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Genetic and physiological characteristics of salt-tolerance in nine rice varieties at seedling stage

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Abstract. Climate change has caused severe saltwater intrusion, significantly affecting rice production in the Mekong Delta. This study aims to evaluate the genotypes and salt tolerance of nine rice varieties, including two control varieties, FL478 (salt-tolerant) and IR29 (salt-sensitive), and seven other varieties: ST3, Lua Tim Can Tho, Lua Tim Vinh Long, ST24, Jasmine85, TNN91, and Vin16. The experiments were conducted under artificial conditions with zero and 100 mM NaCl in the Yoshida nutrient solution for 21 days. The genotypes were analysed with the 15 SSR primer pairs linked to salt-tolerance quantitative trait loci identified in FL478. The measured physiological parameters included Na^+ and K^+ ion contents in shoots and roots, the Na^+-to-K^+ ratio, and the proline content. The results reveal that 8 SSR primer pairs identified salt-tolerant genotypes similar to FL478. Physiologically, the salt-tolerant varieties maintained a low Na^+-to-K^+ ratio and efficiently accumulated proline, although FL478 accumulated less proline than IR29. This indicates that FL478 utilises proline more effectively for osmotic regulation and protecting cells from oxidative stress. The Membership Function Value of Salt Tolerance analysis grouped the 9 rice varieties into three categories according to their salt tolerance levels, with ST3 clustered in the highly salt-tolerant group alongside FL478. The study confirms the correlation between genotype and salt tolerance, providing a scientific basis for breeding rice varieties adapted to increasingly severe salinity conditions in the Mekong Delta.

Keywords: proline, Na^+/K^+, SSR primer, salt-tolerant rice

1 Introduction

The rising sea levels and saltwater intrusion pose serious challenges to the Mekong Delta (MKD), a key region for agricultural and aquaculture production in Vietnam, particularly for rice farming. The Department of Water Resources (Ministry of Natural Resources and Environment in Vietnam) reported that the sea levels in the coastal provinces of MKD have risen by an average of 3–4 mm/year over the past 30 years [1]. If this trend continues, the sea levels could rise by 15–20 cm by 2050 [27, 28], accelerating salinity intrusion and flooding, with severe impacts on coastal provinces such as Ben Tre, Tra Vinh, Soc Trang, Ca Mau, and Kien Giang [2]. Salinity intrusion in the MKD primarily occurs during the dry season (December to April) when the Mekong River flow decreases. The Southern Institute of Water Resources [3] noted that the 4‰ salinity boundary has penetrated 50–90 km inland at major river mouths, affecting over 1.7 million hectares of agricultural land. The 2019–2020 dry season marked the most severe salinity intrusion in history, causing significant agricultural losses and disrupting livelihoods [4].

Because of the urgent needs for agricultural production under increasing salinity intrusion caused by climate change, scientists have actively applied marker-assisted selection (MAS) in rice

breeding to improve salinity tolerance. This approach enables the identification of quantitative trait loci (QTLs) associated with salt tolerance traits, thereby facilitating faster, more accurate, and more efficient selection compared with traditional methods. In recent years, single nucleotide polymorphism (SNP) markers have gradually replaced SSR markers because of their genome-wide coverage and compatibility with genome-wide association studies (GWAS), allowing the discovery and utilisation of novel QTLs associated with salinity tolerance at different growth stages.

Accordingly, at least 18 major QTLs related to salt tolerance have been identified and validated across various genetic backgrounds, highlighting their broad potential application in breeding programs [5]. In addition, Platten et al. identified and demonstrated the effectiveness of the Saltol gene located on chromosome 1, a key locus helping rice plants regulate Na^+ under saline conditions, which has been widely used in MAS-based breeding programs [6]. Furthermore, Hoang et al. discovered novel QTLs, such as qSKC-1 and qNaK-1, that are associated with the regulation of Na^+/K^+ homeostasis, a crucial physiological indicator of salinity tolerance [7]. Advances in next-generation sequencing (NGS) technologies, combined with tools such as genotyping-by-sequencing (GBS) and high-throughput SNP chips, have further supported the rapid detection and application of molecular markers in rice salt tolerance improvement.

Based on studies related to QTLs and using SSR molecular markers combined with an artificial salinity purification method, researchers are actively developing rice varieties with high adaptability to saline conditions while ensuring stable yields. This approach is considered the most sustainable and urgent solution to maintain rice production and food security in affected areas. Recent studies have evaluated rice salt tolerance through artificial saline screening models, such as embryo culture in NaCl-supplemented media, soil-based salt treatments, or Yoshida nutrient solution with added NaCl.

This study employed the Yoshida nutrient solution to create a controlled environment for evaluating the salt tolerance of popular rice varieties. The results can provide a scientific foundation for breeding rice varieties suitable for saline-affected regions.

2 Materials and methods

2.1 Materials

The experiment included nine rice varieties: two controls (salt-tolerant FL478 and salt-sensitive IR29) and seven local varieties (ST3, Lua Tim Can Tho (LT Can Tho), Lua Tim Vinh Long (LT Vinh Long), ST24, Jasmine85, TNN91, and Vin16), preserved in the seed bank of the Department of Genetics and Plant Breeding, Can Tho University.

2.2 Methods

Evaluation of phenotype for salt-tolerance

The DNA of the 9 rice varieties was extracted by means of Doyle and Doyle's CTAB method [8]. The DNA extraction solution was 2X CTAB (2% CTAB, 100 mM Tris pH 8.0, 20 mM EDTA pH 8.0, and 1.4 M NaCl). Additionally, supporting reagents were added to enhance extraction efficiency, including β-mercaptoethanol, chloroform/isoamyl alcohol (24:1), RNase, isopropanol, and 70% ethanol. After extraction, the DNA was dissolved in 35 μL TE (pH 8.0) and stored at –20 °C.

The salinity-tolerant genotype was assessed with the linkage of 15 SSR primer pairs associated with 12 chromosomes by means of PCR technology. The PCR reaction was performed in a

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10 μL PCR mixture with 5x Taq PCR Buffer (Meridian Bioscience), containing dNTP, My-Taq DNA Polymerase, purified water, primers, and DNA. All components were thoroughly mixed before being loaded into the GeneAmp PCR Mastercycler X50s (Eppendorf). The PCR reaction was carried out over 35 heating cycles: 5 minutes at 95 °C, 30 seconds at 95 °C, 30 seconds at 55–65 °C (depending on the primer's annealing temperature), 30 seconds at 72 °C, and 5 minutes at 72 °C. The PCR products were then held at 10 °C for 20 minutes. The sequences of the 15 primer pairs are listed in Table 1.

After electrophoresis, the gel was stained with ethidium bromide (10 μL stock ethidium bromide 1% with 100 mL dH_2O). The PCR results were assessed by means of the electrophoresis bands, comparing their sizes with those of the salinity-tolerant reference varieties or the susceptible control varieties.

Table 1. List of 15 primers used to evaluate phenotypes

Primers	Forwards sequences	Reverse sequences
RM3412	AAAGCAGGTTTCCTCCTCCC	CCCATGTGAATGTGTCTTC
RM140	GGCATGCCGAATGAAATGCATG	TGCCTCTTCCGCTGCCCCTG
RM8132	GAGAGGAATGCGAGATCGGTG	AAACCAACTTATATACTCCC
RM581	GAGTAGCTTTCCCACCCCC	GAGCTGTTTGGACTACGGC
RM8070	GAAATGGACTCCTCAAATGGG	ACGAATCAGGATGGCATCC
RM493	TAGCTCCACAAGTAGACC	GTACGTAAAGCGGAAGGTG
RM3810	ACGAAGGAACTACCCGTGTG	CGCACATGTTACTAGCGG
RM211	CCGATCTCATCAACCACTG	CTTCACGAGGATCTCAAAGG
RM6329	CCCTGGATGAAAGCAAG	GAAGTTGTAGTCCCCTATC
RM127	GTGGGATAGCTGCGTCG	AGGCCAGGGTGTTGAGTGC
RM17749	ACGCACATCACCAACTGC	TCTCTTGCACACACTTACATCC
RM253	TCCTTCAAGAGTGCAAACC	GCATTGTCACTGTCAGCC
RM214	CTGATGATAGAAACCTTCTTC	AAGAACAGCTGACTTCAA
RM6369	CAAGCTAGGGCTGCATAGC	GCTTCACCTACCTCACC
RM24330	AATCGCGGGGAGCAAAACC	CGATGACCAATGACGAGG
RM474	AAGATGTACCGGTGAATTC	TATGAGCGTGTGAATGGG
RM286	GGCTCTCATCTTCTGGTGC	CCGGATTCAAGTCAACTC
RM28102	CACTAATTCTTCCGACTTTAGG	GTGGAAGCTCCGAAGTGC

Salt screening

The rice varieties were screened for salt tolerance with the IRRI [9] method by using Yoshida's nutrient solution [10] with zero mM and 100 mM NaCl for 14 and 21 days. Growth parameters (plant height, root length, and shoot/root biomass), Na^+ and K^+ content (measured with LAQUAtwin ion meters), and proline content (spectrophotometric method at 520 nm) were evaluated.

Parameters monitored

The plant height, root length, shoot, and root biomass were measured. Additionally, pH, Na^+, and K^+ concentrations in the solution, as well as

electrical conductivity (EC), were closely monitored and controlled to ensure experimental consistency.

Evaluation of Na+ and K+ content [11]

The samples were dried at 70 °C for 24 hours until a constant weight was achieved. Then, 15 mg of the dried (DW, dry weight) and finely ground sample was extracted with 1,000 µL of distilled water. The mixture was shaken horizontally on an orbital shaker (C203/BM DTCGCT) for 24 hours and centrifuged at 7,000 rpm for 5 minutes. A 300 µL aliquot of the supernatant was applied directly to the electrode position of a selective ion meter LAQUAtwin Na-11/K-11. The Na^+-to-K^+ ratio was calculated according to the following formula

$$[Na^+ \text{ or } K^+ \text{ mg/g DW}] = \frac{[0.001 \text{ (liter)} \times \text{measured values (mg/liter} \sim \text{ppm)}]}{0.015 \text{ (g)}}$$

Proline content analysis [12]

0.2 g of fresh leaf tissue (FW, fresh weight) uniformly collected from leaves, including midribs, was finely ground in a 2.0 mL tube with 1 mL of PBS (pH 7.8). The mixture was vortexed for 1 minute and centrifuged at 13,000 rpm for 20 minutes at 4 °C. Subsequently, 1 mL of the supernatant was transferred to a new 1.5 mL tube. For proline derivatisation, 50 µL of the centrifuged extract was added to a 2.0 mL tube containing 1 mL of DDPU reagent, prepared by mixing 12 mL of 3% sulfosalicylic acid, 12 mL of glacial acetic acid, and 24 mL of ninhydrin solution in a 50 mL Schott bottle with magnetic stirring for 5 minutes. The mixture was vortexed for 30 seconds and incubated in a water bath at 95–100 °C for 15 minutes, followed by cooling on ice for 5 minutes. Finally, 200 µL of the cooled solution was transferred to a microplate, and absorbance was measured at 520 nm with a Molecular Devices SpectraMax 190 spectrophotometer. The proline content was calculated as µg/g fresh weight according to the formula

Proline (µg/g FW) = ((24.806 × OD520 – 0.0073) × 1000)/(50 × 0.2)

Proline (µmoles/g FW) = ((24.806 × OD520 – 0.0073) × 1000)/(50 × 0.2 × 115.13)

Data analysis

Data analysis was performed by means of Microsoft Excel for initial processing, SPSS with Tukey's test for statistical analysis, and NTSYSpc version 2.1 software for constructing UPGMA dendrograms to assess genetic similarity among the nine rice varieties by means of binary data matrices. The salt tolerance was evaluated according to the Membership Function Value of Salt Tolerance (MFVST) as described by Chen et al. [13] and Afsar et al. [14].

This comprehensive analytical approach enabled both statistical evaluation of experimental results and assessment of genetic relationships among the rice varieties under salt stress conditions.

3 Results and discussion

3.1 Genotyping

In the genotype analysis, the FL478 and IR29 rice varieties served as controls; FL478 carries salt-tolerant genes, and IR29 carries salt-sensitive genes. The evaluation results with the 15 SSR primer pairs revealed that 8 primer pairs (RM211, RM286, RM493, RM3412, RM6329, RM6969, RM8070, and RM8132) exhibited obvious genotypic differences between salt-tolerant and salt-sensitive varieties, while the remaining 7 primer pairs showed no genotypic variation among the rice varieties.

The results with the RM286 primer pair located on chromosome 11 and linked to the QTL

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qSNC11 demonstrated its association with Na^+ exclusion capacity in rice shoots. Effective Na^+ exclusion helps reduce Na^+ concentration in shoots, thus enhancing salt tolerance [15]. The PCR electrophoresis results in Fig. 1A showed that FL478 produced a 115 bp band, and ST3 and ST24 exhibited the same genotype. Jasmine85 displayed a heterozygous genotype, while the remaining varieties matched IR29's genotype (120 bp). These findings indicate that FL478, ST3, and ST24 possess enhanced Na^+ exclusion capacity, contributing to their salt tolerance.

According to Thomson et al., the RM6329 primer pair is linked to QTL qCHL3 on chromosome 3, which affects leaf photosynthetic efficiency under saline conditions [16]. The PCR results in Fig. 1B showed that FL478 produced a 145 bp band, along with four other varieties (Lua Tim Can Tho, Lua Tim Vinh Long, Jasmin, and Vin16), indicating their ability to maintain photosynthesis under salinity stress. In contrast, IR29 (125 bp), Jasmine85, and TNN91 lacked this trait, while ST3 showed a heterozygous genotype.

Lin et al. identified the RM6369 primer pair linked to QTL qTN8.1 on chromosome 8, affecting rice growth under saline conditions [17]. The genotyping results in Fig. 1C revealed that FL478, LT Can Tho, LT Vinh Long, Jasmine85, TNN91, and Vin16 had a 120 bp band, confirming their association with qTN8.1 and salt-adaptive growth. IR29 and ST24 (110 bp) lacked this QTL linkage, while ST3 exhibited a heterozygous genotype with both 120 bp and 110 bp bands. These results demonstrate clear genotypic differentiation among the tested varieties concerning key salt-tolerance mechanisms.

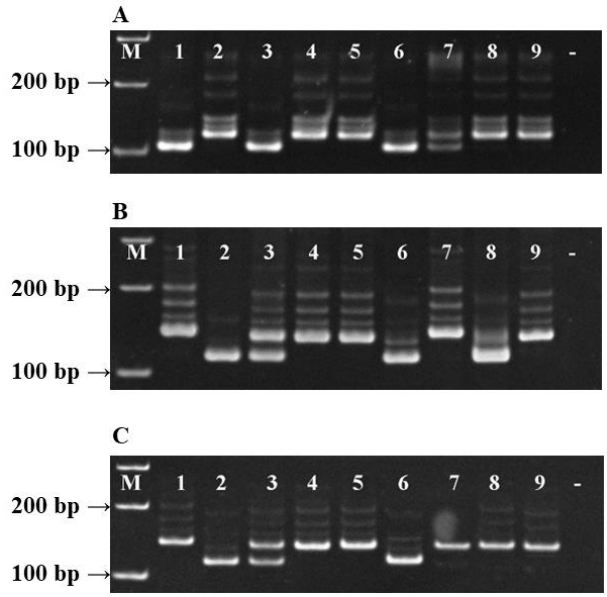

Fig. 1. Electrophoresis spectrum to identify salt-resistant genotypes of 9 rice varieties on 8% acrylamide gel (**A.** RM286; **B.** RM6329; **C.** RM6369. The numbers from 1–9 are the varieties such as FL478, IR29, ST3, Lua Tim Can Tho, Lua Tim Vinh Long, ST24, Jasmine85, TNN91, and Vin16; the "–" sign is the negative sample without DNA. M is the standard scale.)

The UPGMA cluster analysis with 15 SSR primer pairs linked to salt-tolerance QTLs across 12 chromosomes, conducted with the NTSYS pc 2.1 software, revealed distinct genetic differentiation among the nine rice varieties (Fig. 2). The results allowed for the classification of the varieties into three major groups with notable genetic descriptions. Group I with high salt tolerance includes the FL478 and ST3 varieties with a similarity coefficient 0.78, indicating shared genetic traits for salt tolerance. Group II contains the salt-sensitive varieties, including IR29, TNN91, Lua Tim Can Tho, Lua Tim Vinh Long, and Vin16. Among them, IR29 and TNN91 (similarity 0.88) possess nearly identical salt-sensitive genotypes, while Lua Tim Can Tho and Lua Tim Vinh Long share the same genotype despite different collection sites. Group III, which exhibited moderate salt tolerance, included Jasmine85 and ST24 with a 0.67 similarity to Group II and a 0.58 similarity to Group I, suggesting partial salt-tolerance traits. The results

demonstrate that the varieties with high genetic similarity to IR29 lack salt tolerance, while those closely related to FL478 show promising salt-tolerance potential. This genetic clustering provides valuable insights for marker-assisted selection in breeding programs aiming to develop salt-tolerant rice varieties, making FL478-like genotypes specifically promising candidates for further salt-screening studies and breeding initiatives. The intermediate genotypes in Group III may possess beneficial partial tolerance traits that warrant additional physiological investigation.

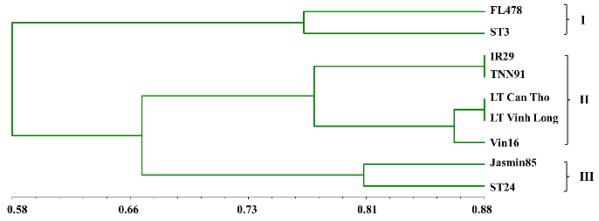

Fig. 2. UPGMA classification diagram based on Nei's genetic distance according to SSR analysis results of 9 rice varieties

3.2 Na^+ and K^+ content analysis in leaves

The accumulation of Na^+ in leaves is an obvious indicator of salt stress, directly affecting cellular structure and photosynthetic function. Excessive Na^+ accumulation disrupts ion exchange, causes cell necrosis and leaf scorching, reduces dry matter accumulation, and impairs plant growth [18, 19].

The Na^+ and K^+ content was analysed with two NaCl concentrations (zero mM and 100 mM) in the Yoshida nutrient solution. The results show significant differences ($p < 0.05$) in Na^+ accumulation among the tested varieties (Fig. 3A and 3B). After 14 days of salt treatment (100 mM NaCl), the Na^+ content in leaves increased significantly compared with the control (zero mM NaCl), ranging from 150 to 550 mg/g DW, with IR29 exhibiting the highest Na^+ accumulation and ST24 the lowest. By the 21st day, the Na^+ level surged to 510–800 mg/g DW, with ST24 showing the highest accumulation. Notably, three salt-sensitive varieties (IR29, Lua Tim Vinh Long, and Vin16) completely died under prolonged 100 mM NaCl stress, preventing further Na^+ or physiological analysis, confirming their lack of salt tolerance.

For the K^+ content, most of the investigated varieties showed reduced K^+ uptake under 100 mM NaCl after 14 days. However, FL478 maintains higher K^+ absorption (260 mg/g DW at zero mM vs. 330 mg/g DW at 100 mM). By the 21st day, K^+ uptake declined across all varieties, although FL478 and ST3 retained relatively higher levels (~125 mg/g DW). According to Anschütz et al., K^+ is vital for enzymatic activation, osmotic regulation, membrane potential maintenance, stomatal control, and cell growth [20]. Thus, the varieties sustaining high K^+ uptake under salinity demonstrate better salt tolerance.

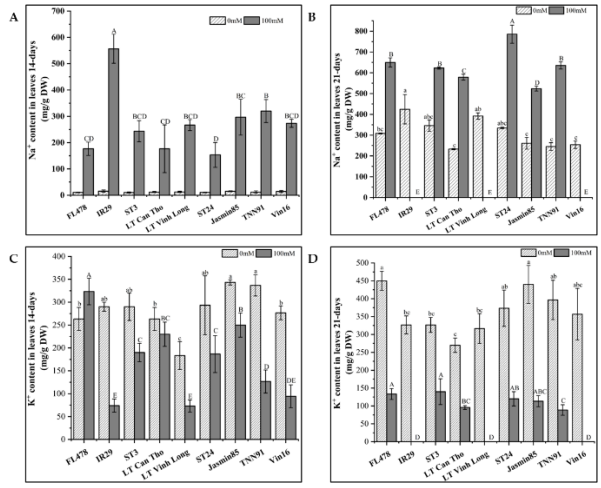

Fig. 3. Na+ and K^+ content in leaves after 14 days and 21 days of salinity treatment. **A:** Na^+ content after 14 days; **B:** Na^+ content after 21 days; **C:** K^+ content after 14 days; **D:** K^+ content after 21 days.

(Note: a, b, c, d, and A, B, C, D: At the same salinity concentration, rice varieties with the same letter followed by the same letter have no statistically significant difference according to Duncan's test ($p < 0.05$).

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3.3 Na^+ and K^+ content in roots

At 14 days after salt treatment (Fig. 4A and 4B), the Na^+ content in roots at 100 mM NaCl concentration increased significantly across all varieties, ranging from 150 mg/g DW with ST3 to over 400 mg/g DW with IR29, while at zero mM NaCl concentration the values ranged between 30–40 mg/g DW with all varieties. Similarly, after 21 days of treatment, three varieties (IR29, Lua Tim Vinh Long, and Vin16) died because of excessive Na^+ accumulation. At this stage, the FL478 variety showed reduced Na^+ uptake (170 mg/g DW) at half its 14-day level. In contrast, other varieties continued accumulating Na^+, three of which (ST3, ST24, and TNN91) showed a similar Na^+ content of 380 mg/g DW.

For the K^+ content in roots (Fig. 4C and 4D), after 14 days of salt treatment with 100 mM NaCl concentration, there was a significant decrease compared with zero mM. Specifically, at zero mM, the K^+ content ranged from 220–310 mg/g DW (lowest in TNN91 and highest in ST24), while at 100 mM, it decreased to 40–190 mg/g DW. FL478 and Lua Tim Can Tho showed the most dramatic K^+ reduction (below 100 mg/g DW).

On the 21st day post-treatment, the K^+ content decreased compared with zero mM. FL478 maintains the highest K^+ level (100 mg/g DW), while the lowest levels were observed in ST3, Lua Tim Can Tho, and ST24 (50 mg/g DW each).

The Na^+ and K^+ content in both leaves and roots demonstrates that Na^+ accumulation increases with salt treatment duration and depends on each variety's ion regulation capacity. Salt-tolerant rice varieties usually employ mechanisms to limit Na^+ uptake in roots, enable selective vascular transport, or sequester Na^+ in vacuoles to avoid cytoplasmic [21]. Maintaining a high K^+-to-Na^+ ratio is essential for sustaining normal metabolic processes under saline conditions. In their study, Ren et al. identified and cloned a quantitative trait locus (QTL) named SKC1 from a salt-tolerant rice variety encoding a member of the HKT-type transporter family, later referred to as OsHKT1;5 [22].

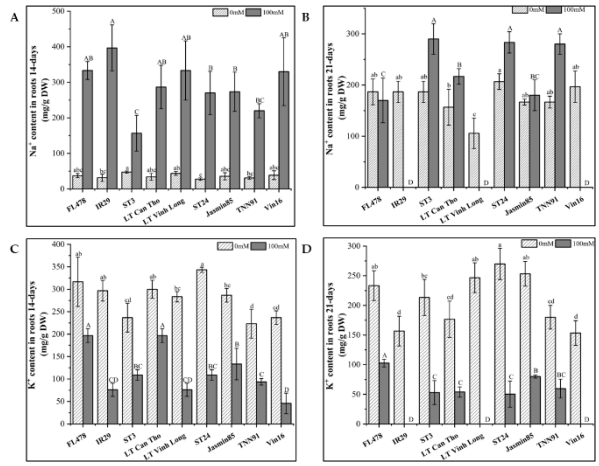

Fig. 4. Na+ and K^+ content in roots on 14th and 21st day after salinity treatment. **A:** Na^+ content on 14th day, **B:** Na^+ content on 21st day, **C:** K^+ content on 14th day, **D:** K^+ content on 21st day.

(Note: a, b, c, d, and A, B, C, D: At the same salinity concentration, rice varieties followed by the same letter have no statistically significant difference according to Duncan test ($p < 0.05$).

3.4 Na^+/K^+ ion balance ability in 9 rice varieties

Maintaining ionic balance, particularly the Na^+-to-K^+ ratio in vegetative organs, is crucial for rice salt tolerance. Under saline conditions, elevated Na^+ levels typically lead to increased Na^+ uptake and reduced K^+ absorption because ion transport competition disrupts physiological processes and exacerbates cellular damage [23].

In the control treatment (zero mM NaCl), the leaf Na^+-to-K^+ ratio remained stable (0.03–0.07) on both the 14th and 21st day (Fig. 5A and 5B), reflecting normal ionic equilibrium. In contrast, 100 mM NaCl treatment sharply increased the ratio across all varieties, indicating salt-induced disruption of K^+ homeostasis and Na^+ accumulation. The salt-sensitive IR29 variety exhibited the most severe imbalance, with a 10-

fold ratio increase (7.5) compared with the control. Three varieties (Lua Tim Vinh Long, TNN91, and Vin16) showed moderate ratios (2.5–4.0), while FL478 and four others maintained only a marginal increase, demonstrating their superior ability to regulate Na^+/K^+ balance under salinity.

Like leaves, roots also reflect rice salt tolerance regarding the Na^+-to-K^+ ratio. A low root Na^+-to-K^+ ratio indicates the plant's ability to maintain a high cellular K^+ concentration, preserving critical physiological functions, such as photosynthesis, protein synthesis, and cell turgor maintenance. Conversely, a high Na^+-to-K^+ ratio may reduce K^+ uptake, negatively affecting growth and yield [24].

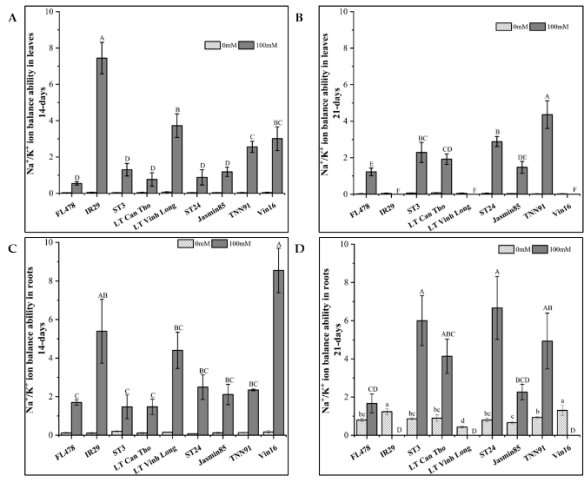

Fig. 5. Na+-to-K+ content ratio in leaves after 14 days and 21 days of salinity treatment. **A:** After 14 days of salinity treatment in leaves; **B:** After 21 days of salinity treatment in leaves; **C:** After 14 days of salinity treatment in roots; **D:** After 21 days of salinity treatment in roots.

(Note: a, b, c, d, and A, B, C, D: At the same salinity concentration, rice varieties with the same letter followed by the same letter have no statistically significant difference according to Duncan's test ($p < 0.05$).

Fig. 5C and 5D show statistically significant differences ($p < 0.05$) in the Na^+-to-K^+ ratio at 100 mM NaCl for both 14-day and 21-day treatments. On the 14th day, Vin16 showed the highest ratio (8.5), while FL478 had the lowest (1.8), comparable with ST3 (1.4) and Lua Tim Can Tho (1.4). IR29 exhibited a high ratio (5.5). By the 21st day, FL478 maintained its ratio (1.8), whereas ST24 peaked at 6.5, followed by ST3 (6.3). Three varieties (IR29, Lua Tim Vinh Long, Vin16) died because of salt sensitivity.

3.5 Proline content in leaves

Leaf proline analysis (Fig. 6) revealed increased accumulation under 100 mM NaCl treatment, indicating osmotic adjustment. IR29 accumulated the highest proline (~1500 μg/g FW), followed by FL478 and Jasmine85. Vin16, TNN91, and ST24 showed lower levels, suggesting weaker tolerance or alternative physiological responses.

After 21 days, the proline content decreased but remained above that of the zero mM NaCl treatment. FL478 maintained the lowest content, yet still higher than controls. Despite lower accumulation, FL478's efficient proline utilisation for osmotic balance, protein or membrane protection, and ROS scavenging [25] underscores its superior tolerance without excessive proline buildup.

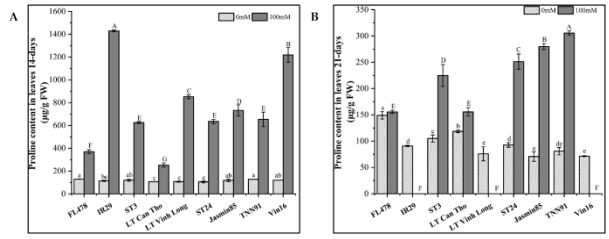

Fig. 6. Proline content in leaves after 14 and 21 days of salinity treatment. **A:** Proline content 14 days; **B:** Proline content 21 days.

(Note: a, b, c, d, and A, B, C, D: At the same salinity concentration, rice varieties followed by the same letter have no statistically significant difference according to Duncan's test ($p < 0.05$).

3.6 Proline content in roots

The increase in root proline content with salinity treatment duration indicates that proline accumulation is a crucial adaptive response to osmotic stress caused by high NaCl levels. On the

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14th day, the proline peak was observed in IR29, Jasmine85, and Vin16 (130 µg/g FW), suggesting that even salt-sensitive varieties initially activate osmotic adjustment mechanisms; however, this response was insufficient to sustain survival under prolonged stress. By the 21st day, the inability to measure proline in the dead varieties (IR29, Lua Tim Vinh Long, and Vin16) further supports the notion that prolonged salinity overwhelms their tolerance capacity. In contrast, the consistently high proline levels maintained in surviving varieties (600–650 µg/g FW) highlight their stronger osmotic adjustment capability, likely contributing to maintaining cell turgor and protecting metabolic functions under stress. Notably, ST24 exhibited the highest root proline content, suggesting a more robust osmoprotective mechanism, whereas Jasmine85 had the lowest among the survivors, indicating variability in tolerance levels even within the surviving group.

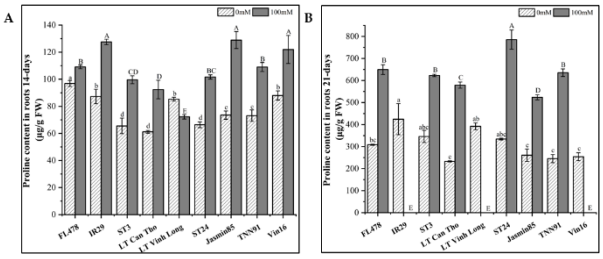

Fig. 7. Proline content in roots after 14 and 21 days of salinity treatment. **A:** Proline content on the 14th day; **B:** Proline content on the 21st day.
(Note: a, b, c, d, and A, B, C, D: At the same salinity concentration, rice varieties followed by the same letter have no statistically significant difference according to Duncan's test ($p < 0.05$).

3.7 Values of functional members equation of salt-tolerance (MFVST)

Evaluation methods for selecting salt-tolerant varieties or lines have been developed for many plant species at the seedling stage. Since salt tolerance is a complex quantitative trait, measuring a single trait alone often does not fully reflect the tolerance of plants to salt stress. To overcome this, researchers apply Membership Function Analysis to integrate multiple physiological and biochemical traits, and this technique helps to screen and evaluate salt tolerance more accurately in different plant species such as rice [26] and wheat [13].

The MFVST (Fig. 8) values allowed for dividing the nine rice varieties into three groups with different levels of salt tolerance. Group I, sensitive to salinity, comprises three varieties, as follows: IR29, Lua Tim Vinh Long, and Vin16, with the MFVST values from 0.216 to 0.311. Group II, moderately tolerant to salinity, consists of four varieties, namely ST24, TNN91, Jasmine85, and Lua Tim Can Tho, with the MFVST values from 0.311 to 0.607. Finally, Group III is tolerant to salinity, constituting two varieties, i.e., ST3 and FL478, with the MFVST values from 0.607 to 0.702.

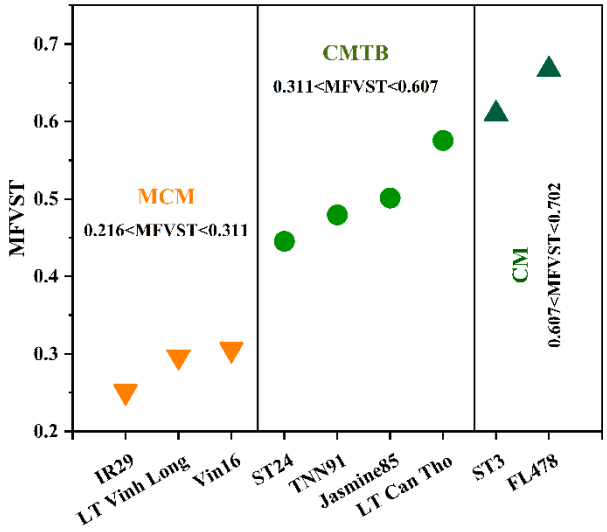

Fig. 8. Membership function values of salt tolerance trait of 9 studied rice varieties.
(*Note: symbols for different salt tolerance subgroups; MCM: salt sensitive; CMTB: moderate salt tolerance; CM: salt tolerance*)

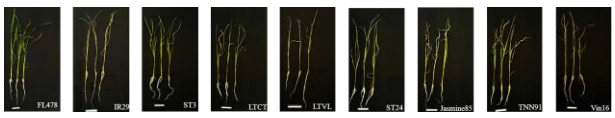

Fig. 9. Effects of salinity on rice varieties after 21 days of salinity treatment

4 Conclusions

In this paper, we identified the most salt-tolerant rice varieties by analysing genotypes with salinity treatment methods in the Yoshida solution. We pinpointed eight primer pairs showing the difference in salinity-resistant and salinity-sensitive genotypes in nine rice varieties, namely RM211, RM286, RM493, RM3412, RM6329, RM6969, RM8070, and RM8132. We divided them into three groups according to the UPGMA values with the SSR marker, reflecting the difference in genetic characteristics or physiological responses among varieties. Additionally, the results of salinity treatment in the Yoshida nutrient solution indicated that three rice varieties were sensitive to 100 mM NaCl salinity conditions, namely IR29, Lua Tim Vinh Long, and Vin16, which died after 21 days of salinity treatment. Variety ST3 has the same salt tolerance as variety FL478; ST24, TNN91, Jasmine85, and Lua Tim Can Tho are classified as having average salt tolerance at an NaCl concentration of 100 mM.

Varieties ST3 and FL478 with similar recovery ability under 100 mM NaCl suggest that they are valuable genetic resources for breeding salt-tolerant rice varieties.

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