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Participatory selection of CWR-derived salt-tolerant rice lines adapted to the coastal zone of the Mekong Delta

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Abstract

Climate change is affecting agricultural production in the coastal areas of the Mekong Delta through the intrusion of salinity into the rice (*Oryza* spp.) fields, where farmers cultivate photoperiod-sensitive rice varieties with long growth duration and low grain yields. A set of 12 stable crop wild relative (CWR)-derived rice lines with introgressions from wild rice *Oryza rufipogon* Griffiths and *O. nivara* S.D. Sharma & Shastry was evaluated for their phenotypic response to salinity tolerance by a participatory selection approach on farm in the Phuoc Long and Gia Rai districts, Bac Lieu province. The evaluation of the results showed that four lines derived from CWR are well adapted to the local environmental conditions, with high grain yield (>6.5 t ha^{-1}), early maturity, and short plant height. These CWR-derived lines were adopted by farmers and proposed for testing on a larger scale in different areas of the coastal zone.

1 | INTRODUCTION

The Mekong Delta is Vietnam's granary, and 4.1 million ha of rice (*Oryza* spp.)-growing land accounts for 55% of the total rice cultivation area and contributes 54% of total rice production (GSO, 2020). Rice production has been on an upward trend since the 1990s, due to improved irrigation systems, improved rice farming practices, and plant breeding programs, including the formal seed sector and participatory plant breeding through international projects (e.g., Sowing Diversity = Harvesting Security, https://www.sdhsprogram.org/country/vietnam/). The formal seed sector can be defined as a framework of institutions linked together by their involvement in or influence on the multiplication, processing, and distribution of improved seed (Cromwell, Friis-Hansen, & Turner, 1992). In contrast, the informal seed system includes all arrangements, such as retaining seed on-farm from previous harvests; farmer-to-farmer seed exchange based on barter, a social obligation; community-level seed production; and distribution, by which farmers can obtain their seed requirements (Cromwell et al., 1992). In the Mekong Delta, ∼80.5% of seed used in the field is from a farm-saved seed source, whereas ∼19.5% of the seed is bought from formal (3.5%) and informal (16.0%) seed systems (Tin, Cuc, Be, Ignacio, & Trygve, 2011). The informal system achieved significantly lower seed prices and a greater variety of rice varieties than the formal seed system (Tin et al., 2011). Participatory plant breeding makes a significant contribution to food and seed security in the Mekong Delta. It is a plant breeding program in which breeders and farmers work together, with farmers making decisions, selecting traits, or conducting on-farm trials at different stages of the process and making selected lines available to their community. In this way, the varieties meet

Abbreviations: CWR, crop wild relative; IRRI, International Rice Research Institute; PC, principal component; PCA, principal component analysis; PVS, participatory variety selection.

market needs. In farmers' fields, crops are not only conserved but also exposed to various selection pressures, both natural and manmade. This leads to the emergence of varieties that are better adapted to specific ecosystems, cropping patterns, and specialized markets, such as red-seeded sticky rice destined for urban markets with high prices. Such adapted varieties exhibit stable yields and resistance to several pests and diseases, as well as highly valued quality criteria including the physical appearance of the grains (uniformity, whiteness, and slenderness), satiety, and aroma (Cuc, Quyen, Huyen, & Ham, 2015; Tin et al., 2011).

The informal seed systems was strengthened by the Community Biodiversity Development and Conservation (CBDC) project in the Mekong Delta, where >320 farmer groups established "seed clubs," which annually cover ∼30% of the total seed requirements for rice production in the delta (Tin, 2018), and in particular 90% of the seed requirements of An Giang province (Tin et al., 2016).

Salinity is one of the main abiotic factors limiting crop production worldwide. Since 2015, rice production in the Mekong Delta has been confronted with climate change, which poses a serious environmental threat, as sea-level rise directly affects coastal areas. In 2016, ∼600,000 ha affected by drought and saline intrusions caused economic losses of up to VND 15 trillion (about US$670 million; Anh et al., 2016). In the Mekong Delta, the government has invested in structures such as dikes and dams to minimize salt intrusion from the rivers and canals. The Ministry of Agriculture and Rural Development (MARD) has promoted changes in the cropping system to adapt to climate change, in particular through plant breeding programs. Some researchers found that low salinity in the early stages of development can have a stimulating effect on plant growth. However, high salinity at each stage of growth inhibits rice growth and yield (Khatun, Rizzo, & Flowers, 1995; Rozema & Flowers, 2008). Most plants suffer from a salt injury at electrical conductivity (EC) values > 4 dS m^{-1}, whereas some tolerant crops can withstand much higher concentrations. The yield of crops decreases significantly with increasing salt concentration, but the threshold concentration and yield decrease vary by plant species and varieties (Be & Hiep, 2000; Phap et al., 2006).

Rice is considered moderately sensitive to salinity. The degree of salt injury depends on salt concentration, pH, temperature, humidity, solar radiation, water depth, duration of exposure, and the growth stage of the plant (Levitt, 1980). The vegetative growth of rice in saline soils is generally better during the wet season than during the dry season, mainly due to a lower vapor pressure deficit and, thus, a lower transpiration rate. Most rice varieties are severely damaged in flooded soils at 8–10 dS m^{-1}. Sensitive varieties suffer damage already at 2 dS m^{-1}. In the past, rice varieties were well adapted to the prevailing saline conditions. However, these traditional varieties are characterized by (a) photoperiod sensitivity (short-day conditions, heading dates from November to December); (b) long growth duration (>6 mo); (c) susceptibility to current pests and diseases; (d) low yield potential (3 t ha^{-1}); and (e) only one harvest per year.

Core Ideas

- Salinity is one of the main abiotic factors limiting crop production worldwide.
- Participatory evaluation is one sustainability strategy to expand the genetics.
- Crop wild relative-derived rice lines were evaluated for tolerance to salinity by PVS.

Thus, several traditional varieties are better adapted to saline conditions than most modern semidwarf varieties such as Doc Do, Doc Phung, Nang Co Do, Nang Keo, Tai Nguyen, Tep Hanh, or Mot Bui (Le, 1999). Although farmers and scientists have succeeded in reducing crop damage caused by salt stress and increasing yields, these strategies had several drawbacks. For example, most current salt-tolerant varieties have low grain quality or long growth duration (e.g., IR42, Doc Phung); moderately tolerant varieties are often severely damaged if saltwater intrudes before the ripening stage or if the salt concentration exceeds 5 dS m^{-1} (Zhu, 2001). The ability to tolerate salinity could be a key factor in plant productivity (Momayezi, Rahman, Mosa, & Ismail, 2009).

The salt content hinders the development of the rice and the adaptation of the plants. High salt content reduces pollen viability at the flowering stage, which in turn determines grain yield (Khatun et al., 1995; Pearson & Bernstein, 1959; Singh, Mishra, & Singh, 2004). Comparisons between crosses with male and female parents developed under different salinity conditions show that the effects on the female plants outweigh the effects on the pollinator plants (Khatun et al., 1995). Salinity delays heading in rice, which negatively affects several yield components. Salinity stress affects the tiller number per plant, the flowering stage, the number of spikelets, the percentage of sterile flowers, and productivity (Gupta & Huang, 2014; Hakim et al., 2010). There is therefore a need to develop varieties that are well adapted to saline soils (4–6 dS m^{-1}; The, Hien, & Cuong, 2018), with shorter duration and higher yield than the traditional varieties.

It is important to introgress genes conferring salt tolerance into locally grown, popular rice varieties, focusing on higher yields to ensure food security under changing climatic conditions. Participatory evaluation and variety selection based on crop wild relative (CWR)-derived rice lines is one strategy to expand the genetic base of current varieties and subsequently identify novel salt-tolerant related loci. This study was conducted to evaluate the salinity tolerance of CWR-derived rice lines based on their yield potential under saline conditions in the coastal areas of the Mekong Delta.

2 | MATERIALS AND METHODS

2.1 | Population development and selection of lines

This study builds on the project "Enhancing utilization of crop wild relatives: capturing genetic value from ancestral populations of wild rice" (2011–2016) within the framework of the CWR Project (https://www.cwrdiversity.org/), which aimed to develop and evaluate introgression lines of *O. rufipogon* Griffiths. Four widely different accessions were selected from the International Rice Genebank Collection at the International Rice Research Institute (IRRI), originating from Bangladesh (IRGC 103837), China (IRGC 100916), Malaysia (IRGC 105491), and Papua New Guinea (IRGC 106276). These were backcrossed over three generations into the modern elite variety IRRI 154 (released in the Philippines under the commercial name NSiC Rc 222) and self-pollinated for two generations (Tin et al., 2020).

Four BC_3F_2 families of nonweedy recombinant inbred lines (RILs) were created and evaluated under well-watered and drought conditions in IRRI's experimental farm at Los Baños, the Philippines, in 2016. A total of 200 BC_3F_{3B} lines were received from IRRI in December 2018 and approved by the Vietnamese quarantine authorities for release into the environment. The 200 lines were distributed to 13 seed clubs in eight provinces in the Mekong Delta region for participatory on-farm selection (Tin et al., 2020).

Details about the first growing season in the Mekong Delta (December 2018–May 2019) and the selected lines are provided by Tin et al., 2020. In total, 1,027 BC_3F_4 lines were selected for the second growing season (April 2019–November 2019) and distributed to 14 seed clubs in nine provinces in the Mekong Delta for further onsite evaluation by farmers. Participatory evaluation trials with technical backstopping support from local advisory officers were carried out during the reproduction phase. The final selection of lines to be advanced in the next generation was made at the maturity stage. A total of 50 stable lines (BC_3F_5) with a sufficient number of seeds were selected after the second growing season (Tin et al., 2020).

Based on the results of the second growing season, a total of 12 stable lines (BC_3F_5) with high yield potential, strong stems, and a sufficient number of seeds were selected and evaluated for relative yield in saline soil conditions on-farm in the coastal area of Bac Lieu province in the winter–spring season of 2019–2020 (hereafter, Crop 1) and during the summer–autumn season of 2020 (hereafter, Crop 2). Additionally, two check varieties were included: (a) the national grain yield check variety OM5451 (Huan et al., 2015; Thuy & Kuniyuki, 2017), and (b) the international salt-tolerant check variety Pokkali (Bohra & Doerffling, 1993; Gregorio, Senadhira, & Mendoza, 1997; Makihara, Tsuda, Morita, Hirai, & Kuroda, 1999) (Supplemental Table S1).

2.2 | The on-farm trial sites and experimental design

In the coastal area, on the farm of Mr. Truong Van Tu (9˚27′10.681″ N, 105˚27′55.404″ E) in the Phuoc Long village, Phuoc Long district, Bac Lieu province, a trial was conducted and implemented in the shrimp–rice production system. This system consists of three growing seasons, such as Shrimp 1 (mid-February to mid-May), Shrimp 2 (early June to mid-September), and Rice in the rainy season from October to the end of January. After harvesting Shrimp 2, rainwater was stored in the field and drained to reduce the salinity of the water until 1–2 dS m^{-1}; then 12-d-old seedlings were transplanted for the trial (Crop 1). The second crop (Crop 2) was conducted at the farm of Mr. Dang Van Canh (9˚25′05,63″ N, 105˚50′85.52″ E) at Lang Tron village, Gia Rai district, Bac Lieu province. Here, rice is grown under saline rainfed conditions in two seasons: (a) Rice 1 (early June to mid-September), and (b) Rice 2 (September to late January). From mid-February to the end of May, nothing can be cultivated due to saline intrusion and lack of freshwater.

The meteorological data of the farm sites during the rice-growing season for Crop 1 (October 2019–January 2020) and Crop 2 (June–September 2020) are given in Table 1.

The evaluation of salinity tolerance of the 14 rice lines (including two check varieties) was performed under a randomized complete block design with three replications. Twelve-day-old seedlings were transplanted in 10-m^2 plots with 20-cm spacing between rows and 15-cm spacing between hills. A total of 330 seedlings were transplanted per line and plot (Supplemental Figure S1). Fertilizer application followed the local agricultural practice, in which commercial fertilizers were applied in three parts (i.e., 7, 20, and 45 d after planting). Fertilizer was applied at 74–18–32 kg ha^{-1} of N–P_2O_5–K_2O. Weed control was carried out by hand. Pesticides were not applied. The physical and chemical properties of the soil samples from the field trials are provided in Table 2.

2.3 | Data collection

The following data were recorded during flowering to maturity: yield components, number of panicles per plant, number of panicles per square meter, seeds per 10 panicles, number of filled grains, number of unfilled grains, 1,000-grain weight, plant height, heading date at 5% (when 5% of panicles per

TABLE 1 Meteorological data from experimental sites during the rice-growing season

	2019			2020				
Parameter	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**June**	**July**	**Aug.**	**Sept.**
Phuoc Long site, Crop 1 (2019–2020)								
Mean temperature, ˚C	27.9	24.4	27.5	26.8				
Total rain amount, mm	214.2	35.8	30.5	0.0				
Mean of relative humidity, %	85.0	82.0	85.0	76.0				
Mean of sunshine duration, h	224.3	229.5	250.0	296.4				
Gia Rai site, Crop 2 (2020)								
Mean temperature, ˚C					28.5	28.6	27.5	27.5
Total rain amount, mm					222.2	175.4	225.5	284.6
Mean of relative humidity, %					83.0	84.0	85.0	87.0
Mean of sunshine duration, h					159.7	208.4	150.5	171.4

Note. These data are provided by the meteorological station of Bac Lieu province, which is measured at Phuoc Long and Gia Rai weather stations.

TABLE 2 Analysis of chemical elements of soils during the on-farm field trials in the Bac Lieu province

Soil property	Unit	Phuoc Long	Gia Rai
pH	–	4.75	5.75
EC	mS cm^{-1}	10.7	8.68
Organic matter	%	4.34	2.56
NH_4^+	cmol kg^{-1}	0.097	0.121
NO_3^-	cmol kg^{-1}	0.00074	0.01342
Na^+	cmol L^{-1}	0.34217	0.32730
Ca^{2+}	cmol L^{-1}	0.06100	0.09185
Mg^{2+}	cmol L^{-1}	0.26667	0.28608
SAR	–	16.7	14.7

Note. Soil sampling in columns was carried out in the field trials. The soil columns were kept undisturbed at room temperature, and to minimize evaporation, the top of the columns was covered with a plastic film. The soil in the columns was air dried and passed through a 2-mm mesh sieve, and the chemical properties including electrical conductivity (EC) and soluble ion concentration (Ca^{2+}, Mg^{2+}, and Na^+) were determined and the sodium adsorption ratio (SAR) calculated (Blake, 1965; Page, Miller, & Keeney, 1982). The chemical properties of the soil, such as EC and SAR {SAR = $Na^+/[(Ca^{2+} + Mg^{2+})/2]1/2$} were analyzed in the laboratory of Can Tho University.

plot flowered), flowering time at 80% (when 80% of panicles per plot flowered), and grain yield. Specifically, grain yield was defined as the dry weight of harvested grains on 5 m^2 per plot and converted from kilograms to tones per hectare on rough rice at 14% of moisture following the Standard Evaluation System for Rice (IRRI, 2014).

2.4 | Farmers' preference ranking of rice lines

Preference analysis was conducted at maturity following the participatory variety selection (PVS) protocol of Witcombe, Joshi, Rana, and Virk (2001). The 27 participating farmers (Crop 1) and 25 farmers (Crop 2) were divided into four groups (6–7 farmers per group), each group being led by local technicians. First, the farmers were asked to move around the field as a group, observe each anonymously labeled line, and use an evaluation form to determine the desired traits of each line. After the review, they were given ballots to vote for the two most preferred and the two least preferred lines. In the second round, farmers and scientists were asked to vote in a group discussion for the two best and two worst lines. After the voting, votes were counted, and preference scores were calculated. Farmers who voted for individual CWR-derived lines were asked to state the reasons for their choice.

2.5 | Statistical analysis

The entire data analysis, visualization, and figure generation were performed with R (R Core Team, 2019). An ANOVA was performed. Duncan's mean comparison test was applied on plant height, days to heading or flowering at 5 and 80%, panicles per plant, panicles per square meter, grains per panicle, number of filled and unfilled grains, the weight of 1,000 grains, and grain yield. Agglomerative hierarchical cluster analysis was conducted to group the genotypes according to their mean values for plant height, days to heading or flowering at 5 and 80%, panicles per plant, panicles per square meter, grains per panicle, number of filled and unfilled grains, the weight of 1,000 grains, and grain yield. All observations were scaled using the scale() function prior to the analysis. The dissimilarity matrix between observations was calculated using the dist() R function and the Euclidean distance method. Then, Ward's minimum variance was used to calculate the dissimilarity between clusters of observations using the hclust() R function. The Cluster dendrogram was visualized using the plot() R function. To determine the relationships between response variables and genotypes and to visualize the overall variance, a principal component analysis (PCA) was performed. The number of principal components, eigenvalues, and factor loads of PCA was calculated using all traits. Correlation coefficients were calculated for 10 traits including plant height, days to heading or flowering at 5 and 80%, panicles per plant, panicles per square meter, grains per panicle, number of filled and unfilled grains, the weight of 1,000 grains, and grain yield using the SPSS version 24 (IBM Corporation, 2019) as $r_{pi.j} = \mathrm{Cov}_{p1.2} / (\sigma_{pi} \times \sigma_{pj})$, where, $\mathrm{COV}_{pi.j}$ is covariance between the variables X_i and X_j, σ_{pi} is the standard deviation of the variable X_i, and σ_{pj} is the standard deviation of the variable X_j.

3 | RESULTS

Analysis of variance reported significant differences between genotypes and the mean values of studied traits of the Crop 1 and Crop 2 seasons. The interaction between genotypes and crop is clearly illustrated in days to heading at 5%, days to flowering at 80%, panicles per square meter, and filled grains per panicle ($p < .01$), whereas grain yield and grains per panicle show significant differences at $p < .05$ (Table 3). All traits were significantly different between genotypes and between crops, whereas all studied traits were nonsignificantly different between replications except for days to flowering at $p = .05$ (Table 3).

Grain yield of the CWR-derived lines under saline conditions was an important trait for farmer's selection criteria in the on-farm trials at both sites. Averaged grain yields ranged from 5.56 (Pokkali) to 6.88 t ha^{-1} (L33-6) (Table 4). Four CWR-derived lines showed high yields >6.5 t ha^{-1}, including L180-3, L93-3, L72-3, and L33-6. Most importantly, seven lines had a statistically significantly higher grain yield than the check varieties Pokkali and OM5451.

Table 4 shows that days to heading at 5% varied from 72 (Pokkali) to 80 d (L188-2, L93-3, and L12-6). Almost all CWR-derived lines reached heading after 75–80 d. For days to flowering at 80%, OM5451 was early and flowered after 82 d, whereas CWR-derived lines flowered after 85–92 d. The suitable time to maturity of rice varieties that can grow in coastal areas is about 110–115 d, and the early-flowering lines L16-2, and L49-3 are the most promising (flowered in <90 d) in this respect. The plant height ranged from 99.8 to 115.6 cm. Five CWR-derived rice lines with a farmer-preferred plant height below 110 cm were identified: L16-2, L55-2, L14-4, L93-3, and L72-3.

The number of panicles per square meter ranged from 223 to 325, and all 12 CWR-derived lines had higher values than Pokkali. Furthermore, nine CWR- derived lines had higher values than the national grain yield check variety OM5451, from which seven CWR-derived lines had >300 panicles per square meter. The number of panicles per plant ranged from 9

TABLE 3 Analysis of variance across 12 stable crop wild relative (CWR)-derived rice lines and two control varieties, crop, replication, and their interaction for the phenotypic characteristics measured

Variance source	HD5%	FT80%	Plant height	Panicles per plant	Panicles per m^2	Grains per panicle	Filled grains per panicle	Unfilled grains per panicle	Weight of 1,000 grains	Grain yield
	d		cm	no.					g	t ha^{-1}
Genotype	17.721**	30.168**	2.572**	1.997*	11.345**	3.632**	3.328**	5.295**	3.281**	2.861**
Crop	453.106**	1,069.65**	174.644**	37.283**	17.396**	ns†	18.217**	ns	ns	17.753**
Replication	ns	3.216*	ns	ns	ns	ns	ns	ns	ns	ns
Genotype x crop	11.557**	13.543**	ns	ns	3.193**	2.307*	4.942**	ns	ns	2.226*

Note. HD5%, days to heading at 5%; FT80%, days to flowering at 80%.
*Significant at the .05 probability level. **Significant at the .01 probability level. †ns, not significant.

TABLE 4 Mean value comparison of traits in the on-farm trials of 14 rice lines in Phuoc Long and Gia Rai districts, Bac Lieu province, sorted by grain yield. Check varieties are indicated in italics

Genotype	HD5%	FT80%	Plant height	Panicles per plant	Panicles per m2	Grains per panicle	Filled grains per panicle	Unfilled grains per panicle	Weight of 1,000 grains	Grain yield
	d		cm	no.					g	t ha^{-1}
Pokkali	72a	83a	101.0b	12ab	223g	131abc	102abc	29abc	24.5bcd	5.56d
L38-4	79cd	90cd	112.2cd	13a	306abc	148ab	87d	61f	25.1abc	5.83cd
L55-2	79cd	90cd	107.1bcd	11abc	247fg	135abc	97bcd	38bcd	24.6abcd	5.9bcd
L12-6	80d	92d	110.8cd	9d	283cde	148ab	94bcd	54def	24.6abcd	5.93bcd
L16-2	78c	89c	109.8cd	11abc	306abc	118cd	91d	27abc	24.2cd	6.04bcd
L49-3	75b	85b	115.6d	10bc	295bcd	137abc	97bcd	40bcde	23.3d	6.18bcd
OM5451	73ab	82a	99.8a	11abc	272def	102d	89d	23ab	24.6abcd	6.19bcd
L188-2	80d	91cd	112.0cd	13a	313ab	126bc	95bcd	31abc	25.7ab	6.3abc
L14-4	79cd	91cd	106.9bcd	12ab	301abc	132abc	103ab	29abc	25.9a	6.39abc
L96-3	79cd	91cd	110.2cd	13a	308abc	132abc	92cd	40bcde	24.6abcd	6.42abc
L180-3	79cd	91cd	110.5cd	12ab	324ab	148ab	95bcd	53def	23.7d	6.52abc
L93-3	80d	91cd	106.1bc	11abc	258ef	154a	96bcd	58ef	24.5bcd	6.52abc
L72-3	78cd	90cd	105.8bc	11abc	271def	136abc	90d	46cdef	24.7abcd	6.56ab
L33-6	79cd	91cd	111.8cd	12ab	325a	125abc	110a	15a	23.3d	6.88a
CV%	15.9	13.9	9.0	5.2	7.3	6.2	7.6	1.9	20.2	9.4

Note. HD5%, days to heading at 5%; FT80%, days to flowering at 80%. Means with a common letter are not significantly different from each other ($P > .05$ ANOVA followed by Duncan's mean comparison).

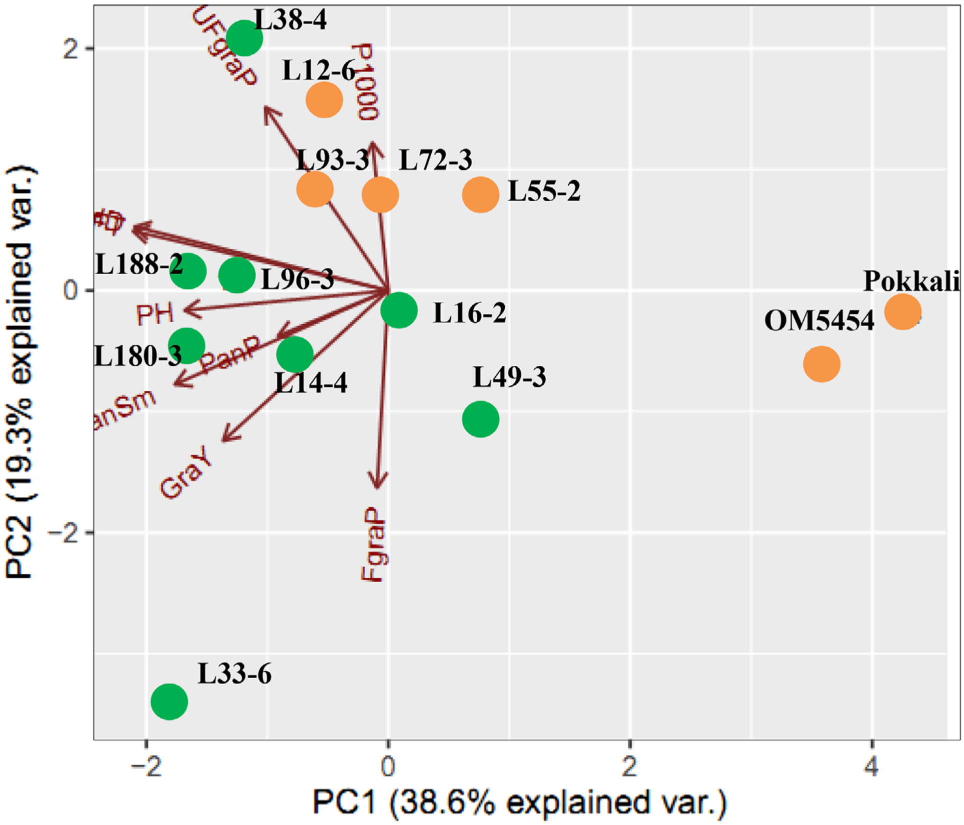

FIGURE 1 Principal component analysis (PCA) plot based on the contribution of the first two principal components (PC1 and PC2) of nine traits for 14 stable rice lines under field conditions in the coastal area in Bac Lieu province. Color codes indicate different cluster membership: green = Cluster 1; orange = Cluster 2 (Figure 2)

to 13. Seven rice lines had 12–13 panicles per plant, including Pokkali and six CWR-derived lines, whereas all rice lines that had 13 panicles per plant are derived from CWR.

The number of filled grains per panicle varied greatly between the rice lines. Besides the check variety Pokkali, two CWR-derived lines showed >100 filled grains per panicle. The number of unfilled grains per panicle ranged from 15 to 61, and L33-6 was the best CWR-derived line for this trait. As a result, the number of grains per panicle ranged from 102 to 154, and L93-3 was the best CWR-derived line for this trait with 154 grains, whereas the minimum value was 102 grain for grain yield check OM5451.

The 1,000-grain weight varied between 23.3 and 25.9 g. Three CWR-derived rice lines with 1,000-grain weight ≥25.0 g were outstanding. Seven CWR-derived lines outperformed the check varieties OM5451 and Pokkali for this trait.

3.1 | Clustering and principal component analysis

Principal component analysis based on Holland (2008) was used to investigate the population structure of the 14 lines and to determine the phenotypic variables. The PCA showed a total of 10 principal components (PCs). The PC1 explained 38.6%, whereas PC2 explained 19.3% of the total variation, which provides a clear idea of the structure underlying the variables analyzed with 57.9% of the total variation. The clustering of CWR-derived lines in the PCA plot based on the first two PCs is shown in Figure 1. All retained phenotypic variables were negatively loaded with the first PC. The clustering of CWR-derived lines in the PCA biplot supported the groupings found by the hierarchical cluster analysis.

The population structure of CWR-derived lines is shown in the hierarchical cluster dendrogram (Figure 2). Two clusters were identified in this study: (a) Cluster 1 comprised eight *Oryza nivara* S.D. Sharma & Shastry and *O. rufipogon*-derived lines, and Cluster 2 consisted of four CWR-derived lines and the two check varieties.

Rice lines belonging to Cluster 1 generally had the highest values for grain yield, number of grains per panicle, plant height, panicles per square meter, panicles per plant, 1,000-grain weight, and grain yield and were late heading and flowering. Lines inferred to Cluster 2 tended to have more filled and unfilled grains per panicle, and lower grain yield values, but included the two check varieties (Figures 1 and 2, Table 4, Supplemental Table S1).

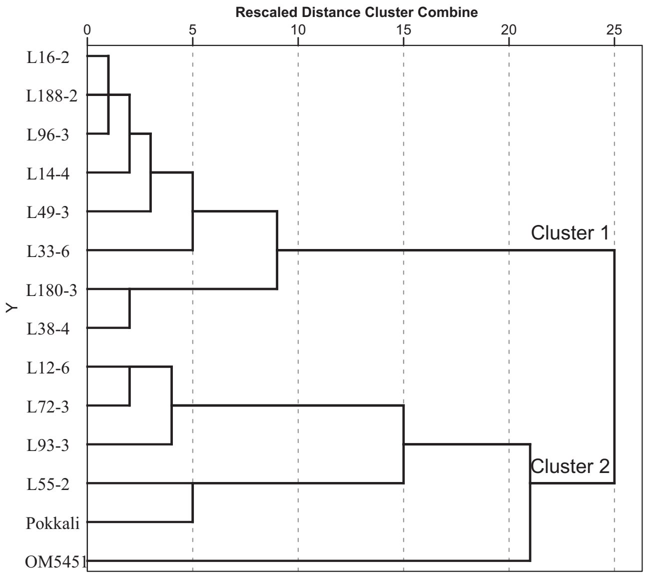

FIGURE 2 Cluster assignment of 12 crop wild relative (CWR)-derived lines and two check varieties. The hierarchical cluster dendrogram showing two major clusters. Rescaled distance cluster combine refers to the value of the distance (similarity) between clusters as per Ward's minimum variance

3.2 | Pearson correlations between the 10 studied traits

The study of the correlation between grain yield and its component traits helps to reveal their salt tolerance in rice. At Phuoc Long site (Crop 1), the correlation analysis showed a highly significant negative correlation between plant height and grain yield ($r = -.54$, $p \leq .01$), and highly significant positive correlations between grain yield and panicle number per square meter ($r = .61$, $p \leq .01$), and number of grains per panicle ($r = .56$, $p \leq .01$) (Figure 3).

Although the correlation between 1,000-grain weight and filled grains per panicle was highly significant and negative ($r = -.44$, $p \leq .01$), the correlation between number of unfilled grains per panicle and the number of grains per panicle was highly significant and positive ($r = .88$, $p \leq .01$) (Figure 3). Results revealed a highly significant and positive correlation between panicle number per square meter and plant height ($r = .44$, $p \leq .01$) and the correlation between days to heading at 5% and days to flowering at 80% ($r = .96$, $p \leq .01$) (Figure 3). Our results suggest that panicle number per square meter and a number of grains per panicle are important yield-related traits contributed to rice grain yield under salt stress conditions in the coastal areas of the Mekong Delta.

Similarly, at the Gia Rai site (Crop 2), the results of the correlation analysis showed a significant and negative correlation between grain yield and days to heading at 5% ($r = -.33$, $p \leq .05$), days to flowering at 80% ($r = -.34$, $p \leq .05$), plant height ($r = -.39$, $p \leq .05$), and a highly significant and negative correlation with the number of unfilled grains per panicle ($r = -.48$, $p \leq .01$) (Figure 3). In contrast, the correlation between grain yield and weight of 1,000 grains and filled grains per panicle was highly significant and positive with $r = .50$ ($p \leq .01$) and .66($p \leq .01$), respectively. Results revealed a significant and positive correlation between grain yield and panicle number per square meter ($r = .40$, $p \leq .05$) and number of grains per panicle ($r = .41$, $p \leq .05$) (Figure 3). Our results suggest that the traits (a) days to heading at 5%, (b) days to flowering at 80%, (c) plant height, and (d) number of unfilled grains per panicle are key traits for grain yield improvement under salt stress conditions during the rainy season in the coastal area of the Mekong Delta.

3.3 | Farmers' preference analysis

To help identify farmer acceptance of an introduced new salt-tolerant rice line, farmer field days were conducted at both on-farm sites. For Crop 1, farmers preferred the following five lines derived from CWR: L96-3, L180-3, L33-6, L93-3, and L188-2. The preference of the 27 participating farmers indicated that the three best-placed lines were L96-3 (70.4% preference), L180-3 (66.7%), and L33-6 (63.0%) (Table 5).

Farmers particularly appreciated their early maturity, short plant height, good grain quality, and grain yield. For Crop 2, the 25 farmers preferred L96-3, L180-3, L33-6, L93-3, and L49-3. The best three lines comprised L180-3 (76.0% preference), L96-3 (68.0%), and L33-6 (64.0%) (Table 5).

4 | DISCUSSION

4.1 | The necessity of salt-tolerant rice varieties in the coastal region of the Mekong Delta

During the dry season (mid-January to early June) in the coastal zone, there is a critical shortage of freshwater for the nonirrigated areas of the Mekong Delta, which covers an estimated 30% of the rice-growing area (Bui & Nguyen, 2004). In this coastal zone, rice can only be grown during the rainy season and depends mainly on the rainfall. In the early (mid-June) or late (early January) rainy season, there is not enough freshwater, whereas saltwater (~5 dS m^{-1}) can penetrate the fields and directly affect rice yields. The frequent intrusion of saltwater can lead to salt accumulation in the soil, which can damage the subsequent crop (Phap et al., 2006). In this study, we conducted participatory on-farm trials in two different rice farming systems representative for the Mekong Delta—(a) shrimp–shrimp–rice system and (b) rice–rice system (saline rainfed condition)—to evaluate the performance of CWR-derived rice lines in these farming systems.

Plants grown in Gia Rai site	Plants grown in Phuoc Long site: HD5%	FT80%	Plant height	Panicle/Pla nt	Panicle/sq uare meter	Grains/pan icle	Filled grains/pani cle	Unfilled grains/ panicle	Weight of 1000 grains	Grain yield (tons/ha)
HD5%		0.96**	0.08	0.29	-0.10	.375*	0.06	0.35*	0.18	0.21
FT80%	0.99**		0.18	0.27	0.00	.354*	0.08	0.32*	0.21	0.33*
Plant height	0.17	0.19		0.19	0.44**	0.02	0.05	0.00	-0.12	-0.54**
Panicle/Plant	-0.16	-0.15	0.34*		-0.08	0.23	0.03	0.21	-0.05	0.11
Panicle/square meter	0.38*	0.39**	0.43**	0.53**		-0.26	-0.16	-0.17	-0.04	0.61**
Grains/panicle	0.39**	0.43**	0.06	-0.48**	-0.06		0.23	0.88**	-0.25	0.56**
Filled grains/panicle	0.13	0.14	-0.09	-0.23	-0.22	0.66**		-0.26	-0.44**	0.17
Unfilled grains/ panicle	0.43**	0.46**	0.15	-0.45**	0.05	0.76**	0.03		-0.04	0.09
Weight of 1000 grains	-0.21	-0.21	-0.20	0.10	-0.10	-0.27	-0.20	-0.18		0.24
Grain yield (tons/ha)	-0.33*	-0.34*	-0.39*	0.31	0.40*	0.41*	0.66**	-0.48**	0.50**	

FIGURE 3 Pearson correlations between the 10 studied traits and the two experimental sites. A heat map is used to visualize the correlations. HD5%, days to heading at 5%; FT80%, days to flowering at 80%, *Significant at the .05 probability value. **Significant at the .01 probability value

Previous studies revealed that the plant growth response in rice was inversely related to the toxic accumulation of NaCl around the root zone (James, Blake, Byrt, & Munns, 2011; Nishimura, Cha-um, Takagaki, & Ohyama, 2011). In addition, rice roots influence the concentration, transport, and distribution of salinity within the root zone due to their selective water uptake (James et al., 2011; Munns, 2005). As a result, grain yield could be affected by the transport and uptake of ions in the soil by plant roots (Khan, Yitayew, & Warrick, 1996). Therefore, a sodium adsorption ratio (SAR) of >10 has a negative effect on cell division and elongation, resulting in reduced growth and yield (Munns, 2002). However, our on-farm experiments were conducted at a Phuoc Long site (SAR = 16.7) and at a Gia Rai site (SAR = 14.7) and demonstrated that some CWR-derived lines were well suited to both sites and both farming systems. The best-performing lines derived from CWR had grain yields higher than those of the two test varieties. At both on-farm sites, the best CWR-derived lines achieved grains yields >6.5 t ha^{-1}. In summary, these best lines are very promising in varying soil conditions in coastal provinces in the Mekong Delta.

4.2 | CWR-derived lines harbor promising traits for variety improvement in saline environments

Under field conditions, most traits such as tiller number, spikelet fertility, number of florets per panicle, or 1,000-grain weight are influenced under both sodicity and salinity (IRRI, 1997). Moreover, the reproductive stage under saline conditions is one of the most sensitive growth stages (Momayezi et al., 2009; Singh et al., 2002). As a result, the ultimate goal is the development of well-adapted new salt tolerance rice varieties with increased grain yield. Crop wild relative-derived materials could play an important role here. Variety improvement based on CWR is feasible by conventional breeding, but reducing the linkage drag is time consuming. This is especially true when salt stress reduces panicle and grain numbers per plant (Ashfaq, Haider, Khan, & Allah, 2012; Bunnag & Pongthai 2013; Flowers, 2004; Piao et al., 2010).

It is clear that under saline conditions, the reproductive stage is one of the most susceptible growth stages (Singh et al., 2004). As far as grain yield is concerned, this is the most critical stage, since effective fertilization at this stage is eventually converted into grain yield. Since the rice genotypes in this experiment were grown during the booting stage under saline conditions, their pollen viability was severely affected (Mohammadi-Nejad et al., 2012). Almost all varieties decreased their pollen viability under stress, but the responsive genotypes for the reproductive process were considered those that dramatically decreased pollen viability in conjunction with a very large decrease in grain yield (Khatun et al., 1995). The grain yield in the reproductive phase under salt stress conditions can be increased by increasing the number of panicles per plant, the number of grains per panicle, and the 1,000-grain weight. We have shown that promising CWR-derived lines harboring such beneficial characteristics are now available for more detailed evaluations.

Furthermore, in this study, we found a significant difference in the number of panicles between the modern check varieties and CWR-derived rice lines under both salt stress conditions sites. These studies suggested to cross the best-performing CWR-derived with modern rice varieties to develop better adapted varieties. It is argued that farmers could be involved in

TABLE 5 A preference analysis of selected lines based on the evaluation before harvesting at the on-farm trial of 27 local farmers (Phuoc Long site, Crop 1) and 25 local farmers (Gia Rai site, Crop 2)

CWR[a]-derived lines	Phuoc Long site (27 farmers)			Gia Rai site (25 farmers)			Farmer's preferred traits	Rank[b]	
	Acceptability		Preference	Acceptability		Preference		Phuoc Long site	Gia Rai site
	Yes	No	%	Yes	No	%			
L96-3	19	8	70.4	17	8	68.0	High yield, short plant height, high number of panicles, fewer unfilled grains, high grain weight	1	2
L180-3	18	9	66.7	19	8	76.0	High yield, short plant height, high number of panicles	2	1
L33-6	17	10	63.0	16	9	64.0	High yield, high number of filled grains	3	3
L93-3	16	11	59.3	14	11	56.0	High yield, high grain weight, short duration	4	5
L188-2	12	15	44.4	X	X	X	High yield, fewer unfilled grains	5	X
L49-3	X	X	X	15	10	60.0	High yield, short duration	X	4

[a]CWR, crop wild relative.
[b]1 = very best; X = nonappliable for unselected lines in these seasons.

the development of such "climate-safe" rice varieties through PVS.

4.3 | PVS in combination with farmer field days is a reliable way to develop and adopt new salt-tolerant lines

It is well documented that PVS contributes not only to the dissemination of farmer-preferred varieties through informal networks such as seed clubs but also to the upscaling of locally adapted technologies through a decentralized technology testing process (Tin et al., 2011). Participatory variety selection is a quick, cost-effective, and reliable way to identify farmer-preferred varieties in real time (Witcombe et al., 2001). In this study, PVS conducted in sodic soils through a cooperation between the Can Tho University and nearby farmers organized in seed clubs has confirmed the relevance and usefulness of PVS to develop and adopt new salt-tolerant lines.

Moreover, PVS on-farm trials are necessary to take into account the voice of local farming communities. Two PVS trials based on preselected CWR-derived lines were conducted in the coastal area in the Mekong Delta. The farmers appreciated some CWR-derived lines, which had high yield, short duration, and low plant height. The most promising CWR-derived lines adapted to different coastal areas of Mekong Delta were L33-6, L96-3, and L180-3. These lines were adopted by farmers and proposed for trials on a larger scale and in all environments of the coastal zone.

All data from this study have been stored in the Germinate-Rice database and are freely accessible from https://ics.hutton.ac.uk/cwr/rice (Raubach et al., 2020).

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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