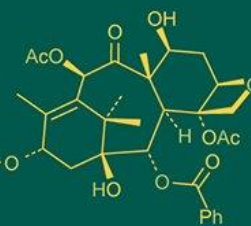
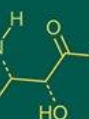
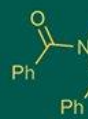
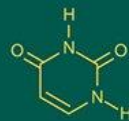
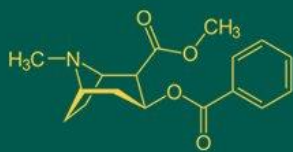


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Effect of increasing salinity levels on growth of rice seedlings in hydroponics

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Abstract

Rice yield is often reduced under saline conditions, especially during the early stages of growth. This experiment examined 100 rice (*Oryza sativa* L.) genotypes under controlled hydroponic conditions. Three replications for each of the three salinity treatments (0, 4, and 8 dS m⁻¹) were used in this factorial completely randomised design. To evaluate genetic variation, key morphological characteristics were recorded, which include germination percentage, root length, shoot length, fresh and dry weight, relative dry weight, seedling vigour index I and II, stress tolerance index and salinity injury score (SES). The statistical analysis showed that noticeable variation existed among the genotypes. Increasing salinity levels resulted in a gradual reduction in growth and vigour, and 4 dS m⁻¹ has been identified to be a key threshold level for genotypic response differentiation. Growth traits exhibited strong positive associations with each other were shown by correlation and principal component analyses, which also highlighted their role in salinity tolerance. While some genotypes revealed sensitivity even at low stress levels, others maintained consistent results despite increasing salinity, reflecting the presence of effective tolerance mechanisms. Overall, the study shows that hydroponic screening is a successful method for identifying salt-tolerant rice genotypes at the stage of seedling. The determination of threshold salinity levels and genotype variability offers an effective basis for breeding and selection programmes targeted at improving paddy's resistance to salinity.

Keywords: Rice, salinity stress, hydroponic screening, seedling vigour, genetic variability

Introduction

Rice (*Oryza sativa* L.) serves as a primary food source for more than three billion people worldwide, a major cereal crop and a primary source of nutrition. The overall number of people on world has reached about 8 billion, and current estimates indicate that 10 billion by 2050. Given that 3.5 billion people worldwide suffer from deficiencies in at least one important nutrient and that over 800 million people are estimated to be hungry, our current ways of producing food are unable to keep up with this growing population [1]. Overcoming the uncertainty of abrupt atmospheric extremes, however, is currently difficult. Growing abnormal climate changes and global warming present a variety of biotic and abiotic difficulties for crops [2].

One of the most important environmental factors endangering rice production is salinity. Salinity is the second crucial abiotic stress that drastically decreases agricultural yield [3]. Due to the high salt content, millions of hectares of suitable rice fields in Asia and Africa are not being used (IRRI, 2020). Salinity poses an existential threat to sustainable agriculture because, according to studies, one-third of all cultivable land globally is impacted by salt, and 50% of all arable land could be salinised by 2050 [4]. Salinity affected 2.1% of India's cultivated area, with 2.96 million hectares of saline soil and 3.77 million hectares of sodic soil. White alkali or saline soil contains EC>4, pH<8.5, and ESP<15; sodic soil has EC<4 dsm-1, ESP>15, and pH = 8.5 to 10 [5]. The adverse effects of salinity stress emphasise how urgent it is to produce rice genotypes that can resist salt to maintain food security, especially in areas where soil salinisation is prominent [6].

Rice plants are more susceptible to salt stress during the seedling and reproductive stages, which can have several damaging effects that significantly reduce production and quality [4]. A trusted strategy for determining how genotypes respond to salt stress is hydroponic cultivation.

Several crops, including rice, have been utilised in hydroponic culture experiments to evaluate how various plant species respond to salinity stress [7]. Yoshida solution served as a nutrient solution for all of these experiments. The macronutrients and micronutrients necessary for healthy crop development are provided in Yoshida solution, which is frequently used to cultivate rice plants in hydroponic culture [8]. By disrupting the physiological mechanism of the seed, salt stress causes an adverse effect on seed germination. It diminishes the growing seeds water potential, food reserve, and protein content [9]. Rice responds and adapts to salt stress in an extremely complex manner. Root growth inhibition, leaf rolling, lowered plant height and tiller number, and spikelet sterility are all consequences of salt stress that eventually result in a lower yield [10]. Raising salinity reduces fresh weight, dry weight, shoot length, root length, and seed germination, according to multiple research [11]. Hence this study aim to understand how the root length, shoot length and morphological parameters reduce in different levels of salinity.

Materials and Methods:

Rice genotypes included 100 varieties from different states of India, including Pokkali (a landrace) as a tolerant check and IR 20 as a sensitive check variety. This research study was conducted in a laboratory at Karunya Institute of Technology and Sciences in 2025. Two distinct treatments and one control were used in the trial. The different concentrations are 0, 4 and 8 EC's. The standard protocol of salinity screening by Yoshida followed the experiment [12] and IRRRI score. We used the trays that have the capacity of holding water up to 5 litres. The Styrofoam sheets were cut according to the depending upon the of the trays. The trays were filled with Yoshida nutrient medium and distilled water. A Styrofoam (polystyrene) sheet was prepared with 100 evenly spaced holes and stitched with mesh below the holes to hold the seeds. The nutrient solution was prepared as a stock as the solution should be changed once in 5 days and the pH and EC's should be maintained perfectly. The PH was adjusted to 5.1 by pH meter using 1N HCL and 1N NaOH when necessary. The standard Yoshida medium. Ammonium nitrate (91.4 grams per litre), potassium sulphate (97.8 grams per litre), potassium dihydrogen phosphate (29 grams per litre), dipotassium phosphate (8 grams per litre), calcium chloride hexahydrate (175 grams per litre), and magnesium sulphate heptahydrate (324 grams per litre) were among the macronutrients. The micronutrients included 1.5 grams of manganese chloride tetrahydrate, 0.074 grams of ammonium molybdate tetrahydrate, 0.93 grams of boric acid, 0.035 grams of zinc sulphate heptahydrate, 0.03 grams of copper sulphate pentahydrate, 10.5 grams of ferric sodium EDTA, and 2.5 grams of ferrous sulphate [13]. The exact amount of chemicals was prepared as a stock. The observed parameters are root length, shoot length, Root and shoot fresh weight, Root and shoot dry weight, Germination percentage, Seedling vigour index, and salinity tolerant

index,

Traits observed

Germination percentage: For each replication and for each treatment, 3 seeds were sown, and so far germination percentage has been taken.

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Shoot length: Shoot length was measured by centimeter scale, which was used for all the replications and the control also.

Root length: Root length was measured using a cm scale, which was used in all the replications and the control also.

Fresh weight: The fresh weight of the seedling was measured using a weighing balance

Dry weight: Dry weight of a seedling was measured by a weighing balance; the seedlings were placed in a hot air oven for 24 hours at 60 degree Celsius.

$$\text{Relative dry weight} = \frac{\text{Dry weight of salinized treatment of the species}}{\text{Dry weight of control treatment value of that species}} \times 100$$

Seedling vigour index (SVI) (%)

SVI-I = Germination (%) × Seedling length (cm)

SVI-II = Germination (%) × Seedling dry weight (mg)

Salt injury score

By visual means, we should give a score to each genotype and all the replications.

Stress tolerance index used to check the tolerance level of rice seedlings: $STI = Y_p \times Y_s / Y_{p2}$

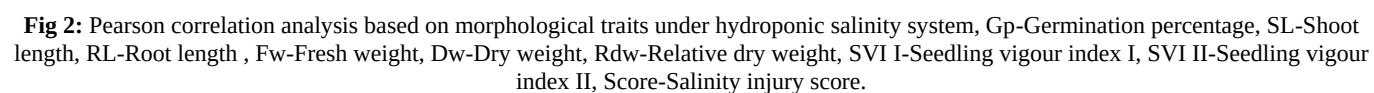
Y_p = Average seedling dry weight of a non-stressed condition.

Y_s = Average seedling dry weight under stressed conditions.

Y_{p2} = Average seedling dry weight of all genotypes at non stressed condition [14].

Statistical analysis

The experiment was carried out in factorial completely randomised block design (FCRD) with the genotypes of 100 and two levels, the experiment was replicated thrice. The data on morphological traits were observed with SES score. The statistical analysis for ANOVA and cluster was done in STAR software, and the PCA and correlation was done in R software. Based on how similar the genotypes were under 4 and 8 EC's circumstances, the dendrogram divided them into multiple clusters. While genotypes in separate clusters performed differently, genotypes within the same cluster performed similarly. While smaller clusters reflected distinct types, larger clusters indicated shared responses across diverse genotypes. The genotypes with varied genetic backgrounds are highlighted by this grouping.



Under 4 and 8 EC conditions, the correlation analysis of the traits under study identified a number of significant connections. Strong and significant correlations were found between seedling length (SL) and root length (RL), fresh weight (FW), relative dry weight (RDW), and seedling vigour index (SVI I and SVI II). This suggests that genotypes with superior growth performance also demonstrated improved biomass accumulation and resistance. Similar to this, root length (RL) demonstrated a strong positive association with salinity and RDW indices, indicating the significance of root characteristics in

determining overall performance under stress. The majority of the growth and salinity-related characteristics are correlated positively with fresh weight (FW) and dry weight (DW), indicating their contribution to the overall vigour of seedlings. Significantly, there was a strong positive correlation between dry weight (DW) and the stress tolerance index (STI), suggesting that genotypes with higher biomass under stress are likely to be more tolerant. The significance of relative dry weight (RDW) as a reliable indicator of salt tolerance was demonstrated by its significant positive correlation with both SVI I and SVI II.

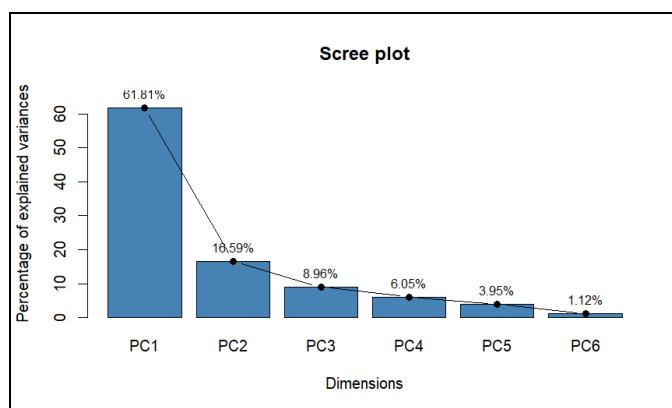


Fig 3: Principal component analysis between morphological characters of salinity under the hydroponic screening system.

Principal component analysis demonstrated that the first component (PC1) contributed significantly to the interpretation of the observed variability, explaining 61.81% of the overall variation among the genotypes. 16.59% of the variation was explained by the second principal component (PC2), followed by PC3, PC4, PC5, and PC6, which explained 8.96%, 6.05%, 3.95%, and 1.12%, respectively. The first two components combined contribution was

78.40%, indicating that a significant amount of the overall variability is successfully contained within these dimensions. The first two principal components are sufficient to represent the diversity among the genotypes, but the following components contribute minimally to the overall variation, as evidenced by the significant decrease in the percentage of variance from PC1 to the subsequent components.

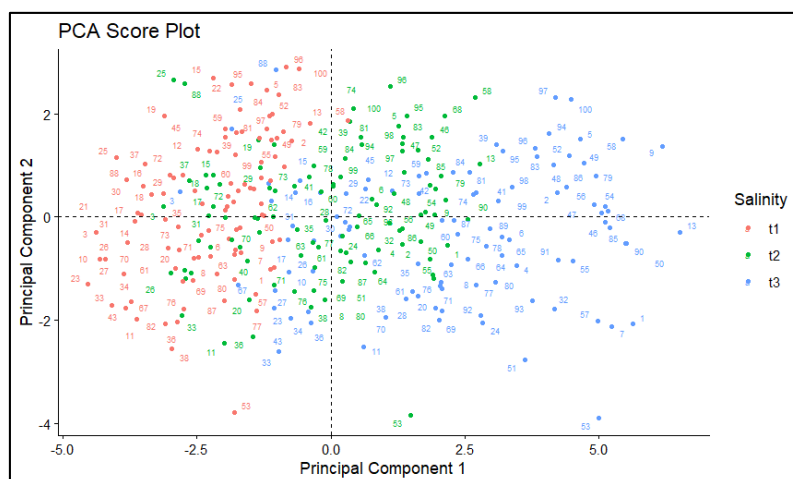


Fig 4: Score plot of principal component analysis among 100 rice genotypes based on morphological characteristics under different treatments.

Genotypes appeared to be distinct according to salt levels in the PCA score plot. The majority of genotypes under t1 (control) were located on the negative side of PC1, whereas t2 occupied an intermediate position and t3 (high salinity) mostly appeared on the positive side.

Variable tolerance among genotypes is highlighted by the broader distribution of genotypes under t2 and t3, which indicates more diversity in response to salinity. While genotypes on the negative side show sensitivity, a few

genotypes located further on the positive side under t3 regarded as salt-tolerant. All things considered, the PCA verifies that salinity has a major impact on trait variation and helps in the identification of genotypes that are resistant. Adequate expression of genotypic variation at the seedling stage proved attainable by maintaining uniform salinity conditions throughout the experiment. Under saline conditions, significant differences were observed among genotypes in different traits. The continuous variations in

response demonstrate that the screening conditions were successful in distinguishing genotypes according to their salinity tolerance.

Results and Discussion

Under hydroponic salinity conditions, rice genotypes performed in a way that clearly distinguished between moderate and severe stress levels. As salinity increased from 4 dS m⁻¹ to 8 dS m⁻¹, there was a noticeable decrease in growth and vigour. Based on the genotypes' overall performance, a threshold level of 4 dS m⁻¹ was identified, at which most genotypes indicated a significant decrease in growth. This suggests that only a few genotypes survive higher stress levels, whereas several genotypes can withstand low salinity. The only two genotypes that remained to grow stably even at 8 dS m⁻¹ were Pokkali and Ezhome-4. Strong tolerance mechanisms that enable them to maintain normal growth without significantly lowering seedling vigour are suggested by their uniform performance under high salinity. A comparatively larger number of genotypes indicated increased adaptation at the threshold level of 4 dS m⁻¹. Genotypes like Ezhome-1, Ezhome-2, Ezhome-3, Kuzhiyadichan, Kullakar, and TRY-3 showed good growth and endured higher vigor, along with Pokkali and Ezhome-4. Genotypes listed above are limited to moderate salinity, however, as demonstrated by their inability to maintain equivalent performance at 8 dS m⁻¹. As a result, these genotypes can be categorized as moderately tolerant. The significance to identify a threshold level in salinity screening is shown by the evident decrease in the

number of performing genotypes above 4 dS m⁻¹. It helps in distinguishing genotypes that show actual tolerance from those that only survive in less stressful conditions. The threshold level successfully differentiated stable performers from genotypes that were susceptible and moderately tolerant in this study. IR 20, TPS-3, and TPS-5, on the other hand, performed weakly even at 4 dS m⁻¹ and were negatively impacted at greater salt levels. These genotypes showed a significant decrease in seedling vigour and growth, indicating a high exposure to salinity stress. The ability of genotypes demonstrating tolerance to maintain root and shoot growth under stressful conditions was another important finding. Better water uptake and internal stability are likely promoted by this balanced growth, which makes it easier for the plant to withstand salinity stress. This classification pattern was also confirmed by the genotypes performance-based grouping. Significant difference based on salt response was demonstrated by the grouping of tolerant genotypes together and the development of separate clusters for susceptible genotypes. Overall, salt tolerance at the seedling stage was successfully determined through the hydroponic screening system. The determination of 4 dS m⁻¹ as a threshold level provides a useful standard for future screening studies. According to the findings, Ezhome-1 to Ezhome-3, Kuzhiyadichan, Kullakar, and TRY-3 were found to be moderately resistant under the threshold level, while Pokkali and Ezhome-4 were found to be highly tolerant under severe salinity. The susceptible response from TPS-3, TPS-5, and IR 20 confirms the screening method's predictability.

Table 1: Modified standard evaluation scoring protocol of IRRI:

Score	Observation	Tolerance
1	Normal growth, no leaf symptoms	Highly tolerant
3	Nearly normal growth, but the leaf tips of a few leaves are whitish and rolled	Tolerant
5	Growth severely retarded, most leaves rolled, only a few are elongating	Moderately tolerant
7	Complete cessation of growth, most leaves are dry, some plants are dying	Moderately susceptible
9	Almost all plants dead or dying	Susceptible

Table 2: ANOVA table for different traits, genotypes and different levels of salinity:

Trait	Mean	Range	CV (%)	SE (Genotype)	SE (Salinity)	SE (G×S)
Germination % (GP)	77.67***	0–100	17.08**	3.83	1.08	10.83
Shoot Length (SL)	23.77***	8.80–33.30	0.68**	0.046	0.013	0.131
Root Length (RL)	8.54***	3.90–12.10	1.06**	0.026	0.007	0.074
Fresh Weight (FW)	0.1609***	0.137–0.182	1.77**	0.0008	0.0002	0.0023
Dry Weight (DW)	0.0380***	0.033–0.043	1.82**	0.0002	0.0001	0.0006
Relative Dry Weight (RDW)	73.94***	31.97–99.60	1.12**	0.240	0.067	0.679
SVI-I	19.75***	4.15–33.20	1.73**	0.098	0.028	0.279
SVI-II	26.81***	6.04–44.30	1.70**	0.131	0.037	0.372
Score	4.35***	1–9	0.81**	0.010	0.002	0.028
STI	0.9787***	0.786–1.22	2.49**	0.007	0.002	0.019

** Significance at 5%, *** Significance at 1%

Conclusion

The research demonstrates that assessing rice genotypes in well-maintained hydroponic settings can give an accurate representation of how various genotypes react to rising salinity stress. Salinity tolerance is a complex trait that cannot be assessed using one example or parameter, as demonstrated by the variation in response among genotypes. Identifying an important stress level improves the precision of choosing potential genotypes and aids in the improvement of screening techniques. The results of this study indicate that rather than depending on a single trait

and a single level of tolerance, future breeding efforts should focus on combining various adaptive traits. The observed genotype diversity provides an opportunity to develop superior cultivars that are more suited to saline environments. Overall, the methodology used in this study can be a useful structure for early screening and selection in breeding programmes.

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