



Morphological and molecular screening of traditional rice varieties for salinity tolerance using the RM3412 Marker

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Abstract

Salinity is an important abiotic stress that adversely affects rice growth and productivity. The present study aimed to screen 42 traditional rice varieties (TRVs) for salinity tolerance using morphological traits and to evaluate the RM3412 marker associated with the Saltol region. Seedlings were grown hydroponically for 30 days and exposed to 2500 ppm NaCl. Root length, shoot length, leaf length, leaf width, number of leaves, number of dry leaves and dry-leaf percentage were assessed. Six varieties (TRV-5, TRV-9, TRV-17, TRV-18, TRV-23 and TRV-26) showed comparatively greater tolerance based on the evaluated traits. The six selected varieties were screened with RM3412, and the expected approximately 211-bp product was observed in five varieties. These traditional rice varieties represent promising genetic resources for further validation and breeding for saline environments.

Keywords: *Oryza sativa*, traditional rice varieties, salinity tolerance, Saltol, RM3412, molecular marker

Introduction

Rice (*Oryza sativa* L.) is a major staple crop and an important component of food security, particularly in Asia. Salinity is one of the major abiotic stresses limiting rice production because excessive soluble salts cause osmotic stress, ion toxicity and nutrient imbalance, thereby reducing plant growth and yield. Rice is particularly sensitive to salinity during the early seedling stage, making seedling-stage screening an important component of breeding programmes for saline environments.

Salinity affects root and shoot development, leaf expansion and leaf survival. The degree of response varies among rice genotypes, providing an opportunity to identify tolerant genetic resources. Traditional rice varieties and landraces are especially valuable because they represent locally adapted germplasm and may contain useful alleles or genomic regions for stress tolerance. Their characterization can therefore contribute to broadening the genetic base of rice improvement programmes.

The Saltol quantitative trait locus (QTL), located on chromosome 1, is one of the best characterized genomic regions associated with seedling-stage salinity tolerance in rice. Molecular markers linked to this region, including RM3412, have been used in screening and marker-assisted breeding. RM3412 is a marker associated with the Saltol region and should not be interpreted as the Saltol gene itself.

Previous marker-assisted breeding studies have demonstrated the usefulness of RM3412 for foreground selection of Saltol-associated genomic regions. Traditional rice germplasm from southern India is of particular interest because several locally cultivated varieties have been reported to possess tolerance to drought, salinity and other environmental stresses. However, phenotypic screening combined with molecular screening of traditional rice varieties from Tamil Nadu remains valuable for identifying promising material for further breeding work.

Therefore, the present study was undertaken to evaluate 42 traditional rice varieties under controlled hydroponic salinity stress using morphological traits and to screen the comparatively tolerant varieties using the RM3412 marker associated with the Saltol region.

Materials and Methods

1. Plant material and study site

Forty-two traditional rice varieties (TRVs) were evaluated in the present investigation. The varieties were obtained from different locations in Tamil Nadu and other parts of India as listed in Table 1. The experiment was conducted under laboratory conditions in the Department of Botany, Kandaswami Kandar's College, Velur, Namakkal District, Tamil Nadu, India (11.1202°N, 78.0031°E).

Table 1: Traditional rice varieties evaluated in the present study.

S. No.	TRV code	Traditional rice variety	Origin	Specific notes recorded in original manuscript
1	TRV-1	Kallundaiyan	Tamil Nadu	Resistance to drought, pests; antioxidant-related note
2	TRV-2	Bhavani	Bhavani, Tamil Nadu	Soft texture, sweet flavour and aromatic
3	TRV-3	Yaanaai Komban	Tamil Nadu	High fibre/mineral content; traditional medicinal use noted
4	TRV-4	Kaalabath	Eastern Uttar Pradesh, West Bengal and Tamil Nadu	Nutri-crop; hardy and disease resistant
5	TRV-5	Karunguruvai	South India, Kerala, Karnataka and Tamil Nadu	Drought and salinity tolerance noted
6	TRV-6	Polanel	Tamil Nadu, India	Pest/disease resistance

7	TRV-7	Thulasi Vasi Siraga Samba	Tamil Nadu	Aroma, fibre and traditional nutritional use
8	TRV-8	Rethasali	Odisha, India	Antioxidants, fibre, minerals and flavor
9	TRV-9	Karudan Samba	Tamil Nadu	Drought/salinity tolerance and antioxidant/anti-inflammatory notes
10	TRV-10	Milagu Samba	Tamil Nadu	Drought tolerance and traditional medicinal use
11	TRV-11	Kichadi Samba	Tamil Nadu	Suitable for dry sowing; pest/disease resistance
12	TRV-12	Karuthakar	Tamil Nadu	Adaptation to limited water; nutritional attributes
13	TRV-13	Nattu Pasmathi	Tamil Nadu	Drought tolerance; pest/disease resistance
14	TRV-14	Velai Rudavali	Tamil Nadu	Abiotic-stress and temperature resilience noted
15	TRV-15	Thanga Samba	Tamil Nadu	Stress tolerance and pest/disease resistance noted
16	TRV-16	Illupai Poo Samba	Tamil Nadu	Water-stress resistance
17	TRV-17	Vellai Ponni	Delta regions of Tamil Nadu	Soft texture and sweet flavor
18	TRV-18	Mali Samba	Tamil Nadu	Drought and temperature tolerance; aroma/flavor
19	TRV-19	Vadan Samba	Tamil Nadu, India	Pest/drought resistance and digestive-health note
20	TRV-20	Valai Poo Samba	South India, delta regions of Tamil Nadu	Traditional nutritional and pest/disease-resistance notes
21	TRV-22	Thuya Mali	Tamil Nadu	Unique flavour and aroma
22	TRV-23	Salem Sanna	Salem, Tamil Nadu	Drought tolerance; traditional nutritional/health uses
23	TRV-24	Milagi	Tamil Nadu	Traditional cancer/diabetes-related use noted
24	TRV-25	Manjal Ponni	Kaveri river delta, Tamil Nadu	Drought resistance; traditional heart-health use
25	TRV-26	Kattu Yaanam	Tamil Nadu	Drought/flood tolerance and antioxidant-related notes
26	TRV-27	Kamban Samba	Tamil Nadu	Disease resistance and antioxidant-related notes
27	TRV-28	Valan Samba	Tamil Nadu	Drought, pest and disease resistance
28	TRV-29	Kallumuthan	South India	Unique aroma/taste and digestibility
29	TRV-31	60 M Kuruvai	Tamil Nadu	Mineral-rich; cholesterol-management note
30	TRV-32	Aarkadu Kichali Samba	Arcot region, Tamil Nadu	Drought/flood tolerance; pest/disease resistance
31	TRV-33	Kullakkal	Tamil Nadu	Drought and pest/disease resistance
32	TRV-34	Sernamaiuri-1	South India	Submergence and fungal-disease tolerance
33	TRV-36	Paal Kudai Vaalai	Tamil Nadu	Climate resilience and traditional digestive-health use
34	TRV-37	Sivan Samba	Thanjavur, Tamil Nadu	Unique flavour and aroma
35	TRV-38	Indirayani	Maharashtra, India	Aroma and fibre-rich
36	TRV-39	Sarvathi	Karnataka, Andhra Pradesh and delta regions of Tamil Nadu	Pest/disease resistance
37	TRV-40	Karuppukavuni	Tamil Nadu	High fibre and antioxidant content
38	TRV-41	Mappilai Samba	Thanjavur, Tamil Nadu	Unique flavour and aroma
39	TRV-42	Aathur Kichali Samba	Athur, Tamil Nadu	Drought/flood tolerance and disease resistance
40	TRV-43	Nei Kichali Samba	Tamil Nadu	Aroma/flavour and pest resistance
41	TRV-44	Poongar	Tamil Nadu	Traditional hormonal-balance claim and aroma/texture
42	TRV-45	Mani Samba	Tamil Nadu and Kerala	Flood tolerance and digestive/gut-health notes

2. Seed germination and hydroponic cultivation

Seeds of each rice variety were soaked in sterile distilled water for 48 h to initiate imbibition and germination. The germinated seeds were transferred to sterilized moist tissue paper and maintained for a further 48 h under laboratory conditions. Five healthy seedlings of each variety were selected based on uniform germination and early growth. Seedlings were transferred to hydroponic containers containing sterile distilled water. The varieties were maintained in two independent rows/sets as described in the original experimental design. Hydrogrow nutrient solution was added on day 1 and day 15. Plants were maintained under controlled laboratory conditions for 30 days with regular monitoring.

3. Salinity treatment

After 30 days of growth, NaCl was added to the hydroponic solution to obtain a final salinity concentration of 2500 ppm (2500 mg L⁻¹). The salt was completely dissolved before application. Untreated plants maintained under the same hydroponic conditions served as controls. Plants were maintained under the salinity treatment and morphological responses were recorded after the treatment period.

4. Morphological assessment

Root length, shoot length, leaf length, leaf width, total number of leaves and number of dry leaves were recorded. Dry-leaf percentage was also determined as described in the original dataset. The morphological response of each variety under salinity was compared with the corresponding control. Based on the overall response across the evaluated traits, varieties showing comparatively better maintenance of growth were considered promising for further molecular screening.

5. Genomic DNA isolation

Young leaf tissue from the selected varieties was used for genomic DNA isolation following a modified CTAB procedure. Fresh tissue was ground in liquid nitrogen and homogenized in lysis buffer containing 2% CTAB, 1% PVP, 10% SDS and 2.5% NaCl. The homogenate was incubated at 55°C for 30 min. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, followed by centrifugation at 16,000 × g for 10 min. The aqueous phase was transferred to a fresh tube. DNA was precipitated using sodium acetate and chilled absolute ethanol and incubated at -20°C for 20 min. Samples were centrifuged at 16,000 × g for 10 min at 4°C, washed with 70% ethanol, air-

dried and resuspended in nuclease-free water. DNA samples were stored at -20°C until PCR analysis.

6. Agarose gel electrophoresis

Genomic DNA quality and PCR amplification products were examined by agarose gel electrophoresis. Genomic DNA was resolved on 1% agarose gels, whereas PCR products were analysed using 1.5% agarose gels prepared in $1\times$ TAE buffer. Ethidium bromide was used for staining as described in the original protocol. Electrophoresis was performed at a constant voltage of 150 mA and DNA bands were visualized using a Bio-Rad gel documentation system.

7. PCR amplification using RM3412

PCR screening was performed using the RM3412 marker associated with the Saltol region. The primer sequences used were: forward primer, 5'-AAAGCAGGTTTTCCTCCTCC-3'; reverse primer, 5'-CCCATGTGCAATGTGTCTTC-3'. The reaction volume was 50 μL and contained template DNA, forward and reverse primers, dNTPs, $1\times$ PCR buffer, MgCl_2 , Taq DNA polymerase and nuclease-free water. The cycling programme reported in the original manuscript consisted of an initial denaturation at 95°C for 3 min, followed by 30 cycles of denaturation at 94°C for 1.5 min, annealing at

55°C for 30 s and extension at 72°C for 45 s, with a final extension at 72°C for 5 min. Amplified products were separated by agarose gel electrophoresis and the expected approximately 211-bp product was assessed against a 100-bp DNA ladder.

8. Statistical analysis

The inferential statistical analysis, replication-based variance estimates or a statistical test. Therefore, the conclusions are presented as comparative morphological screening observations based on the original experimental dataset. If replicate-level raw data are available, a formal statistical analysis should be added before submission.

Results

1. Morphological responses to salinity stress

The 42 traditional rice varieties showed considerable variation in their morphological responses to NaCl-induced salinity stress. Root length, shoot length, leaf length, leaf width, total number of leaves, number of dry leaves and dry-leaf percentage varied among varieties when control and salt-treated plants were compared. The overall response indicated that salinity affected the varieties to different degrees, demonstrating phenotypic variation for salt tolerance at the seedling stage.



Fig 1: Roots of different rice (*Oryza sativa* L.) varieties observed before salt treatment (30th day).



Fig 2: Morphological growth stages of *Oryza sativa* during the study period: (a) early seedling stage recorded on the first day after sowing and (b) 30th day of growth. The panel labels correspond to the TRV groups described.

Root length showed substantial varietal variation under salinity. TRV-6 and TRV-7 showed marked reductions in root growth under saline conditions, whereas TRV-14 and TRV-25 showed moderate reductions. TRV-17 and TRV-18 retained comparatively greater root growth under the treatment. Shoot growth also varied among the varieties.

TRV-1, TRV-8, TRV-9 and TRV-34 were reported to show strong reductions in shoot length, while TRV-2, TRV-5, TRV-13, TRV-16 and TRV-45 showed comparatively moderate reductions. TRV-17, TRV-18, TRV-23, TRV-25 and TRV-26 showed relatively lower reductions in shoot growth under the saline treatment.

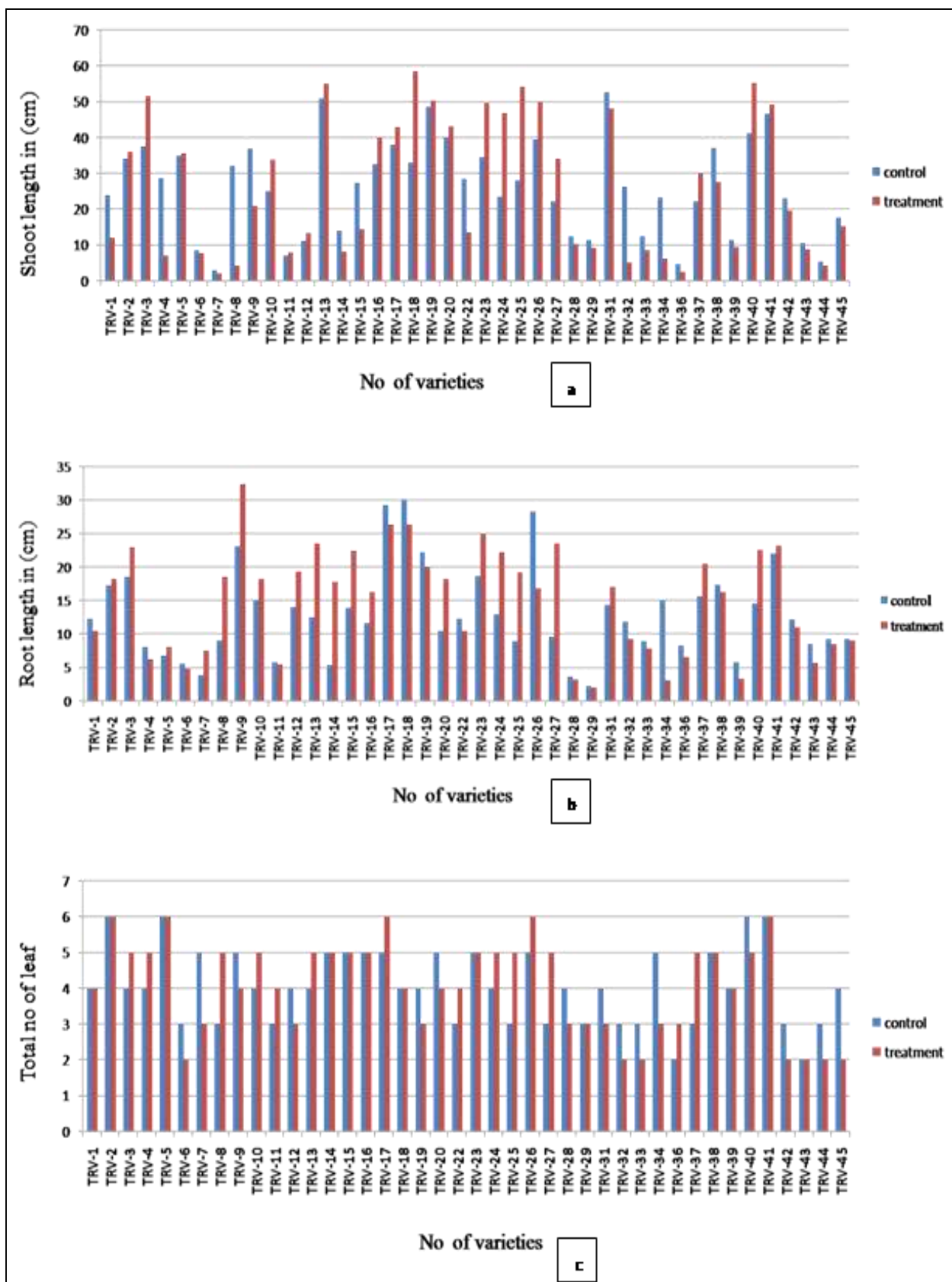


Fig 3: Comparative growth of root length, shoot length and total number of leaves of the traditional rice varieties under control and salinity treatment conditions. (a) shoot length, (b) root length and (c) total number of leaves.

Leaf traits also demonstrated varietal differences. TRV-8, TRV-4 and TRV-34 showed substantial reductions in leaf length, whereas TRV-12, TRV-22, TRV-9 and TRV-45 showed moderate reductions. TRV-17 and TRV-18 maintained comparatively better leaf development.

For total leaf number, TRV-37 and TRV-25 were reported to maintain leaf number similar to the control, whereas TRV-14, TRV-15, TRV-16, TRV-23 and TRV-26 showed comparatively moderate reductions. TRV-7 and TRV-34 showed marked reductions in leaf number.

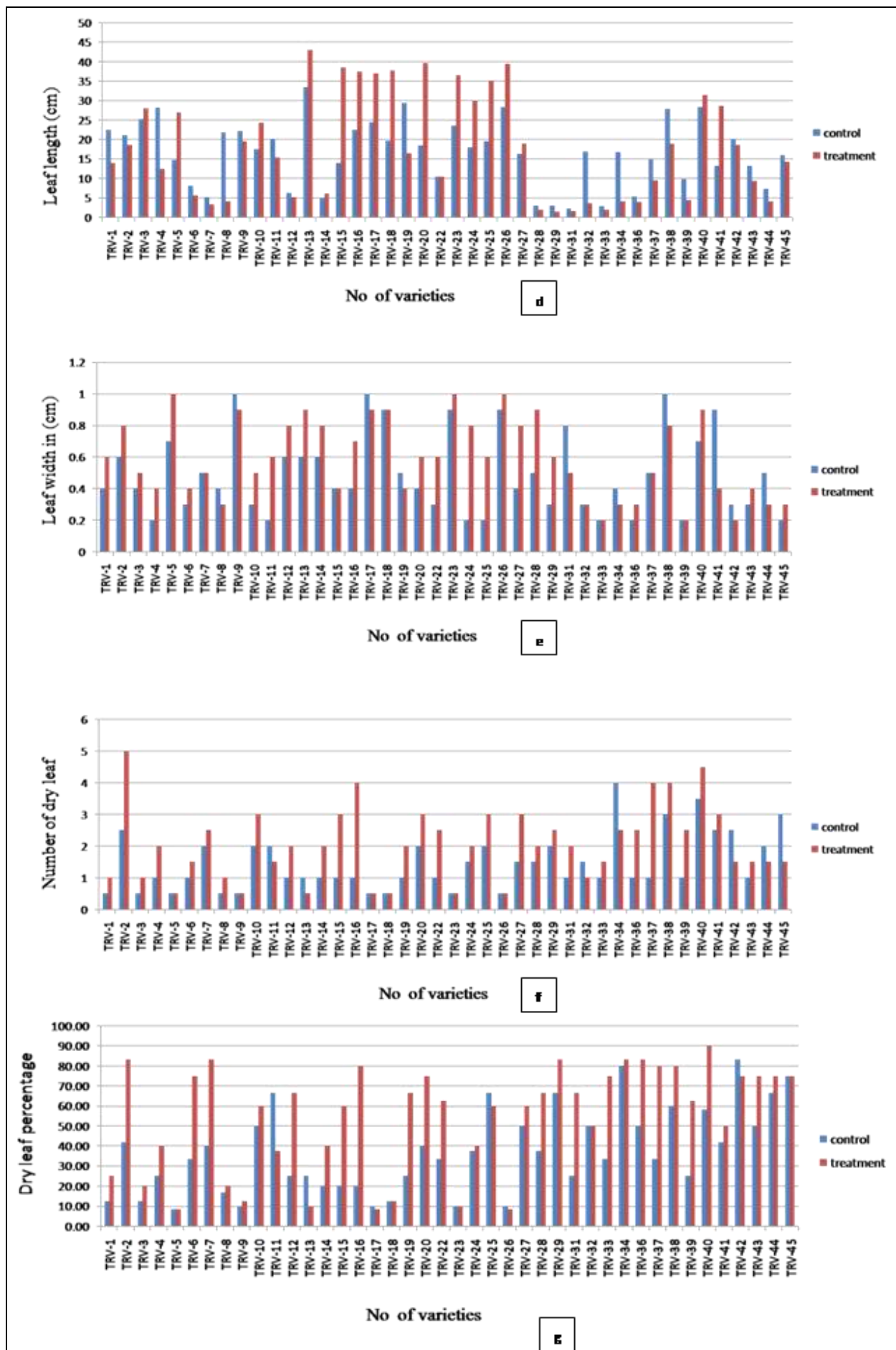


Fig 4: Comparative leaf traits of the traditional rice varieties under control and salinity treatment conditions: (d) leaf length, (e) leaf width, (f) number of dry leaves and (g) dry-leaf percentage.

Dry-leaf responses also differed among the varieties. TRV-45 and TRV-34 showed relatively limited leaf desiccation compared with their controls, while TRV-5, TRV-9, TRV-16, TRV-17, TRV-23 and TRV-26 showed moderate levels of leaf drying. The original dataset further reported increased dry-leaf percentage in TRV-2, TRV-6, TRV-7, TRV-15, TRV-16, TRV-19 and TRV-20, whereas TRV-11, TRV-25 and TRV-42 showed lower dry-leaf percentages. These differences indicate that salinity response was trait-dependent among the traditional rice varieties.



Fig 5: Plant growth after the salinity treatment period. (A) comparatively tolerant growth response; (B) comparatively susceptible growth response.

2. Identification of comparatively tolerant traditional rice varieties

Considering the overall morphological responses recorded across the evaluated parameters, six traditional rice varieties were selected as comparatively tolerant to salinity: TRV-5 (Karunguruvai), TRV-9 (Karudan Samba), TRV-17 (Vellai Ponni), TRV-18 (Mali Samba), TRV-23 (Salem Sanna) and TRV-26 (Kattu Yaanam). These varieties were selected for subsequent molecular screening.

3. Molecular screening using RM3412

Genomic DNA was isolated from the six morphologically selected varieties and screened using the RM3412 marker. The original manuscript reports an expected amplification product of approximately 211 bp in five of the six selected varieties. Based on the lane order stated in the original Fig 6 caption (lanes 2–7 corresponding to TRV-5, TRV-9, TRV-17, TRV-18, TRV-23 and TRV-26, respectively), the visible approximately 211-bp amplification products are consistent with positive amplification in TRV-9, TRV-17, TRV-18, TRV-23 and TRV-26, while TRV-5 does not show the corresponding band in the supplied gel image. This result indicates that most, but not all, morphologically selected varieties carried the RM3412 marker profile detected in the assay.

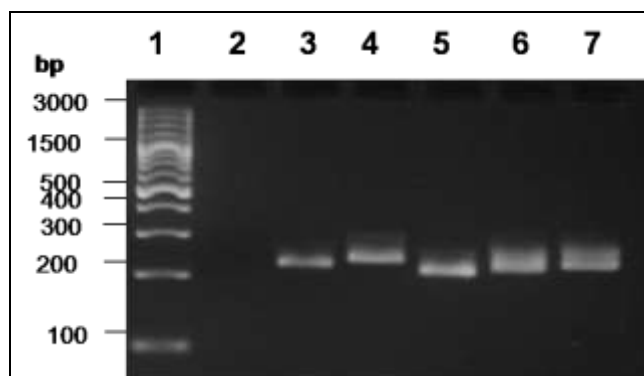


Fig 6: PCR amplification profile of the RM3412 marker in selected traditional rice varieties. Lane 1: 100-bp DNA ladder; lanes 2–7: TRV-5, TRV-9, TRV-17, TRV-18, TRV-23 and TRV-26, respectively. The expected amplification product was approximately 211 bp.

Discussion

Salinity is a major constraint to rice production, and the identification of tolerant germplasm is an important objective of rice breeding. The present screening demonstrated substantial phenotypic variation among 42 traditional rice varieties when grown under hydroponic NaCl stress. Differences in root and shoot growth, leaf development and leaf desiccation indicate that the traditional germplasm evaluated in this study contains variation that can be exploited in further salinity-tolerance studies.

Root and shoot growth are commonly used as early indicators of salinity response in rice. Salinity can restrict water uptake and cause ionic and osmotic effects, leading to reduced growth. In the present study, varieties such as TRV-17 and TRV-18 maintained comparatively better root and shoot growth, whereas TRV-6, TRV-7 and several other varieties showed stronger reductions in specific traits. Such genotype-dependent responses are consistent with previous studies demonstrating wide variation in seedling-stage salinity tolerance among rice germplasm.

The traditional varieties identified here should be considered promising or comparatively tolerant rather than definitively salt-resistant. The experiment was conducted at the seedling stage and evaluated a defined NaCl concentration under hydroponic conditions. Tolerance at this stage does not necessarily predict reproductive-stage performance, grain yield or field-level tolerance. Additional physiological and biochemical traits, including Na^+ and K^+ accumulation, Na^+/K^+ ratio, relative water content, membrane stability, chlorophyll and antioxidant responses, would strengthen the characterization of the selected varieties.

Molecular screening provided additional information about the selected germplasm. Saltol is a major QTL associated with seedling-stage salinity tolerance, and RM3412 is a marker closely linked to this region and has been used for marker-assisted foreground selection. Previous studies have shown that RM3412 can help discriminate salt-tolerant and susceptible genotypes and has been used in marker-assisted introgression of Saltol into elite rice backgrounds. In the present study, the RM3412 amplification profile was detected in five of the six morphologically selected varieties. The agreement between phenotypic screening and marker detection in most of the selected material supports the value of combining morphological and molecular approaches. However, the presence of an RM3412-linked

marker alone should not be interpreted as proof of the complete Saltol QTL or as identification of a specific causal allele. Confirmation would require additional flanking markers, haplotype analysis and/or sequence-based characterization.

The findings are therefore best interpreted as an initial characterization of traditional rice germplasm for salinity tolerance. TRV-5, TRV-9, TRV-17, TRV-18, TRV-23 and TRV-26 can be considered promising candidates for further validation, with particular interest in TRV-9, TRV-17, TRV-18, TRV-23 and TRV-26 because they also showed the RM3412 amplification profile in the supplied gel. These materials may be useful for subsequent breeding, physiological validation and marker-assisted selection studies.

Conclusion

The present study demonstrated considerable variation in salinity response among 42 traditional rice varieties evaluated under controlled hydroponic conditions. Based on the assessed morphological traits, TRV-5, TRV-9, TRV-17, TRV-18, TRV-23 and TRV-26 were identified as comparatively tolerant varieties. Molecular screening using the RM3412 marker associated with the Saltol region produced the expected approximately 211-bp product in five of the six selected varieties. These traditional rice varieties represent promising genetic resources for further investigation of seedling-stage salinity tolerance. However, additional physiological, biochemical, multi-marker and field-level validation is required before their performance can be considered suitable for breeding or cultivation under saline environments.

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Authors' contributions

The authors' contributions should be stated according to their actual roles in the study. The original manuscript reports that all authors contributed to conception/design, acquisition or analysis of data, manuscript preparation or critical revision, and approval of the final version. The authors should verify and personalize this statement before submission.

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Conflict of interest

The authors declare that they have no financial or other conflicts of interest related to this work.

Ethical approval

The study did not involve human participants or animal experimentation.

Data availability

The data generated and/or analysed during the study are available from the authors on reasonable request.

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