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BREEDING RICE (*Oryza sativa.* L) FOR SALT TOLERANCE AND GRAIN QUALITY TRAITS

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SUMMARY

The development of rice cultivars with salinity tolerance, high yield, and good quality is a very essential demand that has been raised to provide poor farmers in the lowland conditions of the coastlines of the Mekong Delta, Vietnam. This study aimed to develop rice cultivars that were tolerant of salinity at the seedling and reproductive stages via molecular marker, single crossing, and backcrossing breeding methods. The salt tolerance of F8 and BC_3F_4 progenies was assessed under salt stress field conditions to select promising lines for improving production by farmers. Salt tolerance alleles on chromosomes 1 and 8 were detected by combining traditional breeding by backcross and generation selection with SSR markers. L1 (OM10252/Pokkali); L5 (OMCS2012/Pokklai); L21 and L22 (OM10252/Pokkali//OM10252); and L24 and L25 (OM8902/Pokkali//OM8902) were identified as high-yielding genotypes with good salinity tolerance. However, many remaining lines failed due to their extended growth duration, high unfilled grain percentage, and poor grain quality. Four lines, namely, *viz.* L1, L5, L24, and L25, exhibited good agronomic characters, such as low amylose content and salt stress tolerance. The typical tolerant genotype Pokkali was successfully exploited into high-yielding rice cultivars via breeding programs. This work is a good opportunity to improve salt tolerance rice cultivars via marker-assisted selection with RM223, RM8094, HATRI02, RM3412, RM493, and RM3252.

Keywords: Chromosome, high-yielding, grain quality, salt, SSR markers

Key findings: The special lines *viz*. L1 (OM10252/ Pokkali); L5 (OMCS2012/Pokklai), L21 and L22 (OM10252/Pokklai//OM10252), and L24, L25 (OM8902/Pokkali/OM8902) were assessed for salinity stress at EC = 8 dS/m under field conditions. This work developed high yielding and good quality rice genotypes with improved salt tolerance for cultivation in various regions in Vietnam.

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INTRODUCTION

Vietnam is the second largest exporter of rice in the world; it exports 23–25 million tons per year (MARD, 2019). The long-term strategy for Vietnam's advancement is balancing exports while ensuring food security before the challenge of climate change. Therefore, the rice breeding strategy is to implement pedigree selection in adverse soil ecosystems, such as salt intrusion areas, which has become a massive challenge for rice breeders.

Water shortage is a main problem encountered in rice production and new cultivar creation. In addition to drought stress, salt stress remains seriously damaging to rice. These factors reduce rice productivity in affected areas, particularly in the Mekong Delta, Vietnam. Salt-water intrusion was identified in early 2016 as a growing risk to agricultural production and is hastened by upstream damming and mangrove decline (Bui 2020).

Salinity intrusion leads to increa ses in the salinity of water in rivers and irrigation canals. Salinity (4 g/L) expanded through the Tien and Hau Rivers by up to 45–65 km and 55–60 km, respectively. Drought has resulted in decreases in groundwater levels and the most extensive salinity intrusion in last 90 years (Bui, 2020). Severe drought and salinity intrusion strongly affect 11 of the 13 provinces in the Mekong Delta. Some 400,000 ha of cropland have been affected, of which 25,900 ha are left fallow (Bui, 2017). Rice areas affected by drought and salinity intrusion have rapidly increased from 139,000 ha in mid-March 2016 to 224,552 ha by mid-April 2016 with the damage value of $US 360 million (Bui, 2017).

Lang *et al.* (2011) reported that tagging salt tolerant genes on the basis of SSR markers identified alleles that are located near chromosome 1 in the advanced backcross populations (BC_2F_2) of IR64/Cheng Hui 448, IR64/OM1706, and IR64/FR13A, whereas in the population of IR68552-55-3-2/OM1706, the alleles are linked with RM223 on chromosome 8 (Table 1).

Tiwari *et al.* (2016) reported a method for the rapid identification of QTLs for reproductive stage salt tolerance in rice via the bulked segregant analysis (BSA) of biparental recombinant inbred lines (RIL). The method was applied to segregating CSR11/ MI48 RILs. The genotyping of the parents and RIL bulks on the basis of salt sensitivity index for grain yield revealed 6,068 polymorphic SNPs and 21 QTL regions showing the homogeneity of contrasting alleles in the two bulks. BSA with 50K SNP chip revealed 5,021 polymorphic loci and 34 QTL regions.

QTL maps for traits related to salinity tolerance at the different stages of rice development have been established by using segregating populations (BC_2F_2) on the basis of SSR markers (Lang *et al* 2011) to coordinate conventional breeding and molecular markers for scientific background and rapid practical

Table 1. SSR markers on chromosomes 1 and 8 used in this study.

SSR markers	Repeat motif	Forward primer sequence	Reverse primer sequence
RM223	(AG) 16	F 5′-GAGTGAGCTTGGGCTGAAAC-3′	R 5′-GAAGGCAAGTCTTGGCACTG-3′
RM8094	(AT) 31	5′-AAGTTTGTACACATCGTATACA-3′	R 5′-CGCGACCAGTACTACTACTA-3′
HATRI 02		F 5′-GGTTAAAGTGGGCAAAAGAGG-3′	R 5′-AGAGGGGGAGAGGTGTGATT-3′
RM3412	(CT) 17	F 5′-AAAGCAGGTTTTCCTCCTCC-3′	R 5′-CCCATGTGCAATGTGTCTTC-3′
RM493	(CTT) 9	F 5′-TAGCTCCAACAGGATCGACC-3′	R 5′-GTACGTAAACGCGGAAGGTG-3′
RM3252		F 5′-GGTAACTTTGTTCCCATGCC-3′	R 5′-GGTCAATCATGCATGCAAGC-3′

application (Lang *et al.,* 2009a, b, 2011). Initial outputs have been obtained via the exploitation of broad-spectrum rice cultivars, such as OM4900 (salinity and water depth of 80 cm), OM6162 (salinity and water depth of 50 cm), and OM6161 (salinity and water depth of 80–100 cm).

The major genes (QTL) for salt tolerance (*Saltol*) were mapped on chromosomes 1 and 8 (Lang *et al.* 1999, Lang *et al.* 2001). In the F_3 population of IR28/Doc Phung, RM223 is linked to the salt tolerance locus at the distance of 6.3 cM on chromosome 8 at the vegetative stage under EC = 10 dS m^{-1} (Lang *et al*. 1999). Bonilla *et al.* (2002) mapped the *Saltol* locus linked to major QTL for Na^+ and K^+ uptake and Na^+/K^+ ratio on chromosome 1; these QTL explained 64.3% of the phenotypic variance.

Ammar *et al.* (2009) reported 25 QTL for salt ion concentrations (Na^+, K^+, and Cl^- measured in leaf tissues at the reproductive stage) on rice chromosomes 1 and 8. The selective screening of some lines with tolerance at the seedling stage remains underway. The current focus on screening and selection for salt tolerance should result in the identification of an increasing number of salt tolerance genes in rice. Takahashi (1974) reported that new improved *indica* and *japonica* cultivars had similar germination trends under 1.5% NaCl but do not germinate at 2.5% NaCl; traditional *indica* cultivars had higher germination percentages than improved cultivars.

MATERIALS AND METHODS

The experiment was carried out with parents from four different crosses from Pokkali mother and father lines. The lines were backcrossed with 11 BC3F4 lines from OM10252/Pokkali//OM10252, and OM8902/Pokkali; OM5451/Pokkali is high-yielding and well-adapting. This high-yielding cultivar was used as the mother and crossed with Pokkali, a tolerant cultivar from India. In total, 1211 progenies were selected from F_2, F_3, F_4, F_5, F_6, F_7, F_8, and BC_3F_4 populations. Under rained lowland conditions in High Agricultural Technology Research Institute for Mekong Delta (HATRI), only 27 lines were selected as good genotypes with salt tolerance. They were continuously evaluated for yield and yield components with checks, i.e., OM10252, OM5451, and OM8902 (the highest yielding and leading cultivars in the Mekong Delta without salinity tolerance). The yield of selected F_8 and BC_3F_4 individuals, which derived their salt tolerance from Pokkali, was the same as that of the checks.

Phenotype analysis

A field experiment with transplanting practice under irrigated lowland field conditions was designed in a randomized complete block with three replications. The experiment was located at Binh Thuy, Can Tho. The agronomic characteristics of 50 BC_3F_4 lines and their parents were evaluated. Genotypic analysis, sensory test, and SSR marker analysis were implemented at HATRI lab. Important agronomic traits were recorded.

Ten randomly selected plants of each genotype were used for agronomic data analysis. Plant height (cm), effective tiller number per plant, panicle length (cm), filled grain number per panicle, 1,000-grain weight (g), days to maturity, and grain yield/plant (g) were recorded and subjected to statistical analyses by using SAS software. After harvesting, the seeds from each genotype were dehulled for the evaluation of grain quality properties and aroma. The grains were classified into different types on the basis of their dimensions (Cruz and Khush 2000). Ten seeds of each cultivar were dehulled and mashed by hand. The rice powder of each cultivar was taken and placed in separate experimental tubes or Petri dishes. A total of 5 mL of KOH 1.7% was added to each petri dish and covered. The samples were stored at room temperature for 30 min. The samples were then scored. The scores corresponded to the absence of aroma, slight aroma, moderate aroma, and strong aroma.

Phenotyping and evaluation of F_8 and BC_3F_4 for salt tolerance

Screening at the seedling stage

A total of 32 lines were screened for salinity tolerance under controlled environmental conditions. The rapid screening method was used. Two pregerminated seeds were planted in each hole of Styrofoam seedling floats and placed in a tray filled with distilled water. After 3 days, the distilled water in trays with seedlings was replaced with salinized nutrient solution. The nutrient solution was prepared in accordance with a protocol by Yoshida *et al.* (1976). Salinization was performed by adding NaCl up to the desired electrical conductivity (EC). Initial salinity was set as EC = 6 dS/m (50 mmol/L) and increased to EC = 12 dS/m (100 mmol/L) 3 days later. The solution was renewed every 8 days, and the pH was maintained daily at 5.0. Parental checks (Pokkali, OM8902, OMCS2012, OM5451, and OM10252) were included in every tray. The screening test was conducted in a HATRI glasshouse maintained at 29 °C/21 °C (day/night temperature) and a minimum relative humidity of 70% during the day under natural daylight.

The modified standard evaluation score system (Table 2) was used to rate the symptoms of salt toxicity. Scoring was done at 21 days after salinization. At this period, the resistant parent (Pokkali) exhibited a score of 3, and susceptible parents (OM8902, OMCS2012, OM5451, and OM10252) exhibited a score of 9.

Table 2. Original F_8 and BC_3F_4 from HATRI (Lang *et al.,* 2016).

No.	Lines	Crosses	No.	Lines	Crosses
1	L1	OM10252/Pokkali	17	L17	OM8902/Pokkali
2	L2	OM10252/Pokkali	18	L18	OM8902/Pokkali
3	L3	OM10252/Pokkali	19	L19	OM8902/Pokkali
4	L4	OM10252/Pokkali	20	L20	OM12025/Pokkali//OM10252
5	L5	OMCS2012/Pokkali	21	L21	OM12025/Pokkali//OM10252
6	L6	OMCS2012/Pokkali	22	L22	OM12025/Pokkali//OM10252
7	L7	OMCS2012/Pokkali	23	L23	OM12025/Pokkali//OM10252
8	L8	OMCS2012/Pokkali	24	L24	OM8902/Pokkali//OM8902
9	L9	OMCS2012/Pokkali	25	L25	OM8902/Pokkali//OM8902
10	L10	OMCS2012/Pokkali	26	L26	OM8902/Pokkali//OM8902
11	L11	OM5451/Pokkali	27	L27	OMCS2012/Pokkali//OMCS2012
12	L12	OM5451/Pokkali	28	OM8902	Parental Check
13	L13	OM5451/Pokkali	29	OM10252	-do-
14	L14	OM5451/Pokkali	30	OMCs2012	-do-
15	L15	OM5451/Pokkali	31	OM5451	-do-
16	L16	OM8902/Pokkali	32	Pokkali	-do-

Shoot Na and K concentration analysis

The confirmed extremes, 27 lines, and parents were tested to determine shoot Na and K concentrations and Na/K ratios. Low shoot Na/K ratios indicated tolerance, and high Na/K ratios indicated susceptibility. The mechanism for salinity tolerance in rice is the capability to absorb less toxic Na^+ and take up more K^+ to maintain a good Na/K balance in the shoot.

The same screening procedure was followed with modifications in the timing of salinization. Plants were grown for up to 14 days in normal nutrient solutions. Salinity was gradually increased by adding NaCl. Salinity was initially set as EC = 4 dS/m for 1 week, increased to EC = 8 dS/m for another week, and then to EC = 12 dS/m for 2 weeks. The test was conducted with a randomized complete block design (RCBD) with three replications. Grouping was done by plant height to prevent tall plants from shading short plants. Shoot sampling for tissue analysis was performed when the susceptible parents OM10252 and OM8902 had a score of 7 and OM5451 and OMCS2012 had a score of 9. These scores were obtained at 21 days after salinization with EC = 12 dS/m. Ten plants per line were sampled and oven-dried for 5 days at 70 °C. Dried samples were ground, and 1 g of powder was taken from each sample for Na and K concentration analysis by using atomic absorption.

DNA extraction

The 31 lines/cultivars were grown in pots. Maximum protection was provided to ensure healthy and disease-free seedlings. The leaves were collected at 2–3 weeks after planting for DNA extraction.

Standard molecular-grade chemicals and general techniques for preparing stock solutions, buffers, reagents, and equipment were used in accordance with Sambrook *et al.*

Table 3. Modified standard evaluation score of visual salt injury at seedling stage.

Score	Observations
1	Normal growth, no leaf symptom
3	Nearly normal growth, but leaf tips or few leaves whitish and rolled
5	Growth severely retarded; most leaves rolled; only a few are elongating
7	Complete cessation of growth; most leaves dry; some plants dying
9	Almost all plants dead or dying

(1989). Molecular work was conducted at HATRI, Vietnam.

DNA suitable for PCR analysis was prepared by using a simplified procedure (McCouch *et al.* 1988). A piece of a young rice leaf (2 cm) was collected and placed in a labeled 1.5 mL centrifuge tube on ice. The leaf was ground in a well of a spot test plate (Thomas Scientific) by using a polished glass rod after adding 400 µL of extraction buffer. Grinding was performed until the buffer turned green, an indication of cell breakage and chloroplast and cell content release. Another 400 µL of extraction buffer was added into the well by pipetting. Approximately 400 µL of the lysate was transferred to the original tube of the leaf sample. The lysate was deproteinized by using 400 µL of chloroform. The aqueous supernatant was transferred to a new 1.5 mL tube, and DNA was precipitated by using absolute ethanol. DNA was air-dried and resuspended in 50 µL of TE buffer (Lang, 2002).

DNA quality checks were performed on 1% agarose, which was prepared by melting 3 g of agarose in 300 mL of TAE buffer. The mixture was heated in a microwave for 5–6 min and then cooled to approximately 55 °C–60 °C. The gel was then poured into a previously prepared electrophoresis box with combs. The gels were prepared, and the combs were removed after approximately 45 min. Seven microliters of DNA sample plus 3 µL of loading buffer (Tris 1 M pH = 8.0, glycerol, 0.5 M EDTA pH = 8.0, 0.2% xylene cyanol, 0.2% bromophenol blue, and distilled water) was run at 70–80 V and 60 mA for 45 min or until the loading buffer dye moved far away from the wells. The gel was then removed, stained with ethidium bromide, and observed under UV light.

Microsatellite analysis

The whole microsatellite analysis included PCR assay, polyacrylamide gel electrophoresis, and band detection and scoring.

PCR assay

Microsatellite primers were used to survey the polymorphism of the samples. These primers were randomly selected from the 6 microsatellite primer pairs that are currently available for rice h (Table 3). The PCR reaction was as follows:
Reactions were overlaid with mineral oil and processed in a programmable thermal controller set for 35 cycles of 1 min at 94 °C, 1 min at 55 °C, and 2 min at 72 °C with a final extension at 75 °C for 5 min. After amplification, 10 µL of stop solution was added to the PCR product, which was then denatured at 94 °C for 2 min. Eight microliters of each reaction was run on polyacrylamide gel.

Data analysis

Analysis of variance**:** Agromorphological data collected were initially analyzed through analysis of variance to verify genetic variation in the measured traits. Several traits with insignificant genetic variation, based on the F-test, were not considered for further analyses.

RESULTS AND DISCUSSION

Lines were developed from four crosses (OM10252/Pokkali, OM5451/Pokkali, OMCS2012/Pokkali, and OM8102/Pokkali). DNA marker data may be useful for several applications in breeding, such as cultivar identification, genetic diversity assessment, parental selection, and hybrid confirmation (Bertrand and Mackill, 2008). Salinity gene screening is based on molecular markers (Lang *et al.* 2015). Molecular values are assessed on the basis of polymorphism targets and the codominant genome of the cultivars. The information of genetic maps (Mackill *et al.* 2006) is recorded with respective molecular markers on chromosomes 1 and 8. Four crosses showed (Table 2) differences between the recorded molecular markers with groups of SSR markers as follows: The line from OM 5451/Pokkali showed polymorphism with the markers HATRI 02 and RM3252. The population from OMCS2012/Pokkali was polymorphic with the molecular markers of HATRI 02, RM223, RM3252, RM493, and RM8904. OM8102/Pokkali was polymorphic with HATRI 02, RM223, RM3252, RM8904, and RM3412 markers. In particular, the combination of OM10252/Pokkali was polymorphic with the six molecular markers HATRI 02, RM223, RM3252, RM493, RM8904, and RM3412 markers belonging to two groups (Figure 1). Some examples showed polymorphisms with the molecular markers.

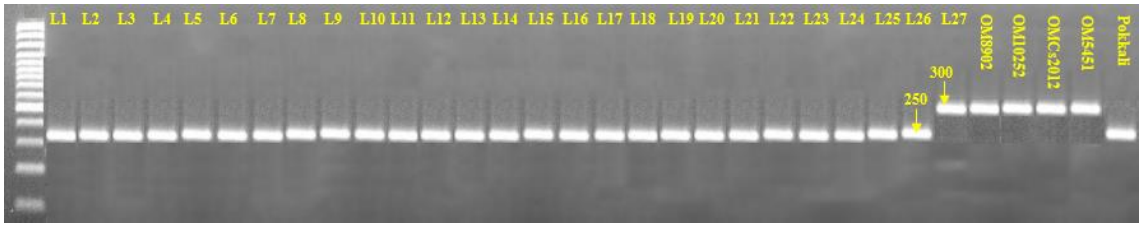
Yield components

Grain yield and 1,000-grain weight: In this study, grain yield from L1L8 reached over 7 t/ha. Most rice lines offered more than 100 grains per panicle, and this character is desirable for breeding programs. The 1,000-grain weight ranged from 23.00 g to 28.78 g (Table 4). This trait is very important for farmers' selection of some lines, such as lines L9, L10, L23, and L27, with large and bold grains.

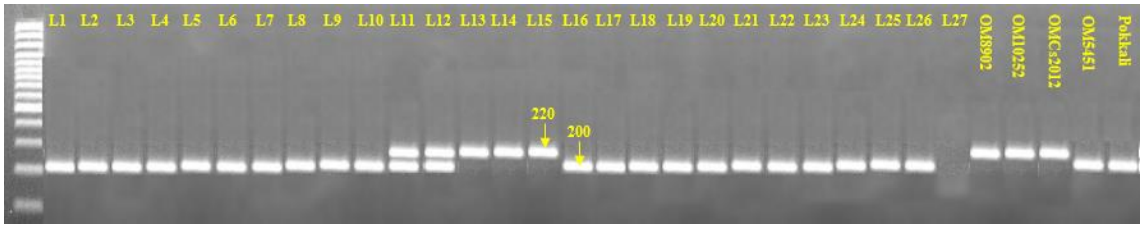
Analyzing and evaluating the salinity targets of 32 promising lines.

Among the 32 lines, six lines, namely, L1, L2, L17, L24, L25, and L27, as well as the Pokkali check, exhibited resistance to salinity at level 1. The ratio of Na^+ and Cl^- were also analyzed (Table 5). Among the 32 lines selected in Ba Tri–Ben Tre, 10 exhibited good adaptability to salinity as compared with their parents (Table 6).

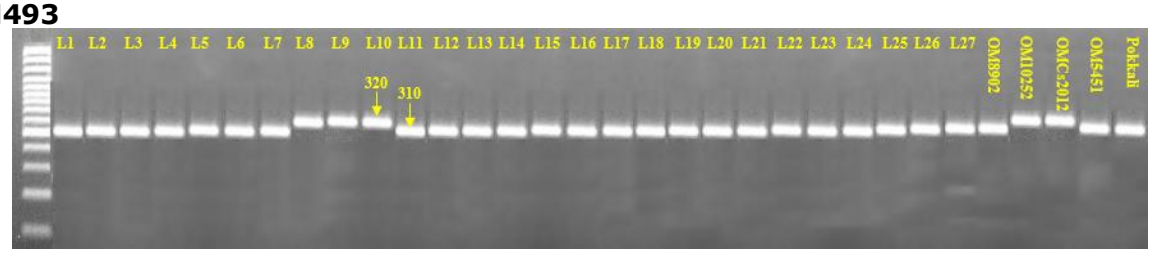
HATRI 02



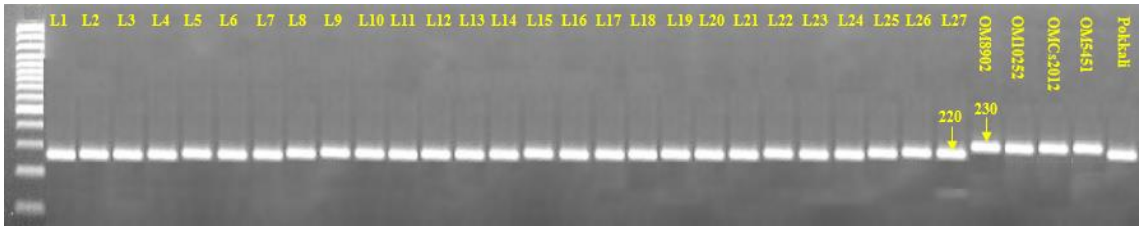
RM223



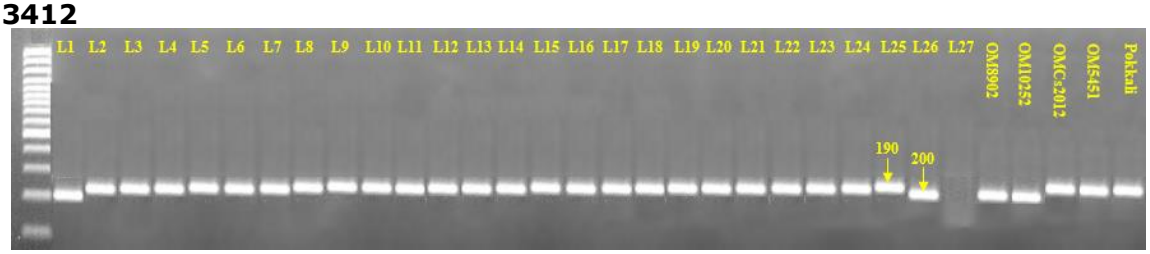
RM493



RM3252



RM3412



RM8094

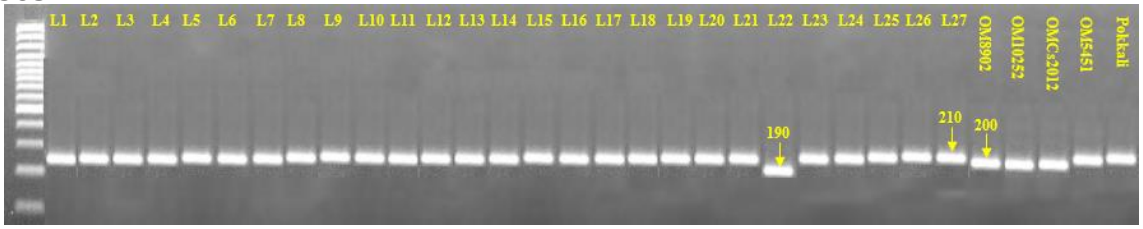


Figure 1. PCR products of the line at HATRI 02 (A), RM223 (B), RM493 (C), RM3252 (D), RM3412 (E), and RM8094 (F). The gene associated with resistance to salinity is located at the positions of chromosomes 1 and 8 on 3% agarose gel.

Table 4. Yield and component yield 32 lines from wet season 2017.

No.	Lines	Dura-tion (days)	Filled panicle	Unfilled (%)	1000-grain weight (g)	Yield (t/ha)	Height (cm)	Salt stress response
1	L1	107	115 ab	17.7 ac	25.63	7.53 a	115	1
2	L2	107	144 a	13.2 d	24.70	7.47 ab	125	1
3	L3	107	117 ab	16.7 ad	27.92	7.43 ab	110	7
4	L4	105	108 b	19.08 a	25.00	7.23 ac	115	5
5	L5	103	122 ab	14.9 bd	24.88	7.13 ad	105	3
6	L6	105	134 ab	14.5 bd	22.53	7.07 ae	107	9
7	L7	105	111 b	16.9 ad	25.23	7.07 ae	110	5
8	L8	104	118 ab	17.2 ab	23.57	7.00 ae	118	5
9	L9	107	115 ab	14.5 bd	28.26	6.97 ae	120	5
10	L10	105	111 b	15.8 ad	28.76	6.90 ae	115	5
11	L11	105	111 b	16.4 ad	23.94	6.87 ae	106	3
12	L12	102	131 ab	16.5 ad	24.32	6.83 ae	115	3
13	L13	103	122 ab	15.5 ad	24.33	6.80 ae	120	7
14	L14	106	119 ab	14.0 cd	25.67	6.67 ae	110	7
15	L15	105	109 b	17.8 ac	24.87	6.67 ae	105	7
16	L16	110	133 ab	15.0 bd	24.03	6.63 ae	116	3
17	L17	100	127 ab	16.7 ad	24.77	6.53 ae	106	1
18	L18	107	107 b	19.3 a	26.75	6.50 ae	114	7
19	L19	103	118 ab	16.6 ad	25.08	6.20 af	105	5
20	L20	105	115 ab	16.4 ad	25.60	6.00 bf	110	7
21	L21	105	128 ab	15.1 bd	24.88	5.90 df	120	3
22	L22	107	132 ab	14.9 bd	23.71	5.80 ef	117	3
23	L23	104	114 ab	16.0 ad	28.01	4.93 fg	118	5
24	L24	104	107 b	18.4 ab	23.93	4.13 g	105	1
25	L25	95	108 b	16.6 ad	22.0	5.8 abc	110	1
26	L26	95	119 ab	19.2 a	27.4	4 ijk	98	7
27	L27	95	125 ab	16.6 ad	28.74	5.8 abc	99	1
Checks								
28	OM8902	100	129 ab	16.2 ad	24.17	3.6 jk	108	7
29	OM10252	100	132 ab	14.1 bd	23.70	3.6 ij	101	7
30	OM5451	90	114 ab	14.3 bd	23.40	4.9 ch	95	9
31	OMCS2012	90	107 b	16.1 ad	27.04	3.8 ijk	105	9
32	Pokkali	120	106 b	18.1 ab	25.07	4 ijk	125	1
CV (%)		17.48	3.97	16.33	2.73	9.55		

Subsequently, yield and yield components were observed, and only two lines, namely, L1 and L5 exhibited high grain yield. Their growth duration was short, i.e., less than 100 days. The grain number per panicle is usually highly proportional to spikelet number. Rice lines L5, L21, L22, and L25 presented high-yielding characters.

Table 5. Extreme tails of salinity tolerance scores, shoot Na and K concentrations, and shoot Na/K ratios identified by screening F8 and BC3F4 under controlled conditions and EC = 8 dS/m.

No.	Lines	Salinity	Na (%)	K (%)	Na/K ratio
1	L1	1	0.533	2.41	0.22
2	L2	1	0.574	2.12	0.27
3	L3	7	1.96	1.52	1.29
4	L4	5	0.612	2.96	0.21
5	L5	3	0.445	2.98	0.15
6	L6	9	1.75	1.32	1.33
7	L7	5	0.745	2.59	0.29
8	L8	5	0.632	2.62	0.24
9	L9	5	0.745	2.43	0.31
10	L10	5	0.669	2.12	0.32
11	L11	3	0.756	2.02	0.37
12	L12	3	0.563	2.16	0.26
13	L13	7	1.75	1.32	1.33
14	L14	7	1.85	1.32	1.40
15	L15	7	1.17	1.32	0.89
16	L16	3	0.532	2.51	0.21
17	L17	1	0.562	2.55	0.22
18	L18	7	1.96	1.32	1.48
19	L19	5	0.612	2.96	0.21
20	L20	7	1.69	2.98	0.57
21	L21	3	0.422	2.89	0.15
22	L22	3	0.745	2.59	0.29
23	L23	5	0.632	2.62	0.24
24	L24	1	0.745	2.43	0.31
25	L25	1	0.669	2.12	0.32
26	L26	7	1.96	1.32	1.48
27	L27	1	0.563	2.16	0.26
Checks					
28	OM8902	7	1.74	1.08	1.61
29	OM10252	7	1.69	1.11	1.52
30	OM5451	9	2.25	1.02	2.21
31	OMCS2012	9	2.1	1.23	1.71
32	Pokkali	1	0.563	2.16	0.26

Evaluation of F_8 and BC_3F_4 lines for grain quality properties

Ten advanced lines along with their parents were subjected to quality analysis. The grain quality of rice consists of several properties, such as milling quality (head rice %) and cooking quality (Lang *et al.* 2005, Lang *et al.* 2012). Cooking and eating qualities are mostly determined by the amylose content and gelatinization temperature (GT) of the grain starch (Lang *et al.* 2005). Appearance quality is mainly specified by grain shape as defined by grain length, grain width, length–width ratio, and endosperm translucency or chalkiness (Tang *et al*. 1989). As mentioned above, some lines were the first IR64 cultivars to possess intermediate amylose content and intermediate GT. These traits are

Table 6. Yield and yield components of 14 lines from dry season 2018–2019 at Be Tri (Ben Tre Province).

No.	Lines	Tillers per hill	Pan. length (cm)	Fertile grain per panicle	Fertile grains (%)	1,000-grain weight (g)	Yield (tons ha^{-1})
1	L1	9 ab	24 bc	127 be	90.24 abc	28.14 bc	6.73 c
2	L2	10 ab	23.2 c	188 a	87.45 abc	24.53 d	7.83 abc
3	L5	11 ab	21.2 d	95 de	89.4 abc	29.29 b	8.13 abc
4	L16	9 ab	24b c	95 de	61.23 d	32.37 a	7.3 abc
5	L17	10 ab	23.5 c	115 cde	83.72 abc	28.76 bc	7.37 abc
6	L21	9 ab	24 bc	97 de	83.07 bc	28.62 bc	8.63 ab
7	L22	9 ab	25 ab	104 de	88.53 abc	27.92 bc	8.27 ab
8	L24	8 ab	26 a	128 b-e	81.86 c	28.48 bc	7.9 abc
9	L25	7 b	24 bc	152 abc	87.56 abc	27.85 bc	8.6 ab
10	L27	9a b	23 c	87 e	83.14 bc	29.22 b	7.13 bc
Checks							
11	OM8012	9 ab	25 ab	162 ab	94.44 ab	27.4 c	6.73 c
12	OM10252	12 a	26 a	141 bcd	94.95 a	29.38 b	6.57 c
13	OMCS2012	13 a	26 a	156 abc	94.55 ab	27.44 c	6.37 c
14	Pokkali	8b	25 ab	140 bcd	89.7 abc	20.58 e	3.5 d
CV%		19.47	3.03	18.92	6.95	3.15	
$LSD_{0.05}$		3.01	1.24	4.55	1.07	1.47	

Note: In the same column, numbers followed by the same letter are not significantly different at the 5% level in Duncan test; **: Significant difference at 1%; ns: not significant statistically.

considered important, especially by many rice consumers in South and Southeast Asia, for the ideal texture of cooked rice (Wand *et al*. 2007). Brown rice percentage varied from 76%–80%. The head rice percentage of the lines ranged from 41% to 55%. Most of the studied lines were found to present nonchalkiness. Three lines (L5, L25, and L27) had lower amylose content than P2 (Pokkali). Most consumers prefer rice with intermediate amylose contents ranging from 18% to 25% (Table 7).

Molecular markers were used to detect 32 lines at genomic screening to assess the salt stress tolerance of the F_8 and BC_3F_4 populations. For self-pollinated crops, an important aim may be to fix alleles in their homozygous state as early as possible. For example, in bulk and single-seed descent breeding methods, screening is often performed on F_8 generations when most loci are homozygous. By using codominant DNA markers, specific alleles can be fixed in their homozygous state by as early as the F_2 generation. However, this approach may require large population sizes; thus, in practical terms, a small number of loci may be fixed at each generation (Koebner and Summers 2003). An alternative strategy is to enrich rather than fix alleles by selecting homozygotes and heterozygotes for a target locus within a population to reduce the required size of the breeding populations (Bonnett *et al.* 2005). For these results, six markers showed the homozygote and Pokkali.

The main features required are tolerance to salinity, high yield, and

Table 7. Grain quality of 14 lines from dry season 2018–2019 at Ba Tri (Ben Tre Province).

Lines	Brown rice (%)	White rice (%)	Head rice (%)	Length (mm)	L/W	Gelatiniza-tion tempera-ture (score)	Chakiness score (%)	Amylose (%)
L1	79.51 ab	74.84 def	29.33 g	7.32 b	3.44 ab	7.12 a	17.67 a	19.35 f
L2	78.34 ab	75.39 cde	41.73 cd	7.06 de	3.27 cde	2.67 def	6.67 ef	24.63 de
L5	77.7 ab	74.62 def	41.90 cd	6.86 g	3.38 abc	6.00 b	4.00 f	19.12 f
L16	79.38 ab	76.72 bc	39.84 de	7.19 c	3.34 abcd	3.33 cd	8.33 cde	24.98 cde
L17	79.32 ab	74.06 ef	39.47 e	6.93 fg	3.46 a	7.00 a	7.67 de	25.67 bc
L21	79.38 ab	77.52 ab	44.15 b	7.30 b	3.34 abcd	3.0 c	10.00 cd	24.63 de
L22	80.04 a	77.65 ab	41.67 cd	7.09 cd	3.33 bcd	2.33 ef	13.00 b	24.98 cde
L24	78.04 ab	73.72 f	43.19 bc	7.05 def	3.32 cd	6.00 b	7.33 de	20.01 e
L25	77.63 ab	75.42 cde	47.38 a	6.95 efg	3.26 de	6.00 b	6.33 ef	19.28 f
L27	79.86 ab	77.69 ab	40.55 de	7.13 cd	3.20 e	6.00 b	11.33 bc	20.00 e
Checks								
OM 8012	79.23 ab	75.73 cd	32.77 f	7.62 a	3.38 abc	5.33 c	8.33 cde	22.15 e
OM 10252	78.54 ab	76.49 bc	48.40 a	6.87 g	3.20 e	6.00 b	8.33 cde	20.27 e
OM CS2012	76.50 b	78.94 a	39.37 e	7.16 cd	3.37 abcd	6.67 b	9.33 cde	20.98 e
Pokkali	77.6 ab	74.61 def	42.90 cd	6.86 g	3.38 abc	2.00 e	4.00 f	26.19 a
CV (%)	2.20	1.30	2.80	0.90	1.80	10.10	18.40	1.90

short growth duration to find the target breeding. The growth duration of rice lines exhibited the standard trait of a ~100-day life cycle. The segregants from four crosses were assessed with 10 lines from each selected individual. The grain yield and yield components of the fixed lines were separately assessed. The grain yield and yield components of the 10 final selected genotypes of the four crosses are exhibited in Table 4. Grain yield is a complex trait that is determined by three components, namely, number of panicles per hill, number of grains per panicle, and 1,000 grain weight, all of which are typical quantitative traits (Xing and Zhang, 2010).

Table 6 shows the yield and yield components of rice genotypes from the 10 lines mentioned above. Only two lines, *viz.* L1 and L5, had significantly higher yield than other lines. They also exhibited short growth durations of less than 100 days. The number of grains per panicle is usually highly proportional to spikelet number. Understanding the basic biological processes of panicle development, as well as the differentiation of meristems into spikelets under salinity stress, is essential to understand the production of the number of grains per panicle in rice. From an agronomic perspective, the number of spikelets per panicle can be attributed to two components: the duration of panicle differentiation and the rate of spikelet differentiation (Huang *et al.* 2006). Six lines, namely, L1, L2, L17, L24, L25, and L27, as well as the Pokkali check, exhibited salt tolerance with a score of 1. They showed good survival days under the condition of EC = 8 dS/m. In farmer fields at Ba Tri (Ben Tre) under natural salt stress, L5, L21, L22, and L25 exhibited higher yield

than others. These lines showed good survival days under the salt stress of EC = 8 dS/m at seedling stage. Field management and improved cultural practices are required for releasing new rice genotypes in saline areas.

As discussed above, evidence for the existence of extensive regions of conserved collinearity among cereal species at the genetic map level is now overwhelming. This knowledge can be exploited to advance molecular marker studies on all grass species and to extend our knowledge of the placement of key genes on genetic maps. Comparative genome research is an excellent tool for gene isolation. The similarity in gene content and order allows finding genes of interest by using the cloned genes of one species to look for similar genes in other species.

CONCLUSION

The effective SSRs used to address 32 rice genotypes indicated obvious polymorphisms that occurred mostly on chromosomes 1 and 8. The genomic screening of F_8 lines and the BC_3F_4 population revealed only one line that was tolerant to salinity with survival days of 30 days and a survival percentage of 95%. Five other genotypes were recognized as having good salt tolerance. The remaining lines exhibited different survival days. Some promising lines, such as L1, L5, L21, and L22, offered good grain shape, high yield, and grain quality. Four lines *viz.* L1, L5, L24, and L25, exhibited good agronomic characters, such as low amylose and salinity tolerance. Their seeds are currently being multiplied by local farmers.

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