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SELECTION OF SALINITY TOLERANT RICE LINES WITH HIGH QUALITY AND RICH IRON CONTENT

Nguyen Thi My Duyen[1], Tran Thi Cuc Hoa[2], Bui Phuoc Tam[3]
[1]*An Giang University, VNU-HCM*
[2]*Cuu Long Delta Rice Research Institute*
[3]*Loc Troi Agricultural Research Institute*

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ABSTRACT

*The demand for rice among producers and consumers is rising in tandem with the advancement of society. Good quality, micronutrient-rich, and cultivation-condition-adapted rice is concerned, especially under the impact of climate change. The study was conducted to select the salinity tolerant rice varieties and crossing rice population with good quality and rich iron content. The rice panel with 36 varieties and the BC3F3 population of OM5451*4/Pokkali were evaluated for salt tolerance by phenotypic screening and genotypic analysis, amylose content, and iron content by biochemical methods. The results showed that among 36 improved rice varieties, six varieties (OM6976, OM109, OM9577, SH1317, SH333-91, and OM108-200) were highly tolerant to the saline condition of 8 dS m-1 at the seedling stage. Meanwhile, OM5451, OM121, OM241-5, OM232, and OM238 had low amylose content and high iron content in both brown and white rice. In the OM5451*4/Pokkali population, among six tolerant lines, two elite lines (BC3F3-42 and BC3F3-53) were good at grain quality and iron content.*

1. INTRODUCTION

Saltwater intrusion is a major challenge for rice farmers nowadays. At the same time, consumers are increasingly demanding higher quality rice products, including rice with good quality (low amylose content) and rich in micronutrients (high iron content).

Rice is most sensitive to salinity during the early seedling stage (Lauchli and Grattan, 2007). Therefore, many studies on the salinity tolerance of rice are conducted at this stage (Mohammadi et al., 2008; Sabouri and Sabouri, 2009). At the seedling stage, high salinity concentrations inhibit the activity of some enzymes, making it impossible for rice plants to use the reserve nutrients in the grain for normal growth (Pongprayoon, 2007). The salinity tolerance plays a crucial role in rice productivity (Tack et al., 2015). Salinity tolerance in rice is a multifactorial trait highly influenced by the environment (Krishnamurthy et al., 2017). A major QTL, Saltol, responsible for salinity tolerance at the seedling stage has been mapped on chromosome 1 (Bonilla et al., 2002). This QTL explained the phenotypic variation of 43% for seedling stage shoot Na+/K+ homeostasis and expressed the tolerance at the seedling stage in rice (Krishnamurthy et al., 2020).

According to the WHO (2008), around 30% of the world's population, mainly women and children, are suffering from malnutrition due to iron deficiency. One of the most common causes is dietary iron deficiency. Genetic engineering of crops to increase iron content has emerged as an alternative to increasing bioavailable iron. Iron-enriched rice grains are expressed by two genes, which produce the enzyme nicotianamin synthase which activates iron and the protein ferritin. These substances store iron and increase the iron content in the rice endosperm more than sixfold. According to the researchers, such enhanced activity of the said protein allows the rice plant to absorb more iron from the soil and store it in the rice grain (Wirth et al., 2009).

Rice grain quality includes physical and chemical characteristics related to grain shape, milling quality, cooking qualities, and nutritional value (Bao, 2014). In particular, physicochemical qualities (amylose content, gelatinization temperature, gel consistency) are one of the most important characteristics that are of interest to researchers, producers, and consumers (Custodio et al., 2019).

In the context of climate change, rice varieties with high quality, rich nutrition, and adaptability to adverse conditions are required to meet the growing demand from customers. With the development of science and technology in genetics as well as the achievements in many previous breeding studies, it provides a broad and effective platform for breeding rice varieties integrating many desirable traits to meet social and economic demands. This study was conducted to select and breed high-quality and iron-rich rice varieties adapted to saline conditions.

2. MATERIALS AND METHODS

2.1 Materials

The group of elite rice varieties including 36 varieties was collected from CuuLong Delta Rice Research Institute (CLRRI) and 18 BC3F3 rice lines of the OM5451*4/Pokkali were bred. The check varieties consisted of Pokkali and IR29 for salinity tolerance testing; KDML105, IR64, and OM5199 for grain quality analysis; and IR68144, IR64, and OM5199 for iron content analysis.

The RM10287 and RM10694 markers, which were found to be tightly linked to the Saltol region on chromosome 1, were used for screening 36 rice varieties and 18 BC3F3 lines.

The experiments were conducted at CuuLong Delta Rice Research Institute, CanTho city, Vietnam in 2018.

2.2 Methods

Evaluation of salinity tolerance: Screening rice for salinity tolerance in the greenhouse at the seedling stage was done according to the method of Gregorio et al. (1997). Salinity screening was conducted with 36 pure rice varieties, 18 BC3F3 elite lines of the OM5451*4/Pokkali, and two checks, tolerant check Pokkali and susceptible check IR29. Germinated seeds of each variety were sown in holes made on 100-hole styrofoam sheets with a nylon net bottom. On each styrofoam, two seeds were sown per hole and 10 holes were for each rice variety or line. The sheets were first floated on distilled water in plastic trays for 3 days, after which a nutrient solution (Yoshida et al., 1976) was used until the plants were 7-day old. Afterward, two nutrient solution treatments were applied including the control treatment with electrical conductivity (EC) of 0 dS m-1 and the salinity treatment with EC of 8 dS m-1 using NaCl (purity of 99%, Emplura). A randomized complete design was designed with three replications. The culture solution was renewed once with the pH adjusted daily to 5.5 by adding either NaOH or HCl. Salinity tolerance was evaluated by salinity injury (SES score) at 3 weeks after the start of the salt treatments (Table 1).

Table 1: Standard evaluation scoring (SES) system based on the visual symptoms of salt stress injury of rice

SES score	Description	Evaluation
1	Normal growth, only the old leaves show white tips while no symptoms on young leaves	Highly tolerant
3	Near normal growth, but only leaf tips burn, few older leaves become whitish partially	Tolerant
5	Growth severely retarded; most old leaves severely injured, few young leaves elongating	Moderately tolerant
7	Complete cessation of growth; most leaves dried; only few young leaves still green	Susceptible
9	Almost all plants dead or dying	Highly susceptible

Source: Gregorio et al., 1997

Analysis of amylose content (AC): AC of the milled rice samples was determined according to the method of Juliano (1971) and Graham (2002). Milled rice flour (100 mg) was soaked in 1 mL of 95% ethanol and 9.0 mL of 1 N NaOH in a 50-mL glass test tube and allowed to stand undisturbed overnight for 16 h. Afterwards, distilled water (90 mL) was added to bring the solution up to 100 mL, and 0.5 mL aliquot was transferred into a 20-mL test tube containing 5 mL distilled water. Then, 0.1 mL of 1 M CH3COOH was added and the solution was mixed thoroughly using a vortex mixer followed by addition of 0.2 mL of iodine solution (0.15% I2 in 1.5% KI). The solution was then diluted to 10 mL using 4.2 mL distilled water. To develop the calibration curves for the determination of amylose content in a rice sample, 40 mg Avebe potato amylose (standard amylose) was put in a 50 mL test tube and proceeded as described above. Then, 0.1, 0.2, 0.3, 0.4, 0.5 ml of the standard amylose sample solution were transferred into 20 mL test tubes and proceeded in the same way as for the test sample. Construction of the calibration curve was carried by converting from the spectral reading to the percentage of amylose content according to the followed formula ($y = ax + b$, where y is the absorbance OD, and x is the amount of amylose in the measured sample (mg/L) (Graham, 2002). AC in rice is classified into high (>25%), intermediate (>20-25%), and low (12-20%), very low (<12%) (Juliano et al., 1981; Yu et al., 2012).

Analysis of gel consistency (GC): Analysis of gel consistency according to the method of Tang et al. (1991). Milled rice flour (100 mg) was put into a glass test tube (13 x 100 mm). Then, 0.2 mL of 95% ethanol containing 0.03% green thymol was placed into the test tube. 2 ml of 0.2N KOH was further added to the test tube and shaken well on a vortex machine. The test tube was covered and placed in a pot of boiling water (100 °C) for 8 min. Test tubes were cooled to room temperature for 5 min and placed in an ice bath for 20 min. Test tubes were removed and placed horizontally for 1 h. The gel consistency is the length of the moved gel measured from the bottom of the test tube to the end of the gel. The classification of gel consistency is applied according to the standard evaluation system for rice of IRRI (2014): soft (> 60 mm), medium (41-60 mm), and hard (< 40 mm).

Analysis of gelatinization temperature (GT): GT was determined using the alkali digestion test (IRRI, 2014). A duplicate set of six whole-milled kernels without cracks was selected and placed in a petri dish (8.0 cm in diameter). Ten mL of 1.7% KOH solution was added. The samples were arranged to provide enough space between kernels to allow for spreading. The dishes were covered and incubated for 23 h at 30°C. The starchy endosperm was rated visually based on a seven-point numerical spreading scale as a standard evaluation system for rice: high (1-2), high or intermediate (3), intermediate (4-5), and low (6-7) (IRRI, 2014).

Analysis of iron content in rice gain: Iron content in rice grains was assessed by the method of Hossain and Virk (2005). Rice grains were sun-dried, dehusked, polished and ground into a fine powder. Rice flour with 0.6 g was weighed and placed in a 15 ml Falcon tube with 2 replicates for each seed sample. Then, 0.5 M HCl was added with 6 ml, mixed well and shaken overnight at about 150 - 200 rpm. The sample solution was centrifuged for 12 min at 5500 rpm, 1 ml of the sample solution was taken out and placed in a new Falcon tube with 2 replicates for each sample. The new sample solution was added with 1 ml of 0.75 M ammonium sulphate and 1 ml of 6.4 mM 2,2' dipyridyl dye. The sample was then loosely capped, mixed well and placed in boiling water for 10 minutes, then kept in ice for about 10 minutes. The Falcon tube was then centrifuged for 12 minutes at 5500 rpm. The sample analysis results were read using a spectrophotometer at 520 nm. Construction of the calibration curve of iron content was carried by converting from the spectral reading to the concentrations of iron content according to the followed formula ($y = ax + b$, where y is the absorbance OD, and x is the amount of iron content in the measured sample (mg/kg).

The formula for calculating the iron content in rice grain was as follows:

The iron content in brown rice (ICB): Fe (mg/kg) = 6 x OD correlation x 0.006 / 0.6 x 1000

The iron content in white rice (ICW): Fe (mg/kg) = 3 x OD correlation x 0.006 / 0.6 x 1000

ICB and ICW were classified according to IRRI (2006) (Table 2).

Table 2: Classification of iron content in rice

Classification of iron content	Iron content in brown rice (mg/kg)	Iron content in white rice (mg/kg)
Low	6.3 - 10.0	< 4.0
Medium	10.1 - 13.9	4.0 - 5.9
High	≥ 14.0	6.0 - 8.0

Source: Hossain and Virk, 2006

DNA extraction and genotyping: Genomic DNA was extracted using the modified cetyltrimethylammonium bromide (CTAB) method based on the protocol of Murray and Thompson (1980). DNA products were air dried and resuspended in 50 µL of TE buffer (10mM Tris-HCl pH 8.0, 1mM EDTA pH 8.0). Then, they were diluted with distilled water to a concentration of 20 ng/µL. PCR amplification of SSR markers was carried out in a Mastercycler (Eppendorf, Germany) in a total volume of 20 µL with the following PCR reaction: 2 µL of

DNA at 20 ng/µL, 2 µL of 10× buffer containing 25 mM MgCl2, 1 µL of 2.5 mM dNTPs, 1 unit of Taq DNA Polymerase (Bioline, England), and 1 µL each of forward and reverse primers (10 µM). All amplifications were performed for a total of 35 cycles of 1 min at 95 °C, 30 s at 55 °C, and 1 min at 72 °C. PCR products are electrophoresed on 2.5% agarose gel in 1X TBE buffer and containing the 1X GelRed fluorescent nucleic acid stain (Merck, Germany). Sample electrophoresis was carried out at 100V for about 120 min. After that, the gel was photographed using a Quantum-ST4 UV gel camera (France).

Statistical methods: Data were collected and stored, and graphs were designed using the Microsoft Office Excel 2013 program. Descriptive statistics of traits were carried using STAR 2.0.1 software (IRRI) (http://bbi.irri.org/products). R-studio software version 3.2.2 (R Core Team, 2015) was used for Pearson's correlation.

3. RESULTS AND DISCUSSION

3.1 Evaluation of salinity tolerance in rice varieties

Salinity screening was conducted with 36 pure rice varieties in Yoshida nutrient solution containing salt at a concentration of 8 dS m-1 with two checks (tolerant check, Pokkali, and susceptible check, IR29) (Figure 1). After 21 days of screening, when IR29 became yellowing and completely died, the salinity tolerance of the varieties was recorded in Table 3. The screening result showed that the high salinity tolerant rice group accounted for 19.4%, the tolerant rice group made up 52.8%, and the moderately tolerant rice group occupied 27.8% (Figure 3a). Of these, seven rice varieties with high salinity tolerance, in the same group as Pokkali, consisted of OM6976, OM9577, OM107, OM109, OM108-200, SH333-91, and SH1317. And then, the tolerant varieties included SH62, OM326, SH514, OM10636, OM40, OM241-7, SH713, and OM11. These 15 good tolerant varieties were used for testing the Saltol gene in the next experiment.

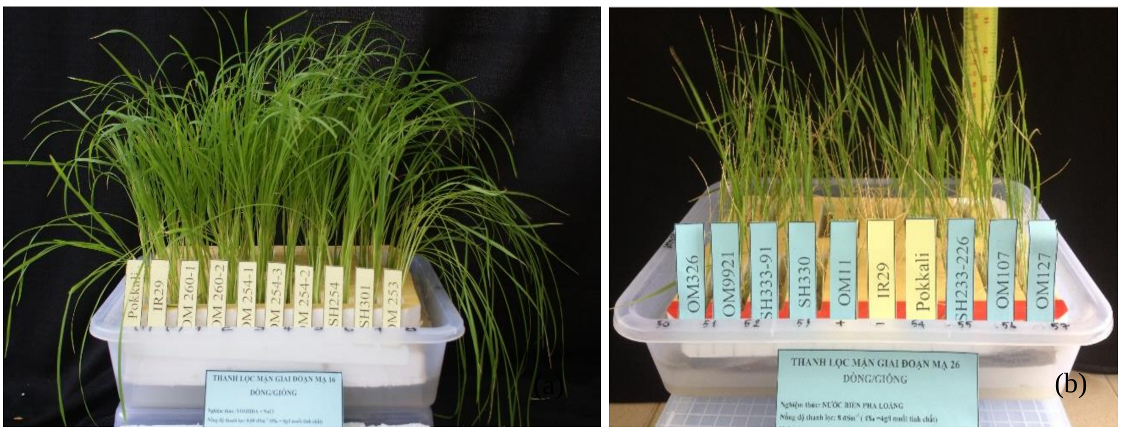
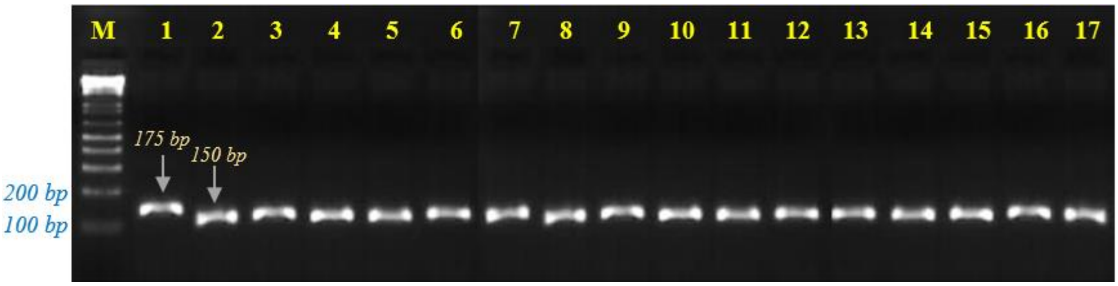


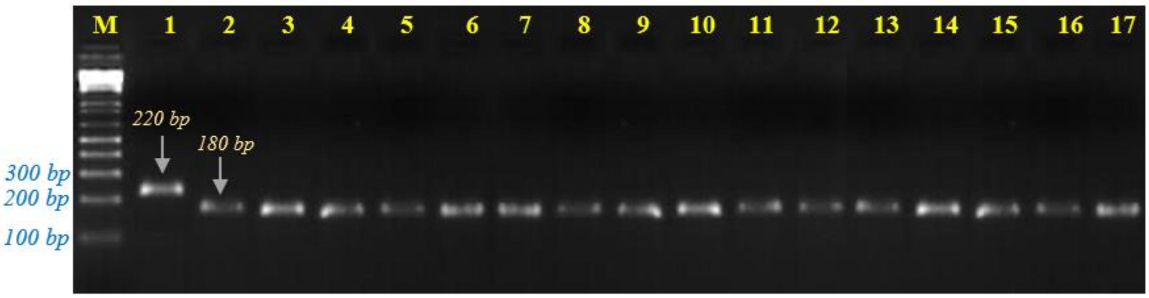
Figure 1: Salinity screening of rice varieties at the control condition (a) and the 8 dS m-1 condition (b) at the seedling stage

Salinity-tolerant rice varieties, according to phenotype, were tested for the Saltol gene on chromosome 1 with two molecular markers RM1287 and RM10694. The results showed that the two markers RM1287 and RM10694 gave polymorphic PCR products and distinguished between the tolerant variety Pokkali and the susceptible variety IR29. With the marker RM1287, the electrophoresis results on 2.5% agarose gel showed two band sizes, 150 bp and

175 bp. The 175 bp position corresponded to Pokkali carrying the Saltol gene and the 150 bp position corresponded to IR29 not carrying the target gene. Similarly, for the molecular marker RM10694, Pokkali had the 220 bp PCR band, carrying the Saltol gene, and IR29 gave a band size of 180 bp, not carrying the target gene. The PCR products of all 15 rice varieties showed 150-bp bands with RM1287 and the 180-bp band with RM10694 (Figure 2). Although the rice varieties had a high tolerance to 8 dS m-1 salinity, these rice varieties did not carry the Saltol gene when evaluated with two markers RM1287 and RM10694.



(a) RM1287



(b) RM10694

Figure 2: PCR products for Saltol gene of material rice varieties with two markers (a) RM1287 and (b) RM10694 on the 2.5% agarose gel

Notes: M: ladder 100-1000 bp (Bioline); 1: Pokkali, 2: IR29, 3: OM6976, 4: OM9577, 5: OM107, 6: OM109, 7: OM108-200, 8: SH333-91, 9: SH1317, 10: SH62, 11: OM326, 12: SH514, 13: OM10636, 14: OM40, 15: OM241-7, 16: SH713, 17: OM11

3.2 Analysis of grain rice quality

The results of AC analysis showed that most rice varieties were in two groups with low and intermediate AC. The soft rice group (AC = 20.1-25.0%) was the majority with 16 varieties (accounting for 44%); followed by the soft and flexible rice group (AC = 10.1-20.0%) with 15 varieties (accounting for 42%). The results recorded 15 rice varieties with low AC (≤ 20%), including OM238, OM231, OM5451, OM241-5, OM326, OM121, OM193, OM232, SH514, OM241-7, OM240, SH713, SH333-91, OM238-2, and OM238-3. All of these varieties had GC with a length from 72.7 - 98.0 mm, belonging to the low GC (soft rice), the same group as Khao Dawk Mali 105. Meanwhile, among varieties with low AC, ten varieties got high GT (score 1 - 3), and four varieties had intermediate GT (score 4 - 5). Only one variety, OM238-3, reached low GT (score 6) (Figure 3d, e, f).

3.3 Analysis of iron content in rice varieties

The iron content in brown rice of 36 material rice varieties ranged from 10.45 - 17.22 mg/kg (Table 3). Among them, 19 varieties with high iron content in brown rice (≥ 14.0 mg/kg) accounted for 52.8% (Figure 3b). The rich iron varieties with ICB ≥ 16.0 mg/kg consisted of OM178, SH62, OM259, OM11, OM107, and OM238.

The iron content of white rice of 36 rice varieties ranged from 3.04 to 6.74 mg/kg. Through classification, 12 varieties had high iron content in white rice (ICW = 6.0 - 8.0 mg/kg), grouped with IR68144 (international check), accounting for 33.3% (Figure 3c). OM121 had the highest iron content at 6.74 mg/kg. Other rice varieties rich in iron in white rice (≥ 6.0 mg/kg) included OM121, OM5451, SH514, OM6976, OM11, SH62, SH1-12, OM232, OM241-7, OM109, OM259, and OM10636.

Thus, through iron content analysis, it was shown that the varieties OM121, OM5451, OM6976, OM11, SH62, SH1-12, OM109, and OM259 were rich iron content in both brown and white rice. These varieties are proposed as potential materials in breeding programs for nutritious rice varieties.

Comparing the results of the iron analysis in brown and white grain rice showed that ICW was always lower than ICB. Because the milling process to convert brown rice into white rice destroys 67% of vitamin B3, 80% of vitamin B1, 90% of vitamin B6, ½ of phosphorus, 60% of iron, all of the fiber and essential fatty acids (Babu et al., 2009). Based on the results, it could be concluded that ICB is about 2.6 times higher than ICW.

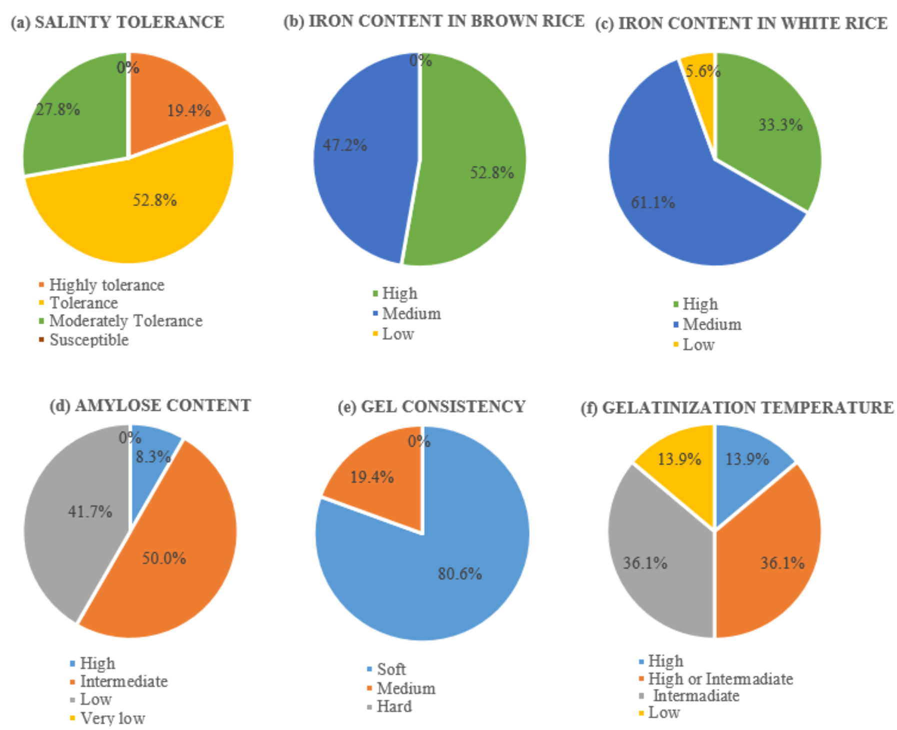


Figure 3: Group classification of rice varieties with (a) salinity tolerance, (b) iron content in brown rice, (c) iron content in white rice, (d) amylose content, (e) gel consistency, and (f) gelatinization temperature

Table 3: Characteristics of salinity tolerance, grain quality, and iron content in rice varieties

No.	Name of variety	SAL	AC	GC	GT	ICB	ICW
		score	%	mm	scale	mg/kg	mg/kg
1	OM6976	2.6	24.0	60.0	4	14.45	6.60
2	OM9577	2.6	25.3	57.7	4	12.51	4.83
3	OM107	2.7	20.9	80.0	4	16.06	4.61
4	OM109	2.7	20.5	86.3	3	14.19	6.29
5	OM108-200	2.7	24.2	63.7	6	10.94	4.65
6	SH333-91	2.8	19.7	81.3	3	11.62	4.27
7	SH1317	2.9	22.5	74.7	7	12.43	4.47
8	SH62	3.1	23.5	78.7	3	16.79	6.49
9	OM326	3.1	17.7	90.0	2	15.03	4.98
10	SH514	3.2	18.8	84.0	5	13.32	6.63
11	OM10636	3.5	24.5	57.7	4	13.63	6.19
12	OM40	3.8	21.2	79.7	3	15.11	5.68
13	OM241-7	3.9	18.3	96.0	3	13.01	6.42
14	SH713	3.9	16.5	98.0	4	11.63	5.77
15	OM11	4.0	24.4	61.3	3	16.23	6.54
16	OM240	4.1	17.9	90.0	2	12.00	4.61
17	OM5451	4.1	18.0	78.0	3	15.23	6.64
18	SH1-12	4.3	25.6	43.3	4	15.66	6.49
19	OM9581	4.3	23.9	66.7	4	10.45	4.74
20	OM239	4.5	23.6	73.3	3	11.18	4.56
21	OM121	4.7	18.6	91.0	2	14.94	6.74
22	OM241-5	4.7	16.4	99.7	4	15.15	5.76
23	SH200	4.8	24.8	60.7	3	15.87	5.53
24	OM232	4.9	19.2	81.3	2	13.53	6.43
25	SH891	4.9	24.7	55.7	3	11.31	4.61
26	OM9584	4.9	22.3	72.3	3	13.43	3.82
27	OM238-2	5.1	18.5	93.0	4	14.98	4.45
28	OM236	5.2	20.3	82.7	5	13.40	3.04
29	OM238	5.2	16.4	97.3	3	16.00	5.37
30	OM231	5.3	19.4	72.7	2	15.40	4.46
31	OM259	5.4	21.2	87.7	6	16.42	6.21

32	SH893-4	5.5	24.8	59.7	5	13.71	4.74
33	OM178	5.6	23.2	74.3	3	17.22	5.24
34	OM193	6.2	19.3	82.7	3	14.80	5.20
35	SH921	6.2	25.1	51.7	6	11.54	4.23
36	OM238-3	6.3	18.4	88.0	6	15.42	4.95
37	Pokkali	2.1	26.6	32.0	5	12.23	5.72
38	IR29	8.5	18.0	75.0	3	15.01	6.44
39	KDML105	-	16.7	98.3	6	-	-
40	IR64	-	23.5	63.7	4	-	-
41	OM5199	-	27.2	24.0	7	-	-
42	IR68144	-	-	-	-	16.38	7.00
43	IR64	-	-	-	-	13.91	6.21
44	OM5199	-	-	-	-	16.30	5.53

Notes: SAL: Salinity tolerance; AC: Amylose content; GC: Gel consistency; GT: Gelatization temperature; ICB: Iron content in brown rice; ICW: Iron content in white rice.

3.4 Correlation of salinity tolerance, grain quality, and iron content in rice

Correlations between characteristics of salinity tolerance, AC, GC, GT, ICB, and IBW were analyzed using Pearson's statistics. The results showed that salinity tolerance was non-significantly correlated with grain quality and iron content. Regarding grain quality characteristics, AC was very closely associated with GC, with a correlation coefficient of r = -0.94, a negative correlation. This meant that the lower the AC, the greater the GC, the more flexible the rice, and vice versa. This result was similarly recorded in previous studies (Ritika et al., 2010; Zhang et al., 2020; Tam et al., 2023). Regarding iron content in grain, ICB and ICW were recorded to have a moderate and positive correlation with each other, with a correlation coefficient of r = 0.41. Iron content in grain was not correlated with other quality traits.

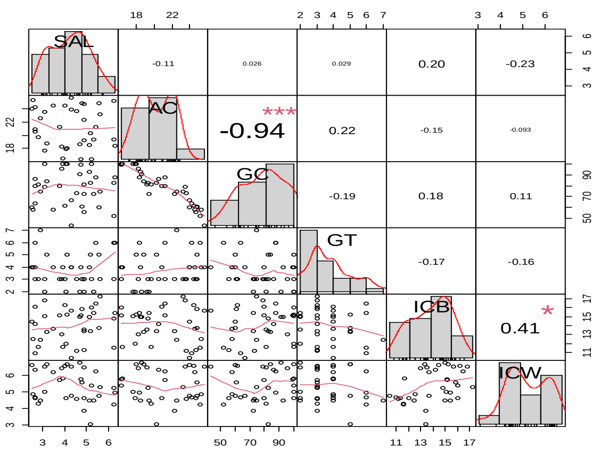


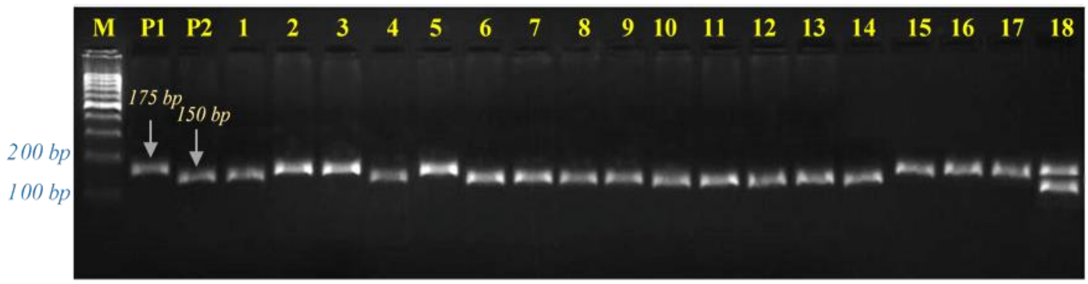
Figure 4: Correlation among salinity tolerance, quality characteristics, and iron contents in the improved rice varieties

3.5 Selection of salinity tolerant rice lines with high quality and rich iron content in the BC3F3 population of OM5451*4/Pokkali

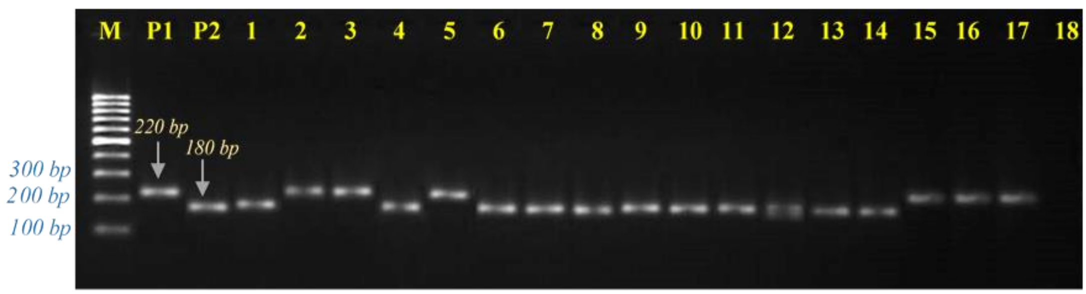
The maternal and paternal rice varieties had opposite traits that could cross with each other. Kumar et al. (2007) argued that a rice cross combination with more genetically diverse parents will have higher hybridization efficiency and more variation in successive segregating populations. Based on characteristics of salinity tolerance, grain quality, and iron content in rice varieties in Table 3, Pokkali is highly tolerant to salinity but low quality (high AC, hard GC, and intermediate GT) and medium iron content in grain. Vice versa, OM5451 is moderately tolerant to salinity but high quality (low AC, soft GC, and intermediate GT) and rich iron content in grain. Moreover, OM5451 is the most popular rice variety in the Mekong Delta with wide adaptability. OM5451 and Pokkali were crossed in order to combine the Saltol region from Pokkali into OM5451.

In the BC3F3 generation, 18 rice lines of the hybrid combination OM5451*4/Pokkali were evaluated for salinity tolerance at a salt concentration of 8 dS m-1 in the seedling stage. The evaluation results showed that 6/18 lines had high salinity tolerance (SAL score < level 5), grouped with the Pokkali variety, namely lines BC3F3-39, BC3F3-40, BC3F3-42, BC3F3-52, BC3F3-53, and BC3F3-54 (Table 4).

Testing the Saltol gene for salinity tolerance of 18 hybrid lines identified six lines carrying homozygous target gene, the same band position as Pokkali variety, including BC3F3-39, BC3F3-40, BC3F3-42, BC3F3-52, BC3F3-53, and BC3F3-54 (Figure 5).



(a) RM1287



(b) RM10694

Figure 5: PCR products for Saltol gene of the BC3F3 rice individuals with two markers (a) RM1287 and (b) RM10694 on the 2.5% agarose gel

Notes: M: ladder 100-1000 bp (Bioline); P1: Pokkali; P2: OM5451; 1-18: hybrid individual in the BC3F3 generation; 1: BC3F3-38; 2: BC3F3-39; 3: BC3F3-40; 4:BC3F3-41; 5: BC3F3-42;

6:BC3F3-43; 7: BC3F3-44; 8: BC3F3-45; 9: BC3F3-46; 10: BC3F3-47; 11: BC3F3-48; 12: BC3F3-49; 13:BC3F3-50; 14: BC3F3-51; 15: BC3F3-52; 16: BC3F3-53; 17: BC3F3-54; 18: BC3F3-55

Table 4: PCR products of the BC3F3 population of OM5451*4/Pokkali with RM1287 and RM10694 markers

No.	Lines	Level of salinity tolerance	Marker		Notes
			RM1287	RM10694	
1	BC3F3-38	5.00 cde	-	-	Non-selected
2	BC3F3-39	4.33 def	+	+	Selected
3	BC3F3-40	4.33 def	+	+	Selected
4	BC3F3-41	7.00 ab	-	-	Non-selected
5	BC3F3-42	3.00 f	+	+	Selected
6	BC3F3-43	5.00 cde	-	-	Non-selected
7	BC3F3-44	5.00 cde	-	-	Non-selected
8	BC3F3-45	6.33 abc	-	-	Non-selected
9	BC3F3-46	6.33 abc	-	-	Non-selected
10	BC3F3-47	5.67 bcd	-	-	Non-selected
11	BC3F3-48	5.00 cde	-	-	Non-selected
12	BC3F3-49	5.00 cde	-	-	Non-selected
13	BC3F3-50	5.00 cde	-	-	Non-selected
14	BC3F3-51	5,67 bcd	-	-	Non-selected
15	BC3F3-52	3.67 ef	+	+	Selected
16	BC3F3-53	4.33 def	+	+	Selected
17	BC3F3-54	3.00 f	+	+	Selected
18	BC3F3-55	6.33 abc	±	0	Non-selected
	Pokkali	3.00 f	+	+	
	OM5451	5.67 b-d	-	-	

Notes: (+): homozygous gene related to salinity tolerance; (±): heterozygous gene related to salinity tolerance; (-): no target gene; 0: missing data. Within the same column, numbers followed by the same letter were not statistically different.

Combining the results of salinity screening and genotyping related to salt tolerance, six rice lines including BC3F3-39, BC3F3-40, BC3F3-42, BC3F3-52, BC3F3-53, and BC3F3-54 were selected because these lines are high tolerant to salinity and also carry the Saltol gene. These elite lines were further recorded for grain quality.

Characteristics of grain quality and iron content of OM5451*4/Pokkali population in the BC3F3 generation were analyzed and shown in Table 5. Among six elite lines, two lines, BC3F3-42 and

BC3F3-53, gave a result of good quality with AC lower than 20%, soft GC, and intermediate GT. For iron content in grain, BC3F3-42, BC3F3-52, BC3F3-53, and BC3F3-54 were rich in ICB, while BC3F3-40, BC3F3-42, BC3F3-53, and BC3F3-54 were recorded rich ICW. Therefore, two lines, BC3F3-42 and BC3F3-53, were selected because of their high quality and rich iron content in the grain.

Table 5: Characteristics of grain quality and iron content in the BC3F3 population of OM5451*4/Pokkali

No.	Lines	AC	GC	GT	ICB	ICW
		%	mm	scale	mg/kg	mg/kg
1	BC3F3-39	25.72	63	4	12.22	5.33
2	BC3F3-40	26.14	58	5	13.57	6.15
3	BC3F3-42	18.99	79	5	14.02	6.20
4	BC3F3-52	22.89	69	4	16.34	5.48
5	BC3F3-53	19.87	77	5	15.20	6.22
6	BC3F3-54	26.72	55	5	15.36	6.54
	Pokkali (♂)	26.56	32	5	12.23	5.72
	OM5451 (♀)	17.98	75	3	15.01	6.44

Notes: SAL: Salinity tolerance; AC: Amylose content; GC: Gel consistency; GT: Gelatization temperature; ICB: Iron content in brown rice; ICW: Iron content in white rice.

4. CONCLUSION

Through salinity screening and biochemical analysis, improved rice varieties with a good combination of characteristics such as high quality, rich grain iron content, and moderate salinity tolerance consisted of OM5451, OM121, OM241-5, OM232, and OM238. These are good sources of materials for breeding salt-tolerant and high-quality rice varieties. Correlations between characteristics showed that AC was very closely and negatively related to GC (r = -0.94) and ICB and ICW were moderately and positively correlated (r = 0.41). In the OM5451*4/Pokkali population at the BC3F3 generation, two elite lines, BC3F3-42 and BC3F3-53, with high salinity tolerance, good quality, and rich iron content in the grain, were proposed to develop further.

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