



Estimates of Genetic Variability and Interplay of Germination and Seedling Traits Conferring Salinity Tolerance in Rice (*Oryza sativa* L.)

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ABSTRACT

This study estimates genetic variability and correlations among germination and seedling traits conferring salinity tolerance in rice accessions. Five rice accessions were screened under salinity levels of 0, 50, 100, and 200 mM NaCl in a controlled laboratory setting. Traits such as germination energy, capacity, shoot and root length, and biomass were measured. Data were analyzed for variance and correlations to assess variability and trait relationships. Significant genetic variability was found among accessions for all traits. Germination energy showed the highest coefficient of variation (CV) at 22.29% under control conditions, while fresh shoot weight had the highest CV (34.35%) under 200 mM salinity. Accessions ACC2 and ACC5 consistently demonstrated higher performance in germination energy (23.33 to 53.33% and 10.00 to 41.67%), germination capacity (40.00 to 60.00% and 28.33 to 46.67%), and shoot length (0.67 to 2.97 cm and 0.40 to 3.93 cm) under various salinity stress levels. ACC1, ACC3, and ACC4 showed more variability but maintained some consistency in specific traits, with ACC4 generally showing lower performance across most traits. Genetic parameter estimates indicated high heritability (>60%) for all traits, with the highest in germination capacity (96.88%). High genetic advance (GAM) was observed for all traits (>20%), with germination energy showing the highest (107.00%). Traits with high heritability and genetic advance, such as germination energy, germination capacity, and root length, suggest strong genetic control and potential for improvement through selective breeding. Significant correlations were found between germination energy and capacity ($r= 0.89$ to 0.96) and between shoot length and leaf length ($r= 0.92$) under stress conditions. Stress tolerance indices identified accessions ACC2 and ACC5 as the most tolerant, with ACC1 showing consistent performance across traits. This study underscores the importance of identifying resilient traits and accessions to enhance salinity tolerance in rice, contributing to improved productivity in saline-affected regions.

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Introduction

Rice (*Oryza sativa* L.) is the preeminent cereal crop globally, playing a vital role in Nigeria's food landscape and presenting a potential catalyst for economic growth in the current challenging circumstances [1]. As the primary sustenance for over half of the world's population, rice is a cornerstone of global nutrition and the most crucial natural energy source worldwide [2]. Rice is an excellent food source rich in vitamins, fiber, and essential micronutrients such as vitamins, minerals, and secondary metabolites [3 – 5]. The dietary minerals in rice include calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, manganese, and selenium [6 – 9]. These minerals are crucial for maintaining human health, and their deficiencies can lead to various pathological conditions [7, 10]. Beyond its culinary significance, rice plays a critical role in multiple industries. It is a vital source of starch, alcoholic beverages such as rice wine, and confectionary flour [7]. Additionally, rice is integral to the pharmaceutical sector for producing phytin and vitamin B, and its oil is extensively utilized in manufacturing soap and candles [11, 12]. Consequently, rice is established as a superior choice for a nutritious and cost-effective energy source [13].

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Rice cultivation has a longstanding history in Nigeria, primarily led by smallholder farmers employing rudimentary technology and traditional methods, contributing to over 80% of the national production [14]. The adaptability of rice to diverse ecological conditions across the country, including rain-fed lowlands, irrigated lowlands, and upland areas, further enhances its widespread growth [15]. As of 2009, Nigeria ranked 12th globally in rice consumption, securing the 17th position in global production, the third in Africa, and the foremost in West Africa [16]. Notably, by 2021, Nigeria emerged as Africa's leading rice producer, with a production volume of approximately 8.3 million metric tons, surpassing Egypt and Madagascar, which produced around 4.8 million and 4.4 million metric tons of rice, respectively [17]. Despite the abundant potential of rice in Nigeria, challenges persist in meeting current demand and accommodating a growing population [13]. Various constraints, encompassing climatic, economic, and environmental factors, hinder rice production. Notably, soil salinity stands out as a significant limitation, adversely affecting the survival, biomass production, and yield of rice—an issue posing a substantial threat to global food security [18].

Soil salinity triggers complex morphological and physiological stress in plants, leading to ion imbalance, oxidative stress, and reduced metabolic activity, ultimately decreasing crop productivity [19, 20]. It affects germination, growth, and overall plant vigor [19, 21]. Research shows that over 1000 million hectares of land could become saline, with 25–30% of irrigated areas affected, and about 50% of the global population already living in regions with severe salinity [22 – 24]. Rising salinity due to climate change, sea level rise, and poor irrigation practices threatens to reduce agricultural lands by 50% by 2050 [23, 25].

Global efforts have focused on understanding salinity tolerance and developing salt-tolerant rice cultivars, but the complexity of plant responses to salinity, including osmotic, ionic, and oxidative stress, makes this challenging [26]. Given significant production losses in rice due to saline soils and climate change, cultivating salt-tolerant upland rice in Nigeria is crucial [27]. Breeding programs rely on genetic diversity and germplasm response to salt stress, highlighting the need to evaluate genetic variability in rice [28]. Although several salt-tolerant rice lines exist globally [13, 29–33], many Nigerian varieties remain unexplored. Identifying salinity-tolerant traits is essential for improving rice yields under salt-stress conditions.

The germination phase is one of the most affected by salinity in many crops, with traits like germination percentage, root and shoot length, and dry weight negatively impacted by high salinity levels. A plant's growth and development under salinity stress depend largely on its tolerance at the germination stage [34]. Therefore, assessing variability in salinity tolerance traits during germination is crucial for rice breeding. Screening at this stage is advantageous as it is quicker and ensures uniform salt distribution, offering valuable data for salinity tolerance in crops [21].

The objectives of this study were to rigorously estimate the extent of genetic variability and elucidate the correlations among germination and seedling traits that confer salinity tolerance in selected rice accessions. These accessions were systematically screened under varying levels of salinity stress in a controlled laboratory environment.

Materials and Methods

Plant materials

Five rice accessions were obtained from the National Cereals Research Institute (NCRI) in Badegi, Nigeria, and all these accessions were affiliated with the forest transition/derived savanna Agroecology. The specific details of each genotype are provided in Table 1.

Table 1 List of rice accessions and their origins

S/N	Accession	Origin	Code
1	FARO26	NCRI, Nigeria	ACC1
2	FARO16	NCRI, Nigeria	ACC2
3	FARO21	Philippines	ACC3
4	FARO61	Africa Rice, Nigeria	ACC4
5	FARO15	NCRI, Nigeria	ACC5

Experimental procedure

In February 2023, the study was carried out at the Plant Science and Biotechnology Laboratory, Adekunle Ajasin University, Nigeria, located at a latitude of 7.20°N, longitude of 5.44°E, and an altitude of 423 meters above sea level. The experimental design involved a factorial experiment with a 5 × 4 treatment combination, utilizing a completely randomized design (CRD). Uniform-sized seeds were surface sterilized in a 10% (v/v) sodium hypochlorite (NaClO) solution for 30 minutes, followed by three rinses in distilled water. Seeds of

each accession were exposed to sodium chloride solutions at concentrations of 0 (pH: 7.0), 50 (pH: 7.22), 100 (pH: 7.38), and 200 mM (pH: 7.51) in three replicates. Sterile plastic containers lined with two filter papers each and soaked with 5 ml of NaCl solution were used for seed incubation in the dark for 48 hours, followed by exposure to a photoperiod of 12 hr./12 hr. (day/night) at room temperature for three weeks.

Data Collection

During data collection, germination energy, representing the percentage of germinated seeds on day four after sowing, was determined by dividing the number of germinated seeds by the total planted and multiplying by 100. Similarly, germination capacity, calculated on day ten, followed the same formula. Other measurements included shoot length, assessed from the base of the plant to the first leaf at week three, and the length of the main root per shoot, measured from the base to the tip of the taproot at the same period. Leaf length, number of roots, and leaves per shoot were also recorded in week three. Furthermore, fresh shoot weight was determined by separating seedlings into shoots and roots and recording the shoot weight, while fresh root weight was measured by weighing the separated fresh roots. Dry shoot and root weights were obtained after oven-drying plant shoots and roots for 48 hours at 60°C.

Data analysis

The data underwent analysis of variance (ANOVA) using SPSS version 21, and mean values were separated utilizing Duncan's multiple range test (DMRT) at a significance level of $P \leq 0.05$. Salinity tolerance indices for all traits were determined using the stress tolerance index (STI) as per Ajayi [35] as follows: $STI = \frac{C \cdot S}{(GM)^2}$, where C, S, and GM represent the mean values for an accession under control, mean value under stress, and grand mean over all accessions under control, respectively. Accessions were ranked into different classes of salinity tolerance ranging from 1 (most tolerant) to 5 (least tolerant) for each trait. Genetic parameter estimates were conducted following the methodology outlined by Ajayi *et al.* [36]. Pearson's correlation analysis was conducted using SPSS, and significance levels were compared using Pearson's correlation Table at $P \leq 0.05$ and $P \leq 0.01$.

Results

ANOVA results indicated significant differences among accessions for all traits across treatments (Table 2), demonstrating ample genetic variability in rice accessions under differential salinity stress. In the control treatment, germination energy showed the highest coefficient of variation (CV) at 22.29%, followed by the number of leaves at 17.79%, with the lowest in dry root weight at 1.05%. In the 50 mM treatment, the number of roots per shoot exhibited the highest CV (33.83%), while root length had the lowest (4.31%) (Table 2). Similarly, the 100 mM treatment showed the highest CV in the number of roots (32.07%) and germination capacity (28.2%), while fresh shoot weight had the lowest (1.09%). However, in the 200 mM treatment, fresh shoot weight had the highest CV (34.35%), while the number of leaves had the lowest (1.922%). Across all factors, accession, treatment, and their interaction, significant effects were observed for all traits, with the highest CV in the number of roots (7.21%) and the lowest in fresh root weight (0.44%).

Table 3 displays mean trait values under control and salinity stress (50, 100, 200 mM). In the control, ACC1 and ACC2 had the highest germination energy (28.33%), while ACC4 had the lowest (6.67%). Across stress levels, ACC2 consistently showed the highest germination capacity (40% to 51.67%), while ACC4 had the lowest (11.67% to 5%). Shoot length's highest mean was 3.73 cm in ACC1 under control and varied from 1.01 cm (ACC1, 200 mM) to 3.93 cm (ACC5, 50 mM) under stress. ACC1 and ACC5 had the highest mean root lengths under control and 50 mM, while ACC4 had the lowest. The number of leaves varied, with ACC1 having the highest under control and ACC4 the lowest across stress levels. Leaf lengths peaked at 8.13 cm (ACC5, control) and dropped to 0.13 cm (ACC5, 200 mM). ACC5 consistently had the highest root lengths, peaking at 10.93 cm (control) and 7.93 cm (50 mM). Fresh root weights ranged from 0.005 g (ACC1, control) to 0.001 g (ACC4, 200 mM). ACC1 consistently had the highest dry root weights, while ACC4 had the lowest across conditions. Dry shoot weights ranged from 0.05 g (ACC5, control) to 0.001 g (ACC4, stress conditions). Table 4 summarizes the stress tolerance indices (STI) and accession ranks based on germination traits of rice accessions. ACC2 excelled under 50 mM stress, with high values in germination energy (2.49) and germination capacity (2.70), while ACC5 performed well in root length (1.53) and shoot length (1.37). Under 100 mM stress, ACC2 maintained strength, while ACC5 exhibited resilience, particularly in germination energy (2.51) and number of roots (1.46). ACC1 consistently performed well, especially in dry root weight (1.16) and germination capacity (1.31). Under 200 mM stress, ACC2 showed resilience, ACC5 displayed adaptability, and ACC1 exhibited moderate performance. Overall, ACC2 and ACC5 demonstrated strong tolerance across stress levels, while ACC1 showed consistent performance, indicating potential resilience to salinity stress in rice accessions.

Table 2 Mean square values of germination and seedling traits of rice accessions screened under differential salinity stress

Source of variation	DF	GE (%)	GC (%)	SL (cm)	NL	NR	LL (cm)	RL (cm)	FRW (g)	FSW (g)	DRW (g)	DSW (g)
Combin e												
Accession (A)	4	1892.8 2*	2269.7 92*	2.067 *	0.60 8*	28.97 5*	9.856 *	9.585*	0.00075 *	0.00029 *	0.000051 *	0.000014*
Treatment (T)	3	1293.2 61*	684.86 *	23.26 7*	3.88 3*	52.15 6*	87.45 6*	100.75 2*	0.004*	0.003*	0.000098 *	0.000061*
A × T	12	206.02 9*	72.014 *	0.525 *	0.23 1*	8.475 *	3.155 *	5.753*	0.00039 *	0.00011 *	0.000018 *	0.00000034*
Error	40	37.817	36.667	0.082	0.2	2.267	0.385	0.505	0	0	0	0.00000019
CV (%)		6.767	4.98	2.889	2.918	7.213	3.825	4.165	0.663	0.439	1.719	
Control												
Accession	4	237.50 0*	377.5*	0.924 *	0.43 3*	10.93 3*	6.152 *	14.659 *	0.00018 *	0.00024 *	0.000092 1*	0.0000041 *
Error	10	23.333	28.333	0.014	0.2	0.6	0.126	0.509	0.000023	0.000029	0.00000071	0.0000006
CV (%)		22.29	9.24	5.68	17.79	11.9	6.13	9.48	12	13.5	1.05	1.54
50 Mm												
Accession	4	526.66 7*	735.00 0*	1.158 *	0.10 0*	22.10 0*	4.599	3.908*	0.00137	0.00017 *	0.000009 *	0.00000054*
Error	10	41.667	45	0.061	0.133	4.4	0.979	0.081	0.00026	0.00001018	0.00000079	0.00000012
CV (%)		21.28	20.33	7.6	17.2	33.83	18.83	4.31	4.48	10.64	4.68	5.77
100 mM												
Accession	4	1296.7 67*	800.00 *	1.346 *	0.26 7*	16.43 3	8.154 *	3.575*	0.0000296*	0.000124*	0.00000024*	0.000014*
Error	10	66.267	38.333	0.161	0.133	3.467	0.454	1.103	0.0000368	0.0000162	0.00000017	0.000000055
CV (%)		28.2	16.36	14.23	16.36	32.07	1.5	24	20.33	1.09	10.31	12.36
200 mM												
Accession	4	450.00 *	573.33 8	0.214 *	0.50 *	4.933 *	0.414 *	4.707*	0.0000102*	0.0000686*	0.00000023*	0.000000027*
Error	10	20	35	0.096	0.33	0.6	0.011	0.327	0.0000205	0.0000118	0.000000027	0.000000027
CV (%)		44.7	26.11	48	43	31.17	1.923	33.73	64.29	34.35	26	26

*: Significant at $P \leq 0.05$. DF: Degree of freedom; CV: Coefficient of variation

GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.

Table 3 Mean performance of germination and seedling traits of rice accessions screened under differential salinity stress

Accession/Treatment	GE (%)	GC (%)	SL (cm)	NL	NR	LL (cm)	RL (cm)	FRW (g)	FSW (g)	DRW (g)	DSW (g)
Control											
ACC1	28.33±3.33 ^b	30.00±2.89 ^{bc}	3.73±0.06 ^c	3.00±0.10 ^b	9.00±0.58 ^d	5.90±0.23 ^b	6.30±0.57 ^{ab}	0.04±0.02 ^{bc}	0.50±0.006 ^c	0.02±0.006 ^c	0.006±0.006 ^b
ACC2	23.33±1.67 ^b	40.00±2.89 ^c	2.53±0.03 ^a	2.33±0.33 ^{ab}	7.33±0.33 ^c	4.70±0.20 ^a	7.37±0.22 ^{bc}	0.036±0.002 ^{ab}	0.04±0.003 ^{ab}	0.01±0.00 ^b	0.006±0.00 ^b
ACC3	21.67±4.41 ^b	21.67±4.41 ^b	3.53±0.06 ^c	2.00±0.001 ^a	6.67±0.33 ^{bc}	5.67±0.23 ^b	7.97±0.62 ^c	0.03±0.003 ^a	0.04±0.00 ^b	0.005±0.001 ^a	0.004±0.00 ^a

ACC4	6.67±1.67 ^a	11.67±1.67 ^a	2.80±0.10 ^b	2.67±0.33 ^{ab}	4.00±0.58 ^a	4.47±0.06 ^a	5.03±0.29 ^a	0.05±0.01 ^c	0.03±0.002 ^a	0.004±0.001 ^a	0.004±0.00 ^a
ACC5	28.33±1.67 ^b	35.00±2.89 ^c	3.70±0.05 ^c	2.67±0.33 ^{ab}	5.33±0.33 ^{ab}	8.13±0.24 ^c	10.93±0.12 ^d	0.05±0.05 ^c	0.05±0.001 ^c	0.004±0.003 ^a	0.05±0.00 ^{ab}
Grand mean	21.67±2.37	27.67±2.92	3.26±0.13	2.53±0.13	6.47±0.49	5.79±0.24	7.52±0.55	0.04±0.02	0.04±0.0025	0.08±0.0015	0.05±0.0003
50 mM											
ACC1	21.67±4.41 ^a	20.00±5.00 ^{ab}	3.13±0.13 ^b	2.33±0.33 ^a	5.33±0.88 ^a	5.47±0.74 ^{abc}	5.07±0.07 ^a	0.04±0.07 ^a	0.03±0.009 ^{ab}	0.01±0.001 ^b	0.007±0.001 ^b
ACC2	50.00±2.89 ^c	51.67±4.41 ^c	2.70±0.06 ^{ab}	2.33±0.33 ^a	5.33±1.33 ^a	4.07±0.39 ^a	6.50±0.06 ^b	0.02±0.11 ^a	0.02±0.004 ^a	0.01±0.000 ^b	0.007±0.001 ^b
ACC3	26.67±3.33 ^{ab}	30.00±2.89 ^b	3.87±0.20 ^c	2.00±0.00 ^a	7.00±1.15 ^{ab}	6.03±0.58 ^{bc}	6.03±0.22 ^b	0.04±0.02 ^a	0.03±0.001 ^{ab}	0.001±0.001 ^a	0.004±0.00 ^a
ACC4	16.67±3.33 ^a	16.67±3.33 ^a	2.63±0.19 ^a	2.00±0.00 ^a	3.00±0.58 ^a	4.27±0.73 ^{ab}	7.47±0.27 ^c	0.02±0.02 ^a	0.03±0.005 ^{ab}	0.003±0.000 ^a	0.004±0.001 ^b
ACC5	36.67±4.41 ^b	46.67±3.33 ^c	3.93±0.07 ^c	2.00±0.00 ^a	10.33±1.76 ^b	7.03±0.24 ^c	7.93±0.07 ^c	0.07±0.16 ^b	0.04±0.006 ^b	0.01±0.000 ^b	0.005±0.001 ^a
Grand mean	30.33±3.47	33.00±4.02	3.25±0.16	2.13±0.91	6.20±0.26	5.37±0.37	6.60±0.28	0.36±0.01	0.03±0.003	0.006±0.0003	0.006±0.00

Accession/ Treatment	GE (%)	GC (%)	SL (cm)	NL	NR	LL (cm)	RL (cm)	FRW (g)	FSW (g)	DRW (g)	DSW (g)
100 mM											
ACC1	17.67±4.33 ^b	33.33±1.67 ^b	2.37±0.26 ^{ab}	2.33±0.33 ^a	6.33±1.20 ^b	3.07±0.28 ^a	2.80±0.58 ^a	0.026±0.004 ^{ab}	0.037±0.002 ^{bc}	0.004±0.000 ^a	0.008±0.001 ^{ab}
ACC2	53.33±4.41 ^d	60.00±2.89 ^c	2.97±0.12 ^{bc}	2.00±0.00 ^a	8.33±1.20 ^b	4.87±0.17 ^b	5.10±0.70 ^b	0.037±0.002 ^{bc}	0.03±0.003 ^{ab}	0.005±0.001 ^a	0.006±0.002 ^{ab}
ACC3	31.67±7.26 ^{bc}	40.00±5.77 ^b	3.63±0.29 ^c	2.00±0.00 ^a	5.67±0.88 ^b	6.27±0.37 ^c	3.83±0.75 ^{ab}	0.034±0.002 ^{bc}	0.044±0.002 ^c	0.004±0.001 ^a	0.004±0.002 ^{ab}
ACC4	0.00±0.00 ^a	15.00±0.00 ^a	1.93±0.27 ^a	2.00±0.00 ^a	2.00±0.00 ^a	2.60±0.26 ^a	5.40±0.29 ^b	0.015±0.003 ^a	0.028±0.001 ^a	0.003±0.000 ^a	0.0037±0.002 ^a
ACC5	41.67±4.41 ^{cd}	43.33±4.41 ^b	3.17±0.17 ^c	2.67±0.33 ^a	6.67±1.45 ^b	5.90±0.64 ^{bc}	5.03±0.60 ^b	0.039±0.005 ^c	0.042±0.003 ^c	0.003±0.001 ^a	0.0083±0.001 ^b
Grand mean	28.87±5.28	38.33±4.13	2.81±0.18	2.20±0.11	5.80±0.69	4.54±0.42	4.43±0.35	0.03±0.003	0.37±0.02	0.004±0.000	0.006±0.001
200 mM											
ACC1	0.00±0.00 ^a	15.00±0.00 ^{ab}	1.03±0.37 ^b	2.00±0.58 ^a	2.33±0.67 ^{ab}	1.13±0.09 ^c	3.37±0.24 ^d	0.016±0.001 ^b	0.017±0.001 ^c	0.003±0.001 ^c	0.001±0.00 ^a
ACC2	30.00±5.77 ^c	41.67±6.01 ^d	0.67±0.13 ^{ab}	1.33±0.33 ^a	4.33±0.33 ^c	0.47±0.09 ^b	1.43±0.26 ^{bc}	0.007±0.003 ^a	0.013±0.003 ^{bc}	0.002±0.000 ^b	0.002±0.00 ^a
ACC3	10.00±0.00 ^b	23.33±1.67 ^{bc}	0.77±0.88 ^{ab}	1.33±0.33 ^a	3.00±0.00 ^{bc}	0.37±0.03 ^b	1.13±0.18 ^{ab}	0.004±0.003 ^a	0.006±0.003 ^a	0.0023±0.000 ^{bc}	0.002±0.00 ^a
ACC4	0.00±0.00 ^a	5.00±0.00 ^a	0.40±0.00 ^a	1.00±0.00 ^a	1.00±0.00 ^a	0.50±0.00 ^b	0.10±0.00 ^a	0.001±0.00 ^a	0.011±0.00 ^{ab}	0.001±0.00 ^a	0.001±0.00 ^a
ACC5	10.00±0.00 ^b	28.33±4.41 ^b	0.40±0.06 ^a	1.00±0.00 ^a	1.67±0.67 ^{ab}	0.13±0.09 ^a	2.43±0.62 ^{cd}	0.008±0.004 ^{ab}	0.007±0.002 ^{ab}	0.001±0.00 ^a	0.001±0.00 ^a
Grand mean	10.00±3.09	22.67±3.55	0.65±0.09	1.33±0.16	2.47±0.35	0.52±0.09	1.69±0.32	0.007±0.0017	0.01±0.001	0.002±0.000	0.002±0.000

Mean values followed by the same superscript within a column are not significantly different from one at $P \leq 0.05$ significance level using DMRT. GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.

Table 4 Stress tolerance indices (STI) and (accession rank) based on germination and seedling traits of rice accessions screened under differential salinity stress

Accession/Treatment	GE	GC	NL	SL	NR	RL	LL	FSW	FRW	DRW	DSW
50 mM											
ACC1	1.31 (3)	0.78 (4)	1.09 (1)	1.10 (3)	1.15 (2)	0.56 (4)	0.96 (3)	1.00 (2)	0.86 (2)	0.81 (1)	0.35 (2)
ACC2	2.49 (1)	2.70 (1)	0.85 (2)	0.64 (4)	0.94 (4)	0.85 (2)	0.57 (5)	0.45 (5)	0.42 (5)	0.32 (2)	0.43 (1)
ACC3	1.23 (4)	0.85 (3)	0.62 (4)	1.29 (2)	1.12 (3)	0.85 (2)	1.02 (1)	0.73 (3)	0.69 (3)	0.19 (3)	0.32 (3)
ACC4	0.24 (5)	0.25 (5)	0.83 (3)	0.69 (5)	0.29 (5)	0.66 (3)	0.58 (4)	0.47 (4)	0.47 (4)	0.06 (4)	0.21 (5)
ACC5	2.21 (2)	2.13 (2)	0.83 (3)	1.37 (1)	1.32 (1)	1.53 (1)	1.70 (2)	1.23 (1)	1.93 (1)	0.06 (4)	0.27 (4)
Grand mean	1.50	1.34	0.84	1.02	0.96	0.89	0.97	0.77	0.87	0.29	0.32
100 mM											
ACC1	1.07 (4)	1.31 (3)	1.09 (2)	0.83 (5)	1.36 (2)	0.31 (5)	0.54 (4)	1.15 (2)	0.64 (3)	1.16 (1)	2.09 (1)
ACC2	2.65 (2)	3.14 (1)	0.73 (4)	0.71 (4)	1.46 (1)	0.66 (2)	0.68 (3)	0.79 (4)	0.75 (2)	0.81 (2)	1.65 (3)
ACC3	1.46 (3)	1.13 (4)	0.62 (5)	1.21 (1)	0.90 (3)	0.54 (3)	1.06 (2)	1.06 (3)	0.62 (4)	0.30 (3)	0.64 (4)
ACC4	0 (5)	0.23 (5)	0.83 (3)	0.51 (3)	0.19 (5)	0.48 (4)	0.35 (5)	0.50 (5)	0.43 (5)	0.17 (5)	0.58 (5)
ACC5	2.51 (1)	1.98 (2)	1.11 (1)	1.10 (2)	0.85 (4)	0.97 (1)	1.43 (1)	1.29 (1)	1.07 (1)	0.20 (4)	1.69 (2)
Grand mean	1.54	1.56	0.88	0.87	0.95	0.59	0.81	0.96	0.70	0.53	1.33
200 mM											
ACC1	0.0 (4)	0.59 (5)	0.93 (1)	0.36 (1)	0.50 (2)	0.38 (2)	0.20 (1)	0.54 (1)	0.40 (1)	0.81 (1)	0.35 (2)
ACC2	1.49 (1)	2.18 (1)	0.48 (2)	0.16 (3)	0.76 (1)	0.19 (3)	0.07 (2)	0.28 (3)	0.15 (3)	0.32 (2)	0.43 (1)
ACC3	0.46 (3)	0.66 (3)	0.42 (3)	0.25 (2)	0.48 (3)	0.16 (4)	0.06 (3)	0.13 (5)	0.07 (4)	0.19 (3)	0.32 (3)
ACC4	0.0 (4)	0.08 (4)	0.42 (3)	0.11 (5)	0.10 (5)	0.01 (5)	0.07 (2)	0.19 (4)	0.03 (5)	0.06 (4)	0.21 (5)
ACC5	0.60 (2)	1.30 (2)	0.42 (3)	0.14 (4)	0.21 (4)	0.47 (1)	0.03 (4)	0.21 (2)	0.23 (2)	0.06 (5)	0.27 (5)
Grand mean	0.51	0.96	0.53	0.20	0.41	0.24	0.09	0.27	0.18	0.29	0.32

GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.

Table 5 presents the combined rank sum (RkSum), rank means (RkMean), and standard deviation of ranks (SDRk) among rice accessions under varying salinity stress. ACC1, ACC2, and ACC5 exhibited lower RkSum and RkMean values (< grand mean), indicating high tolerance to stress. Conversely, ACC4 had higher values, suggesting susceptibility to stress.

Table 6 presents combined estimates of genetic parameters for germination traits among rice accessions under varying salinity stress. Across traits, germination capacity exhibited higher GV (186.09), PV (192.09), and heritability (96.88%) suggesting a stronger genetic component influencing the trait. Except for the number of leaves, all traits showed moderate to high variability with GCV and PCV ranging from the lowest (15.54 and 18.50%) in fresh shoot weight to the highest (54.73 and 57.69%) in germination energy indicating considerable phenotypic variability among accessions. High broad-sense heritability was observed in all traits ranging from 61.22% in root length to 96.88% in germination capacity, indicating the proportion of total variation attributable to genetic factors and the degree of genetic control over traits. Genetic advance ranged from 0.002

in dry shoot weight to 27.66 in germination capacity, while GAM ranged from 14.82% in the number of leaves to 107.00% in germination energy, indicating the relative improvement potential.

Table 5 Combined rank sum (RkSum), rank means (RkMean), and standard deviation of ranks (SDRk) among rice accessions screened under deferential salinity stress

Accession	RkSum	RkMean	SDRk
ACC1	79	3.59	1.30
ACC2	82	3.73	1.30
ACC3	103	4.68	0.93
ACC4	143	6.50	0.85
ACC5	75	3.41	1.28
Grand mean	96.40	4.38	1.13

In bold: Highly tolerant (values < grand mean); highly susceptible (values > grand mean)

Table 7 summarizes Pearson’s correlation analysis for germination and seedling traits under differential salinity stress. Germination energy showed strong positive correlations with germination capacity under 50 mM (0.89*) and 100 mM (0.96**) salinity stress. The number of leaves correlated highly positively with shoot length (0.88*) under 200 mM salinity stress. Additionally, shoot length was strongly correlated with leaf length (0.92*) under 100 mM salinity stress, while root length showed a strong positive correlation with fresh root weight (0.87*) under 200 mM salinity stress.

Table 6 Combined estimates of genetic parameters of germination and seedling traits among rice accessions screened under deferential salinity stress

Trait	GM	GVT	GV	PV	GCV (%)	PCV (%)	H ² B (%)	GA	GAM (%)
GE	22.72	56.07	154.58	171.75	54.73	57.69	90.00	24.30	107.00
GC	30.42	11.78	186.09	192.09	44.85	45.57	96.88	27.66	90.93
NL	2.05	0.01	0.03	0.05	9.00	11.26	63.91	0.30	14.82
SL	2.50	0.15	0.17	0.21	16.30	18.33	79.07	0.74	29.85
NR	5.23	2.07	2.22	2.93	28.51	32.72	75.91	2.68	51.17
RL	5.06	1.75	0.76	1.24	17.19	21.96	61.22	1.40	27.70
LL	4.06	0.90	0.79	1.05	21.90	25.28	75.01	1.59	39.07
FSW	0.03	0.00002	0.00002	0.00003	15.54	18.50	70.53	0.01	26.88
FRW	0.03	0.0001	0.00006	0.00001	25.62	32.39	62.59	0.01	41.76
DRW	0.005	0.000005	0.000004	0.000006	41.94	49.29	72.43	0.004	73.53
DSW	0.004	0.0000005	0.000001	0.00001	22.14	25.16	77.40	0.002	40.12

GM: Grand mean; GVT: Genotype × treatment variance; GV: Genotypic variance; PV: Phenotypic variance; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; H²B: Broad sense heritability; GA: Genetic advance; GAM: Genetic advance as percent over mean; GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DSW: Dry shoot weight, and DRW: Dry root weight.

Discussion

Increasing salt tolerance is crucial for enhancing the growth, development, and yields of crops on saline soils and for reintroducing cropping on salinized lands [36]. Screening and identifying salinity-tolerant traits in rice are essential for developing salt-tolerant cultivars and improving yields under salt-stress conditions [21]. The germination phase, significantly affected by salinity, is critical for plant performance under stress [32]. Traits such as germination percentage, root and shoot length, and dry weight are negatively impacted by high salinity levels. Understanding the variability of these traits among rice genotypes during germination and seedling stage is vital for breeding salinity-tolerant rice. Screening at the germination and seedling stage is efficient and ensures even salt distribution, unlike pot and field techniques, providing valuable data for salinity tolerance [21]. Several studies have shown that using germination and seedling traits for screening can effectively identify genotypes for further field evaluation [36].

The observed significant genetic variability among rice accessions in response to different salt treatments suggests that there is potential for breeding salinity-tolerant rice varieties. Traits such as germination energy, number of roots, and overall root characteristics displayed notable variability, indicating that these traits could be targeted in breeding programs to enhance salinity tolerance. The significant interaction between accession and treatment for all traits indicated that accessions performed differently under varying salinity conditions, similar to findings reported in rice [37]. By critically observing the performance of accessions across treatments, it was found that while lower concentrations of salinity enhanced the performance of tolerant accessions, increased salt concentration generally reduced seed germination, seedling growth performance, and overall seedling biomass similar to findings in sunflowers [36], wheat [38], and rice [39]. The observed trends in the response of various traits to salinity stress conditions provide valuable information for breeding salinity-tolerant rice varieties. Resilient accessions, such as ACC1, ACC2, and ACC5, consistently excel in crucial traits like germination energy and capacity, shoot length, root characteristics, and overall plant health. These accessions demonstrate high performance across multiple traits, underscoring their potential as key contributors to salinity tolerance. This study emphasizes the importance of trait-specific responses, enabling targeted breeding efforts to enhance characteristics crucial for plant resilience under stress, including germination, shoot length, and root development. By quantifying the impact of salinity on each trait and prioritizing resilient accessions, breeding strategies can be fine-tuned to develop rice varieties with improved overall salinity tolerance efficiently.

Table 6 Combined estimates of genetic parameters of germination and seedling traits among rice accessions screened under deferential salinity stress

Trait	GM	GVT	GV	PV	GCV (%)	PCV (%)	H ² B (%)	GA	GAM (%)
GE	22.72	56.07	154.58	171.75	54.73	57.69	90.00	24.30	107.00
GC	30.42	11.78	186.09	192.09	44.85	45.57	96.88	27.66	90.93
NL	2.05	0.01	0.03	0.05	9.00	11.26	63.91	0.30	14.82
SL	2.50	0.15	0.17	0.21	16.30	18.33	79.07	0.74	29.85
NR	5.23	2.07	2.22	2.93	28.51	32.72	75.91	2.68	51.17
RL	5.06	1.75	0.76	1.24	17.19	21.96	61.22	1.40	27.70
LL	4.06	0.90	0.79	1.05	21.90	25.28	75.01	1.59	39.07
FSW	0.03	0.00002	0.00002	0.00003	15.54	18.50	70.53	0.01	26.88
FRW	0.03	0.0001	0.00006	0.00001	25.62	32.39	62.59	0.01	41.76
DRW	0.005	0.000005	0.000004	0.000006	41.94	49.29	72.43	0.004	73.53
DSW	0.004	0.0000005	0.000001	0.00001	22.14	25.16	77.40	0.002	40.12

GM: Grand mean; GVT: Genotype × treatment variance; GV: Genotypic variance; PV: Phenotypic variance; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; H²B: Broad sense heritability; GA: Genetic advance; GAM: Genetic advance as percent over mean; GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DSW: Dry shoot weight, and DRW: Dry root weight.

Salinity stress has been reported to inhibit seed germination, decrease plant growth and development, and hinder the survival of seedlings. According to Ali *et al.* [40], the toxic effects of salinity likely caused a significant reduction in germination and seedling trait performance among sensitive accessions, leading to disrupted photosynthesis and increased respiration. This results in a shortage of assimilates essential for the proper growth and development of critical organs, potentially halting growth entirely. According to Zafar *et al.* [41], salinity decreases radicle and plumule growth, worsened at higher levels, by reducing water absorption and osmotic potential, thus hindering cell division and differentiation. It negatively impacts plumule, radicle, biomass, and physiological indices due to ionic toxicity, nutrient uptake issues, and reduced enzyme and hormone biosynthesis. During germination, salinity damages cell membranes, increasing permeability, replacing Ca²⁺ with Na⁺, and causing K⁺ leakage, disturbing osmotic balance. According to Zhang *et al.* [21], salt stress damages cell membranes during seed imbibition, increasing permeability and impairing function. This reduces seed germination, energy, and capacity. Intracellular K⁺ and Na⁺ exosmosis rise, while amylase activity and soluble sugar content drop, delaying starch hydrolysis and causing starch grains to cluster in rice seed storage tissues. Protease activity, protein conversion rate, and dry matter consumption decrease, increasing residual protein in the aleurone cytoplasm.

Table 7 Pearson’s correlations among germination traits of rice accessions screened under defferential salinity stress

Trait	Treatment	GE	GC	NL	SL	NR	RL	LL	FSW	FRW	DRW	DSW
GE	Control	1	0.79	0.02	0.52	0.57	0.53	0.52	0.82	0.23	0.41	0.46
	50	1	0.89*	0.22	0.04	0.26	0.15	-0.01	-0.29	0.04	0.39	0.42
	100	1	0.96**	0.11	0.57	0.78	0.26	0.65	0.38	0.81	0.39	0.28
	200	1	0.85	-0.19	-0.09	0.71	-0.06	-0.30	-0.15	-0.05	0.00	0.23
GC	Control		1	-0.07	0.02	0.42	0.46	0.29	0.49	-0.27	0.32	0.66
	50		1	0.05	0.21	0.45	0.32	0.22	0.01	0.38	0.31	0.2
	100		1	0.00	0.49	0.77	0.09	0.55	0.29	0.80	0.44	0.27
	200		1	-0.02	0.02	0.68	0.28	-0.35	-0.09	0.25	0.05	0.39
NL	Control			1	0.19	0.09	-0.17	0.09	0.15	0.55	0.23	0.18
	50			1	-0.10	-0.22	-0.29	-0.34	-0.61	-0.41	0.31	0.66
	100			1	-0.01	0.43	-0.08	0.05	0.19	0.22	0.20	0.17
	200			1	0.88*	0.31	0.33	0.55	0.68	0.37	0.46	0.29
SL	Control				1	0.23	0.44	0.74	0.69	0.08	0.11	-0.09
	50				1	0.71	0.08	0.82	0.37	0.64	0.09	-0.09
	100				1	0.39	0.004	0.92*	0.79	0.55	0.19	0.04
	200				1	0.43	0.28	0.53	0.56	0.39	0.63	0.26
NR	Control					1	-0.01	-0.02	0.52	-0.39	0.83	0.58
	50					1	0.26	0.69	0.42	0.70	0.29	-0.07
	100					1	-0.06	0.34	0.29	0.57	0.57	0.33
	200					1	0.09	0.04	0.19	0.05	0.39	0.44
RL	Control						1	0.83	0.58	-0.09	-0.23	0.01
	50						1	0.14	0.2	0.27	-0.25	-0.43
	100						1	0.09	-0.23	0.03	0.01	-0.27
	200						1	0.42	0.37	0.87*	0.38	-0.04
LL	Control							1	0.64	0.22	-0.16	-0.05
	50							1	0.56	0.67	0.12	-0.08
	100							1	0.79	0.62	0.01	0.12
	200							1	0.71	0.52	0.58	-0.1
FSW	Control								1	-0.07	0.51	0.35
	50								1	0.78	0.19	-0.3
	100								1	0.51	0.09	0.32
	200								1	0.55	0.49	-0.09
FRW	Control									1	-0.19	-0.12
	50									1	0.26	-0.22
	100									1	0.31	0.19
	200									1	0.47	-0.09
DRW	Control										1	0.55
	50										1	0.77
	100										1	-0.27
	200										1	0.01
DSW	Control											1
	50											1
	100											1
	200											1

*: Significant at $P \leq 0.05$; **: Significant at $P \leq 0.01$

GE: Germination energy; GC: Germination capacity; NL: Number of leaves; SL: Shoot length; NR: Number of roots; RL: Root length; LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.
LL: Leaf length; FSW: Fresh shoot weight; FRW: Fresh root weight; DRW: Dry root weight; DSW: Dry shoot weight.

Consequently, rice seedlings show stunted shoot and root length, number of roots, fresh plant weight, and seedling vigor index. Therefore, identifying specific genotypes with favorable traits under different salt concentrations provides valuable information for developing rice varieties that can thrive in varying salinity conditions, contributing to the resilience and productivity of rice crops in salt-affected regions.

Understanding the genetic variability and heritability of germination and seedling traits allows for focused efforts on traits that offer the greatest potential for improvement. The results from estimates of genetic parameters of germination and seedling traits under differential salinity stress have several implications in breeding for salinity tolerance in rice such as identifying high-performing traits, targeted breeding efforts, utilization of genetic resources, and accelerated breeding programs. In the present study, all traits consistently exhibited a high level of genetic variability under varied salinity stress except the number of leaves, shoot length, root length, and fresh shoot weight with low to moderate variability. Traits with high heritability and high genetic advance as a percent of the mean (GAM) such as germination energy, germination capacity, shoot length, number of roots, leaf length, fresh shoot and root weights, and dry shoot and root weight indicate additive gene effects and their suitability for targeted breeding. Mohammadi *et al.* [42] reported high broad-sense heritability estimates for seven seedling traits in rice, with high values for salinity score and shoot height, moderate values for shoot and root dry weight, and low values for Na^+ , K^+ , and K^+/Na^+ ratio, indicating varying levels of genetic and environmental influence. Hence, breeders can prioritize these traits for selection to develop rice varieties with improved salinity tolerance. In a similar study on the rice recombinant inbred line (RIL) population [25] evaluated under salinity stress based on seed germination traits, it was reported that these traits are controlled by two or three major genes plus polygenes, with major genes exhibiting high heritability values. By selecting germination traits with high heritability and GAM, breeders can expedite the development of salinity-tolerant rice varieties. Accessions such as ACC1, ACC2, and ACC5 exhibiting favorable trait performance under varied salinity stress will serve as valuable genetic resources for the breeding program. They can be used as parents in breeding crosses to introduce salinity tolerance into elite rice varieties. Employing germination trait STIs and ranking genotypes also have multifaceted implications for breeding salinity-tolerant rice varieties. These include the identification of high-performing accessions exhibiting higher STIs and superior rankings which can serve as valuable genetic resources, targeted selection of parental breeding lines, accelerated breeding programs, enhanced breeding strategies, and improved crop resistance thereby contributing to sustainable agriculture and food security in salinity-affected regions. One of the biggest strengths of STI is its capacity to pinpoint high-performing and consistent genotypes under both normal and stressed conditions an attribute that has been proven successful in several crops such as rice for salinity tolerance [36], and cowpea for aluminum stress [38]. Also, the effectiveness of various salt tolerance indices has been reported in several crop species [40, 42, 43]. Based on the rankings of accessions in the results, where 1 indicates a high ranking and above-average performance of trait across salinity stress conditions and 5 indicates high susceptibility, ACC2, ACC1, and ACC5 were pinpointed as highly tolerant which may be utilized in a breeding program. At the same time, ACC3 and ACC4 were highly susceptible. The tolerant accessions may be used as tolerant breeding stock for further crop improvement programs.

The considerable connections between germination and seedling characteristics at various degrees of salt stress have many major implications for developing saline-tolerant rice varieties. Strong positive associations between germination energy and germination capacity, especially under low to moderate salt stress, suggest that selecting for high germination energy can enhance overall germination success in saline conditions. This finding is consistent with reports by Zhang *et al.* [23] and Ding *et al.* [43]. Breeders should prioritize rice lines with high germination energy due to its strong correlation with germination capacity, crucial for seedling establishment in salty environments. Additionally, the significant link between the number of leaves and shoot length under high salt stress indicates that plants continuing vegetative growth (more leaves and longer shoots) are better adapted to high-salinity conditions. Selecting rice cultivars that produce more leaves and longer shoots under severe salt stress may improve plant resistance and overall development in saline soils. Moreover, the strong association between shoot length and leaf length under salt stress implies that longer shoots correspond to longer leaves, potentially enhancing light uptake and photosynthetic efficiency. Breeding for longer shoots may indirectly select for longer leaves, boosting the plant's ability to thrive under mild salt stress through increased photosynthetic capacity and growth. Lastly, the substantial correlation between root length and fresh root weight under severe salt stress suggests that longer roots accumulate more biomass, improving water and nutrient uptake in saline environments. Focusing on root traits such as root length and fresh root

weight can enhance salt tolerance, as plants with robust root systems are more efficient at absorbing water and nutrients, providing a significant advantage in saline soils. These results align with findings by Zhang *et al.* [23] in rice and Wu *et al.* [34] in oilseed rape. Therefore, breeding programs should take a holistic approach, selecting for high germination energy, vigorous vegetative development (more leaves and longer shoots), and strong root systems. Understanding trait correlations enables breeders to pick numerous desirable qualities simultaneously, increasing breeding program efficiency. The differences in responses under differing salt levels highlight the necessity for focused breeding techniques adapted to specific salinity circumstances (for example, moderate vs. high salinity). By focusing on these essential qualities, breeders can create rice varieties better suited to establishing, growing, and producing yields under salty circumstances, thereby contributing to food security in salinized areas.

Conclusion

This study successfully estimated genetic variability and correlations among germination and seedling traits conferring salinity tolerance in rice. Screening five rice accessions under various salinity levels in a controlled laboratory setting revealed significant genetic variability across all traits. Notably, germination energy and fresh shoot weight exhibited the highest coefficients of variation under control and 200 mM salinity conditions, respectively.

Accessions ACC2 and ACC5 consistently outperformed others, showing higher values for germination energy, germination capacity, and shoot length under different salinity levels. In contrast, ACC4 generally showed lower performance, indicating higher susceptibility to salinity stress. Estimates of genetic parameters indicated high heritability and genetic advance for all traits, with germination capacity and germination energy standing out, suggesting strong genetic control and potential for improvement through selective breeding. Significant correlations between germination energy and capacity, as well as between shoot length and leaf length under stress conditions, were observed. Stress tolerance indices further identified ACC2 and ACC5 as the most tolerant accessions, highlighting their potential for breeding programs aimed at enhancing salinity tolerance in rice.

This study underscores the importance of identifying resilient traits and accessions to improve salinity tolerance in rice, ultimately contributing to increased productivity in saline-affected regions.

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Competing Interest

None declared by the authors.

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