sup>-1</sup>). During treatment, EC and pH (5.5) of the culture solution were checked daily by EC and pH meters. The salinity level (EC level) was maintained at 4 dS m<sup>-1</sup>, 8 dS m<sup>-1</sup>, and 12 dS m<sup>-1</sup> by dissolving NaCl powder in the solution. The culture solution was renewed weekly. The treatment was carried out in 21 days, and the experiment was tri-replicated.

### 2.2. Phenotypic Evaluation

After 21 days of treatment, salinity tolerance of 10 rice materials was scored by standard evaluation score (SES) [13], as shown in Table 2. Survivability was determined by the percentage of survived plants after treatment. Root lengths and plant heights of rice samples were measured in millimeters. After drying in a hot air oven for 5 days at 40 °C, each individual plant was weighed and expressed in

grams. Reductions in root length, plant height, and dry weight caused by salinity stress were calculated by the following formula:

$$\% \text{ reduction} = (P_{\text{sample}} - P_{\text{control}}) / P_{\text{sample}} \times 100$$

where  $P_{\text{sample}}$  is the value of the sample growing in salt concentration (average data of levels 4 dS m<sup>-1</sup>, 8 dS m<sup>-1</sup>, and 12 dS m<sup>-1</sup>) and  $P_{\text{control}}$  is the value of the sample in non-salinity.

**Table 2.** Modified standard evaluation score (SES) of visual injury at the seedling stage.

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but leaf tips or a few leaves whitish and rolled	Tolerant
5	Growth severely retarded, most leaves rolled, only a few are elongating	Moderately tolerant
7	Complete cessation of growth, most leaves dried, some plants are dying	Susceptible
9	Almost all plants dead or dying	Highly susceptible

### 2.3. Genotypic Analysis

The total DNA of rice was extracted by the cetyl trimethylammonium bromide (CTAB) method [35]. Before applying for polymerase chain reaction (PCR), 0.8% agarose gel electrophoresis was used to check the quality of DNA. Forty-two SSR markers linked to *Saltol* were collected from the Gramene database [36] and previous studies [13–34]. DNA was amplified using a Thermal Cycler Gen Atlas S machine. Each reaction contained 0.75 µL genomic DNA (100 ng), 1.5 µL PCR buffer (10 X), 1.2 µL MgCl<sub>2</sub> (25 mM), 0.15 µL dideoxynucleotides (dNTPs) (1 mM), 0.75 µL each of forward and reverse SSR primers (5 µM), and 0.05 µL Taq DNA polymerase (5 U). The volume was increased up to 15 µL by nuclease free water. PCR conditions included initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55–62 °C for 1 min (based on each primer annealing temperature), extension at 72 °C for 2 min, and finished by a final extension at 72 °C for 7 min. The amplification products were resolved on 3 % agarose gel in TBE buffer (0.5 X) and 2.5 µL of safe view under room temperature, at a constant voltage of 50 volts for 75 min. After running, the gel was visualized by AMZ System Science limited STAGE system ECX-F15M (Vilber Lourmat, Eberhardzell, Germany).

### 2.4. Data Analysis

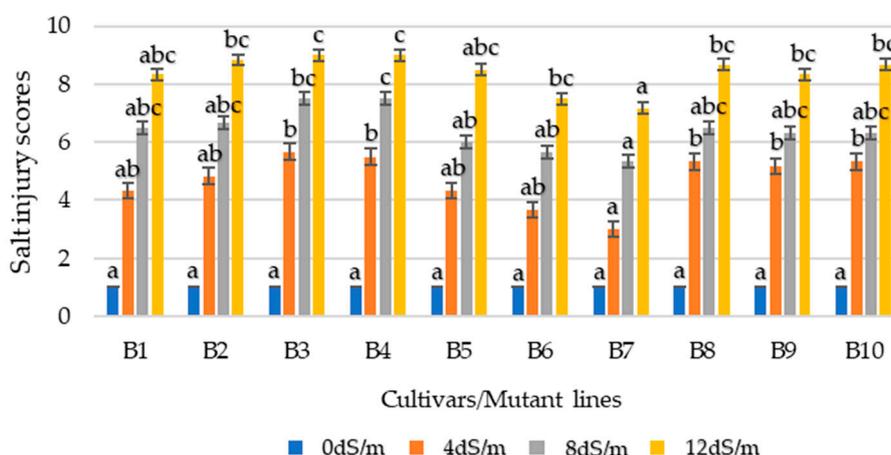
Thirty plants from the three replications were selected in a completely randomized design to evaluate the phenotypic measurements. Phenotypic data were analyzed by one-way analysis of variance (ANOVA), analysis of variance for salt-tolerance parameters was conducted by two-way ANOVA, Minitab software version 16. Tolerant and susceptible lines were evaluated for genotypic identification by SSR markers. Genetic banding patterns were scored based on the presence and absence of a particular band. Heterozygosity (He) and polymorphic information content (PIC) values were calculated with a PIC calculator [37].

## 3. Results

### 3.1. Phenotypic Performance

Reactions of rice plants to salinized solution showed prominent differences at the seedling stage (Figure 1). All the rice materials grew healthily in non-salinized conditions (0 dS m<sup>-1</sup>). In salinized culture solutions, there was a classification of salt tolerance among 10 examined rice cultivars and lines. Results of measurements show that B7 had the lowest injury score in all three levels of salt stress (4 dS m<sup>-1</sup>, 8 dS m<sup>-1</sup>, and 12 dS m<sup>-1</sup>) (3.00, 5.33, and 7.17, respectively); followed by B6 with 3.67, 5.67, and 7.50, respectively. In contrast, the high scores were recorded in B3 (5.67, 7.00, 9.00) and B4

(5.50, 7.50, 9.50) at 4 dS m<sup>-1</sup>, 8 dS m<sup>-1</sup>, and 12 dS m<sup>-1</sup>, respectively. Based on these results, B7 and B6 were determined as tolerant genotypes with lower scores than other samples, while B3 and B4 were susceptible.



**Figure 1.** Salt injury scores of rice samples at different levels of salinity. Values represent mean of scores; different letters in a salinity level indicate a significant difference ( $P < 0.05$ ) by Tukey's test.

Table 3 shows an extreme decrease in survivability of 10 genotypes after treatment. At all three levels of NaCl 4 dS m<sup>-1</sup>, 8 dS m<sup>-1</sup>, and 12 dS m<sup>-1</sup>, survivability of B7 was the greatest (100%, 96.67%, and 66.6%, respectively); followed by B6 (100%, 93.33%, and 63.33%, respectively). B4 was found as the most salt-sensitive genotype, with only 3.33% plants survived at 12 dS m<sup>-1</sup>. B3 was also susceptible to salinity condition, with 16.67% survived plants observed at the same salt level of 12 dS m<sup>-1</sup>.

**Table 3.** Survivability of rice samples under different levels of salinity stress.

Samples/ Salinity Levels	Survivability (%)			
	0 (dS m <sup>-1</sup> )	4 (dS m <sup>-1</sup> )	8 (dS m <sup>-1</sup> )	12 (dS m <sup>-1</sup> )
B1	100	100	90.00 ± 0.00 <sup>ab</sup>	50.00 ± 0.00 <sup>bc</sup>
B2	100	100	86.67 ± 3.33 <sup>abc</sup>	46.67 ± 3.33 <sup>c</sup>
B3	100	100	73.33 ± 3.33 <sup>c</sup>	16.67 ± 3.33 <sup>de</sup>
B4	100	100	86.67 ± 3.33 <sup>abc</sup>	3.33 ± 3.33 <sup>e</sup>
B5	100	100	86.67 ± 3.33 <sup>abc</sup>	56.67 ± 3.33 <sup>abc</sup>
B6	100	100	93.33 ± 3.33 <sup>ab</sup>	63.33 ± 3.33 <sup>ab</sup>
B7	100	100	96.67 ± 3.33 <sup>a</sup>	66.67 ± 3.33 <sup>a</sup>
B8	100	100	83.33 ± 3.33 <sup>abc</sup>	46.67 ± 3.33 <sup>c</sup>
B9	100	100	80.00 ± 0.00 <sup>bc</sup>	43.33 ± 3.33 <sup>c</sup>
B10	100	100	83.33 ± 3.33 <sup>abc</sup>	26.67 ± 3.33 <sup>d</sup>

Values represent mean ± standard errors (SE). Different letters in a column indicate a significant difference ( $P < 0.05$ ) by Tukey's Test.

Salt stress significantly impacts root length, plant height, and dry weight of examined rice (Table 4). In detail, the root length of B3 and B4 decreased, while root length of remaining rice increased. The largest root length reduction was recorded in B4 with 3.17%, followed by B3 with 2.9%. Conversely, roots of B7 and B6 developed by 7.92% and 7.91%, respectively. An extreme reduction was observed in plant height of rice materials under salinity stress (Table 4). The minimum decline was found in B7, followed by B6 (5.65% and 8.57%, respectively). Plant height reduction of B3 was largest, with a percent of 20.34%, remarkably high compared to B4 (16.31%), as well as remaining samples. Similar phenomena were observed in dry weight decrease. Particularly, B3 and B4 presented noteworthy reductions in dry weight with 40.70% and 29.76%, respectively. On the contrary, B6 and B7 performed much lower percentages, in which B7 had the lowest value, at 14.42%.

**Table 4.** Reductions in root length, plant height, and dry weight caused by salinity stress.

Samples	Reduction (%)		
	Root Length	Plant Height	Dry weight
B1	−5.88 ± 1.04 <sup>cd</sup>	11.15 ± 2.17 <sup>ab</sup>	29.66 ± 6.01 <sup>ab</sup>
B2	−2.21 ± 0.92 <sup>c</sup>	13.69 ± 3.68 <sup>ab</sup>	24.85 ± 4.18 <sup>ab</sup>
B3	2.90 ± 0.93 <sup>ab</sup>	20.34 ± 3.80 <sup>a</sup>	40.70 ± 4.90 <sup>a</sup>
B4	3.17 ± 0.67 <sup>a</sup>	16.31 ± 1.82 <sup>ab</sup>	29.76 ± 2.78 <sup>ab</sup>
B5	−5.51 ± 1.19 <sup>cd</sup>	10.83 ± 1.76 <sup>ab</sup>	17.07 ± 4.34 <sup>b</sup>
B6	−7.91 ± 1.19 <sup>d</sup>	8.57 ± 1.33 <sup>b</sup>	15.93 ± 3.56 <sup>b</sup>
B7	−7.92 ± 1.57 <sup>d</sup>	5.65 ± 0.78 <sup>b</sup>	14.42 ± 3.19 <sup>b</sup>
B8	−3.49 ± 0.88 <sup>cd</sup>	13.77 ± 1.95 <sup>ab</sup>	28.44 ± 5.34 <sup>ab</sup>
B9	−4.57 ± 1.42 <sup>cd</sup>	15.35 ± 1.41 <sup>ab</sup>	23.64 ± 3.64 <sup>ab</sup>
B10	−1.83 ± 0.39 <sup>bc</sup>	15.79 ± 2.97 <sup>ab</sup>	21.42 ± 2.80 <sup>ab</sup>

Values represent mean ± standard errors (SE). Different letters in a column indicate a significant difference ( $P < 0.05$ ) by Tukey's Test.

Under salt stress, variation of all parameters was highly significant in different samples at different salinity levels (Table 5). Results of analysis also show that salinity level is the most influential factor in the disparity of salt injury score, survivability, plant height, and dry weight reduction. Root length reduction was most affected by samples factor. Noticeably, there was a remarkable discrepancy observed in root length and plant height reductions of rice plants caused by the interaction of samples and salinity levels. No disparity decrement was recorded in salt injury score, survivability, and dry weight reduction of examined rice, because of this combination.

**Table 5.** Analysis of variance for salt-tolerance parameters of rice.

Source of Variation	Df	Salt Injury Score	Survivability	Root Length Reduction	Plant Height Reduction	Dry Weight Reduction
Samples	9	91.93 *	5298.90 *	1255.35 *	1447.31 *	5155.20 *
Salinity levels	2	128.67 *	47426.00 *	334.36 *	2103.27 *	10942.60 *
Interaction	18	7.92	4902.20	87.12 *	608.56 *	1047.80
Error	60	5.16	2074.10	402.64	1254.22	723.30
Total	89	233.68	59701.20	2079.48	5413.36	17868.80

Values with \* are significantly different at  $P < 0.05$  by Tukey's Test.

Results of correlation analysis among five parameters are presented in Table 6. There are noticeable negative correlations between survivability and remaining parameters in which the maximum value was observed between survivability and root length reduction (−0.95). Additionally, the tolerant score was significantly positively correlated with root length, plant height, and dry weight reductions. The analysis also recorded the positive correlations between plant height and root length reductions, dry weight, and root length reductions, and dry weight and plant height reductions.

**Table 6.** Correlation matrix among tolerant parameters.

Parameters	Parameters				
	TS	SUR (%)	RLR (%)	PHR (%)	DWR (%)
TS	1				
SUR (%)	−0.93 **	1			
RLR (%)	0.90 **	−0.95 **	1		
PHR (%)	0.94 **	−0.87 **	0.89 **	1	
DWR (%)	0.79 **	−0.70 *	0.78 **	0.82 **	1

\*, \*\*: correlation is significant at 0.05 and 0.01 levels, respectively; TS: tolerant score; SUR: survivability; RLR: root length reduction; PHR: plant height reduction; DWR: dry weight reduction.

### 3.2. Genotypic Analysis

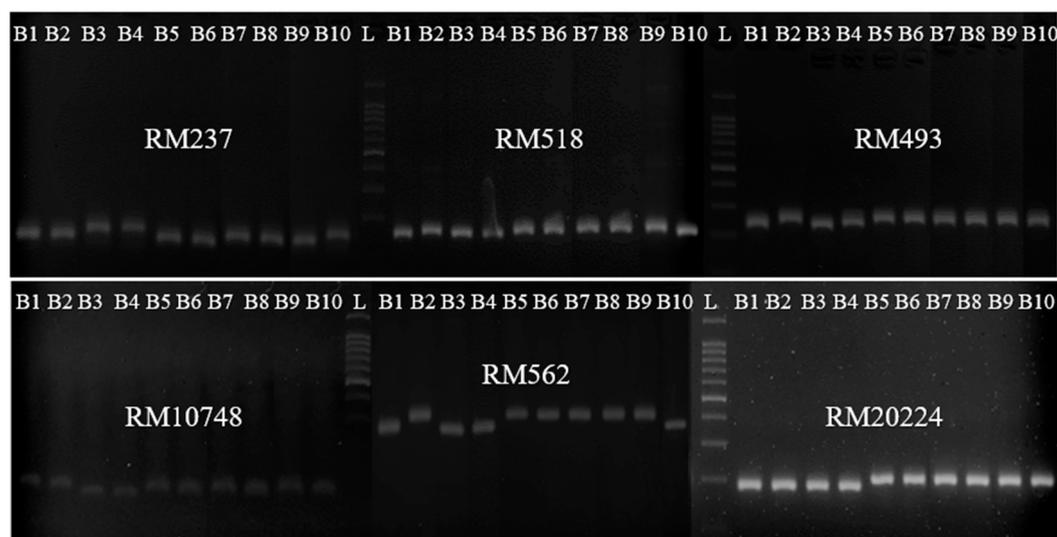
A set of 42 SSR markers were used to identify the differences between tolerant and sensitive genotypes. Among them, twenty-two markers showed clear DNA bands, and six markers were found to be polymorphic with amplified SSR loci, including RM 237, RM 518, RM 493, RM 10748, RM 562, and RM 20224. All these markers could determine the difference between tolerant groups (B6 and B7) and susceptible groups (B3 and B4), as shown in Figure 2. SSR markers, location, number of alleles, heterozygosity (He), and polymorphism information content (PIC) are presented in Table 7.

**Table 7.** Polymorphic markers information.

SSR Markers	Chromosome	Sequences *	Number of Alleles	He	PIC
RM 237	1	CAAATCCCGACTGCTGTCC TGGGAAGAGAGCACTACAGC	3	0.65	0.58
RM 518	4	CTCTTCACTCACTACCATGG ATCCATCTGGAGCAAGCAAC	2	0.49	0.37
RM 493	1	TAGCTCCAACAGGATCGACC GTACGTAACGCGGAAGGTG	2	0.41	0.33
RM 10748	1	CATCGGTGACCACCTTCTCC CCTGTCATCTATCTCCCTCAAGC	3	0.65	0.58
RM 562	1	CACAACCCACAAACAGCAAG CTCCCCCAAAGTTTAGCC	2	0.41	0.33
RM 20224	2	AGTATGAAAGTCGGTGACGATGG GAGATGTCACGTCTTCACTTAGGG	3	0.65	0.58

He: heterozygosity; PIC: polymorphism information content. \*: Gramene.com.

Among six polymorphic markers, four markers were located on chromosome 1 (RM 237, RM 493, RM 10748, and RM 562) while RM 518 was located in chromosome 4, and RM 20224 was found in chromosome 2. Three markers, RM 237, RM 10748, and RM 20224 had three alleles; other markers, RM 518, RM 493, and RM 562 had two alleles. Heterozygosity values of these SSR markers ranged from 0.41 to 0.65, and PIC values varied from 0.33 to 0.58. Three markers, RM 237, RM 10748, and RM 20224, shown the highest heterozygosity and PIC values at 0.65 and 0.58, respectively.



**Figure 2.** PCR products of simple sequence repeat (SSR) markers RM 237, RM 518, RM 493, RM 10748, RM 562, and RM 20224 (L: ladder 100 bp).

#### 4. Discussion

Salinity stress caused variations in survivability, plant height, root length, and dry weight of ten examined parameters. However, observed responses to salinity were different in different samples. Based on phenotypic performance, ten rice cultivars/lines can be classified into three groups: tolerant (B6 and B7), moderate-tolerant (B1, B2, B5, B8, B9, B10), and susceptible (B3 and B4) genotypes. The classification was carried out based on their performances in all measurements, including root length, plant height, and dry weight surveys, in which salt injury score and survivability are conclusive parameters. Notably, root length reductions of examined rice variably fluctuated. Negative values in root length reduction indicate that roots of these variety/mutant lines were elongated under saline treatment. This phenomenon maybe occur because of their tolerant mechanisms adapting to salt concentrations. Previous researchers indicated that each rice variety has one or two salt tolerance mechanisms, not all, and the response of plants to salt tolerance is a complex combination of individual factors [38]. Although  $\text{Na}^+$  and  $\text{K}^+$  contents in shoots and roots are different between tolerant varieties and sensitive varieties, these traits are mainly controlled by additive genes [38]. Therefore, the heritability of these traits is very low [39]. On the other hand, the *Saltol* QTLs located on chromosome 1, which are tightly correlated with chlorophyll content and  $\text{Na}^+$  and  $\text{K}^+$  concentrations, as well as  $\text{Na}^+/\text{K}^+$  ratios in rice shoots and roots [23], were used for the genetic analysis. The results showed a clear distinction between tolerant genotype and susceptible genotype. Since phenotype represents the consequence of genotype–environment interactions ( $P = G \times E$ ) in all living organisms, phenotype is the physical representation that positively correlates with genotype (under the same controlled environment). In this case, genes with specialized functions play a key role in a series of transcriptions and translations. Therefore, tolerant plants are able to protect themselves from saline damages by various physiological processes. Correlation analysis reflects that all morphological assessments contributed to salt injury score. Explanation of high correlation may be attributed to pleiotropic or linked genes [40]. Pleiotropism occurs when the expressions of more than one character are influenced by the same gene. In case of genes linkage, several genes located in the same chromosome are inherited together. This information is effective in rice breeding, due to the simultaneous selection of various expected characters based on the individual.

Although rice yield is adversely affected by salinity, rice has been considered as a suitable crop for reclamation of saline and sodic soil [41], therefore, developing salinity tolerance in rice is a crucial key to ensure sustainable rice production. Studies on rice tolerance mostly focus on seedling stage, because of its significant interaction with productivity [8,38,41]. Salt-tolerant rice at the seedling stage has a lower sodium concentration in maturity, compared to sensitive genotypes [41]; the desirable plants can be easily and quickly selected. On the other hand, rice salt-tolerant investigation in this stage is considered as a rapid method based on simple principles [42].

Salinity tolerance is a complex physiological trait, it is related to other traits [21] and controlled by different mechanisms [8]. Therefore, it is essential to evaluate rice genotypes. Application of molecular markers helps the breeders to select target plants in early stages by classifying genotypes based on the presence or absence of a particular marker locus and determine whether significant differences exist between them [43]. Among these markers, SSR is considerable in accelerating rice breeding time [44]. Therefore, SSR is the most widely-used marker in major cereals [45–47]. Former research used SSR markers linked to the *Saltol* gene in chromosomes 1, 2, and 4, to identify tolerant as well as sensitive genotypes. In this study, genetic analysis was conducted by application of 42 *Saltol*-linked SSRs, distributed along 12 chromosomes. Among them, six markers, RM 493, RM 562, RM 10748, RM 20224, RM 237, and RM 518 are polymorphic, as they can classify tolerant and susceptible genotypes (others were not shown). Therefore, these markers are useful for classification of salt tolerance of rice. Similar results were observed in earlier research [22,24–30,48]. The low polymorphic ratio may be because of the high genetic similarity between examined rice. Analysis pointed out that these SSR markers expressed moderate polymorphic values. In particular, we found that they distinguish tolerant and sensitive rice in both mutant lines and cultivars. Although SSRs are co-dominant, they

were scored as presence or absence markers, because examined cultivars (homozygous genotypes) are the result of breeding programs through various generations. On the other hand, homozygosity can be also obtained in rice mutants when they complete segregation.

It is significant to note that the mutant line originating from SKLo and BC15TB had higher yield than their parents [33]. Also confirmed were their widely adapted abilities, as well as better characteristics, compared to parental performances [33]. Furthermore, this mutant line was originated from elite cultivated parents in Northern Vietnam which are indicated to have good quality, high yield, and abiotic resistance [49]. Haplotypes developed from them are promised as valuable inheritances for crop improvement. However, the cross SKLo/BC15TB needs to be compared its characteristics with BC15TB, especially the production under saline stress. The further goal is to breed a new cultivar generation with higher quality, productivity, and wider adaptation to feasible conditions.

## 5. Conclusions

In this study, rice mutants were classified into three groups of salinity tolerance, including tolerant (SKLo/BC15TB, BC15TB), moderately tolerant (Bao Thai, DT84, SKLo, Khang dan/Wild rice, Bao Thai DB, BC15TB/TBR1), and susceptible groups (Bao Thai/DT84, DT84 DB). The markers RM 493, RM 562, RM 10748, RM 518, RM 237, and RM 20224 are the most polymorphic in salinity tolerance. Of them, RM 237, RM 10748, and RM 224 show the highest polymorphism information (PIC = 0.58). The results suggest that BC15TB and its progeny BC15TB/SKLo are valuable sources for breeding rice tolerant to salinity. Findings of this study may help to simplify the breeding of salinity-tolerant rice to adapt to climate change.

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