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RESEARCH ARTICLE

Assessment of morphological and biochemical traits for the development of new salt tolerant lines derived from biparental cross in rice (*Oryza sativa* L.)

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Abstract

More than half of the world's population is fed by rice, a significant staple crop, yet its production is extremely susceptible to stress. Saline environments impair vital physiological functions of rice which lowers production and growth. To ensure food security in areas affected by salt and climate stress, it is imperative to develop rice cultivars that can withstand salinity. This study assessed the morphological and biochemical characteristics of 25 rice lines resulting from biparental crossings in both normal and salinized environments. ANOVA (Analysis of variance), correlation, PCA (Principal Component Analysis) and biochemical profiling were among the statistical analyses that were carried out. Under normal conditions, NDRK-CS-22 produced a high grain yield of 31.08 g, but under saline conditions, NDRK-CS-19 produced a high grain yield of 19.84 g. Significant trait variability was found using PCA; in normal conditions, five principal components explained 77.81 % of the overall variance, but under saline conditions, four principal components explained 71.45 %. Under normal conditions, PC1 explained 21.56 % variance (eigenvalue 2.4), while under salinity, PC1 explained 23.46 % variance (eigenvalue 2.61). Interestingly, under stress, NDRK-CS-2 showed higher yield, proline and superoxide dismutase (SOD) concentration, indicating that it may be used in breeding for salinity tolerance. These results demonstrate how different rice lines are genetically and how they adjust to salinity. In order to maintain sustainable rice production in the face of climate change, top-performing lines (NDRK-CS-2, 19, 22, CSR10) need to be further validated in a variety of saline conditions using genomic methods to create resilient, high-yielding rice cultivars.

Keywords: chlorophyll content; proline; protein content; rice; saline; superoxide dismutase

Introduction

Rice stands as the paramount global food crop, sustaining over half of the world's population. In rice-producing regions such as Asia, Africa and South America, more than 400 million individuals still derive a significant portion of their energy and its byproducts. Projections indicate a further 38 % surge in food demand within the next three decades (1, 2). However, rice productivity in numerous areas faces a significant threat from salinity stress. This stress arises from the accumulation of subterranean salts, a problem intensified by activities such as salt mining, deforestation and irrigation (3). While rice is generally classified as a salt-sensitive crop, the degree of this sensitivity fluctuates across different growth and developmental phases. Notably, rice exhibits tolerance to salinity stress during germination and active tillering stages. Conversely, it increased sensitivity is noted during the early vegetative and reproductive stages (4, 5).

Salinity, a prevalent abiotic stress, poses a significant constraint on crop growth, development and overall productivity. It also contributes to the ongoing loss of fertile land, ultimately leading to desertification in arid and semi-arid regions globally (6). Across the world, surveys indicate that over 800 million hectares of land are negatively impacted by elevated salinity levels (7). Saline

soils are distinguished by an excess of sodium ions with chloride and sulfate as the predominant anions resulting in a high electrical conductivity (greater than 4 dS m^{1}) (8). Generally, salinity stress triggers an initial osmotic stress, followed by toxicity stemming from ion accumulation. However, damage can also arise from the overproduction of reactive oxygen species (ROS), such as superoxide radicals (O), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•). These ROS commonly accumulate in plant tissues due to ion imbalance and hyperosmotic stresses. The buildup of ROS leads to lipid peroxidation and exerts a detrimental effect on cellular metabolism and physiology, thereby compromising membrane integrity (9).

A popular and widely grown rice variety, Sarjoo-52 is renowned for its excellent yield potential, tolerance to a variety of agroclimatic conditions and resistance to common pests and illnesses (10). Conversely, CSIR-10 is a rice variety that can withstand salinity due to advanced breeding techniques, which means it can be cultivated in salt-affected soils (11). There is a great deal of promise for creating excellent salt-tolerant rice lines when the agronomic perfection of Sarjoo-52 and the salt tolerance of CSIR-10 are combined.

This study intends to create superior salt-tolerant rice lines that combine resilience and productivity in saline environments by combining the genetic pathways of salinity tolerance found in CSIR-10 with the high yield features and adaptability of Sarjoo-52. To increase food security and guarantee sustainable rice production in areas that are at risk, such improved lines would be crucial in extending rice agriculture to marginal lands impacted by salt. The production of genotypes with both excellent agronomic performance and abiotic stress resistance is supported by the strategic use of these two complimentary kinds, addressing important issues brought on by soil degradation and climate change.

Experimental material and site

An experiment was conducted in the *Kharif* season 2023-24 at the GPB farm in Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, India to develop the salt tolerant lines. In this experiment, 25 lines derived from biparental cross viz. CSR-10 (tolerant)* Sarjoo- 52 (susceptible) were considered for evaluation. Seed bed nursery was raised primarily and raised seedlings were transplanted after 21 days in the field by employing Randomised Complete Block Design (RCBD) with three replications under both normal and salinity conditions. The field condition for salinity was maintained by dissolving common salt (NaCl) in irrigation water to reach desired salinity levels (e.g. EC 4, 8 or 12 dS/m). The Electrical Conductivity (EC) meter was used to check and adjust the salt concentration accurately before application. pH of the farm is typically 7.6 while the EC of the saturation extract is generally above 4.7 dS/m.

Observations of parameters

The observations were recorded on randomly selected five plants from each line with replication. Such as days to 50 % flowering, plant height, number of tillers/plants, biological yield/ plant, harvest index, grain yield/plant, total protein content, chlorophyll content, amylase, superoxide dismutase and proline.

Estimation of total protein content

The total protein content in grains was estimated using a modified Lowry method (12). Proteins were extracted by treating the dried pellet with 2 mL of 1 N NaOH at 80 °C for 1 hr, followed by centrifugation at 5000 rpm for 10 min to obtain the supernatant as the stock solution. A fivefold dilution was prepared, mixed with alkaline copper reagent and incubated before adding diluted Folin-Ciocalteu's reagent. After 15 min in the dark, absorbance at 650 nm was measured and protein concentration determined using a BSA standard curve.

Estimation of total chlorophyll content

The total chlorophyll content in mature leaf tissue was estimated following Arnon's method (13). About 50 mg of leaf tissue was homogenized in 5 mL of methanol and kept at 4 °C in the dark for 16-24 hr to ensure complete pigment extraction. After centrifugation at 5000 rpm for 10 min, the supernatant was collected and absorbance was measured at 645 nm and 663 nm using a spectrophotometer. Total chlorophyll (mg/g FW) was calculated using Arnon's formula with results expressed as milligrams per gram fresh weight (mg/g FW).

Estimation of amylase activity

Amylase activity was estimated using a modified method (14). About 20 mg of fresh leaves was homogenized in ice-cold 10 mM $CaCl_2$ and incubated overnight at 4 °C. The homogenate was centrifuged at 5000 rpm for 20 min and the supernatant served as the enzyme extract. The extract was incubated with starch substrate at 37 °C for 15 min and the reaction stopped with DNSA reagent. Absorbance at 540 nm was measured and activity calculated using a maltose standard curve.

Estimation of SOD activity

SOD was extracted from 0.2 g fresh leaf tissue by homogenizing in 2 mL ice-cold 0.05 M phosphate buffer (pH 7.0) with acid-washed sand, followed by centrifugation at 30000 × g for 30 min at 0 °C—4 °C. Activity was assayed using a previously known method, based on inhibition of nitro blue tetrazolium (NBT) photoreduction (15). Absorbance at 560 nm was measured and SOD activity expressed as the enzyme amount causing 50 % NBT inhibition per gram fresh weight or per mg protein.

Estimation of proline content

Proline content in leaf tissue was estimated using a modified method (16). About 0.1 g of fresh leaf was homogenized in 5 mL of 3 % sulfosalicylic acid and 2 mL of the extract was reacted with 2 mL acid ninhydrin and 2 mL glacial acetic acid. After incubation at 100 °C for 1 hr, the reaction was stopped on ice, extracted with toluene and absorbance measured at 520 nm. Proline concentration was determined from a standard curve and expressed as µg/g FW.

Statistical analysis

The experiments were conducted using a RCBD with three replications. Data collected were analyzed through Pooled-ANOVA to identify significant differences among treatments and mean comparisons were made using the Least Significant Difference (LSD) test at a significance level of $p < 0.05$. All statistical calculations were performed using OPSTAT. Pearson's correlation coefficients were then computed to evaluate interrelationships among traits. Further statistical analyses including boxplot, correlation and PCA were

conducted using Past version 4.03, Agri Analyze and SRplot to ensure an accurate and comprehensive interpretation of the data.

Results and Discussion

ANOVA

The ANOVA of pooled analysis of RCBD results showed highly significant differences in all lines for traits such as days to 50 % flowering (42.744), plant height (26.064), protein (21.129), chlorophyll (376.137), amylase (59.543), SOD (176.218) and proline (33.281) while for traits number of tillers per plant (2.453) harvest index (55.111) and grain yield per plant (7.162) indicating substantial genetic variability was noted. The significant differences among the genotypes for traits such as protein, amylose, SOD and proline are primarily due to genetic factors, as there were no significant differences for these traits across environments. In contrast, traits such as number of tillers (NOT), harvest index (HI) and grain yield per plant (GYP) showed significant differences only across environments and not among genotypes, suggesting that these traits are highly influenced by environmental factors. The environment significantly influenced traits like days to 50 % flowering, plant height, tillers, yield attributes and chlorophyll content while environment × salinity interactions were significant for tillers, yield traits and proline, indicating variable genotypic responses under both (normal and salinity) conditions (Table 1). Replication within the environment showed highly significant effects only for the NOT per plant (16.16), HI (191.767) and GYP (64.304). The pooled error values were low across traits (0.898 to 4.693) except HI (19.804) indicating high experimental precision and reliability. These findings are in agreement with earlier reports (17, 18).

Mean performance of morphological and biochemical parameters

The mean performances of 25 lines and 2 check data were presented in Table 2 and variations were observed within the lines. In days to 50 % flowering in normal conditions and salinity conditions ranged from 88.00 to 99.00 and 92.00 to 103.00 respectively. In maximum days to 50 % flowering were found in NDRK-CS-14 (99.00) and (103.00) in both normal and salinity conditions respectively. While range for plant height in both normal and saline conditions are (97.48-105.98) and (95.16-101.63) respectively. For NOT per plant ranges in both normal and saline conditions are (7.00-11.00) and (5.00-9.00) respectively. While biological yield per plant range is (45.36-55.49) and (37.48-49.38) respectively for both normal and saline conditions. For HI and grain yield per plant range between (46.59-58.54 and 22.16-31.08) and (27.82-49.44 and 11.03-19.84) respectively in both normal and salinity conditions. This finding aligns with previous studies that have documented the detrimental effects of salinity on plant height and grain yield per plant (19-21).

Under normal conditions, grain protein content varied from 5.02 to 13.26 mg/g with CSR-10 (13.26 mg/g), NDRK-CS-17 (12.04 mg/g) and NDRK-CS-23 (11.49 mg/g) showing the highest values while NDRK-CS-14 (5.02 mg/g) recorded the lowest. Traditional cultivars exhibited higher protein content than enhanced lines. Under salinity stress, protein levels ranged from 4.03 to 10.69 mg/g, highest in CSR-10 (10.69 mg/g), Sarjoo -52 (10.32 mg/g) and NDRK-CS-16 (10.30 mg/g) and lowest again in NDRK-CS-14. Coefficients of variation (CV) were 23.22 % (normal) and 27.94 % (salinity) with significant critical differences (CDs) at 5 % and 1 %. Chlorophyll content ranged from 13.36 to 46.20 mg/g under normal conditions, highest in NDRK-CS-24, NDRK-CS-19 and NDRK-CS-22. Under salinity, NDRK-CS-24 again showed the highest value (43.66 mg/g). CVs were 28.83 % (normal) and 31.12 % (salinity).

Amylase content ranged from 15.39 % to 29.44 % under normal and 13.74 % to 27.89 % under salinity with NDRK-CS-18 consistently highest. SOD activity under normal conditions ranged from 64.26 to 83.64, peaking in Sarjoo-52 while proline ranged from 9.14 to 17.60, highest in NDRK-CS-5. Under salinity, SOD and proline ranged from 66.36-85.30 and 9.30-19.67, respectively. CVs for SOD and proline were low to moderate with statistically significant CDs indicating variation among genotypes in both environments.

The boxplot provides a visual depiction for a better understanding of how characters are affected by salinity conditions (Fig. 1 a, b). A boxplot is a statistical visual tool used to summarize the distribution of a dataset. It provides a clear picture of data symmetry and spreads by displaying the median, interquartile range (IQR) and possible outliers. Boxplots can be used to overlay for comparison, but they do not offer the actual mean value. These finding aligns with previous studies that have documented the detrimental effects of salinity on plant protein and chlorophyll (7, 22).

Phenotypic correlations analysis

GYP to NOT (0.40*), biological yield per plant (0.78**) and harvest index (0.85**) all showed substantial positive relationships under normal conditions suggesting that these features positively affect yield under ideal circumstances. In contrast, under normal conditions, Biological Yield per Plant

Table 1. Analysis of variance for studied traits under normal and saline conditions

Source of Variation	d.f.	Characters										
		DFF	PH	NOT	BYP	HI	GYP	Protein	Chlorophyll	Amylose	SOD	Proline
Environment	8	782.321**	1551.476**	213.556**	4259.109**	10552.876**	5661.757**	61.656	194.588**	119.89	78.984	24.987
Rep within Environment	4	35.654	53.799	16.16**	60.541	191.767**	64.304**	15.518	5.121	27.652	21.871	8.39
Genotypes	26	42.744**	26.064**	2.453	32.04*	55.111	7.162	21.129**	376.137**	59.543**	176.218**	33.281**
Normal x Saline	26	3.154	4.769	3.132**	16.645**	81.947**	14.047**	0.597	1.549	0.678	0.414	1.349**
Pooled Error	104	3.308	3.424	1.532	4.693	19.804	3.305	1.325	2.552	1.166	1.164	0.898

*, ** Significant at 5 % and 1 % probability levels, respectively.

Note- DFF- days to 50 % flowering, PH- plant height, NOT- number of tillers per plant, BYP- biological yield per plant, HI- harvest index, GYP- grain yield per plant, SOD- superoxide dismutase.

Table 2. Mean performance of morphological and biochemical parameters under both normal and salinity conditions

SN	Characters	DFF		PH		NOT		BYP		HI		GYP	
		N	S	N	S	N	S	N	S	N	S	N	S
P1	CSR-10 Tolerant	89	92	100.26	97.58	8	6	52	40.25	57.69	45.27	30	18.22
P2	Sarjoo-52 Susceptible	98	100	99.89	95.46	10	5	48	38.34	52.08	39.8	25	15.26
1	NDRK- CS- 1	94	97	103.27	99.02	7	7	51.96	44.3	54.54	33.61	28.34	14.89
2	NDRK- CS-2	92	96	97.58	95.34	8	8	53.2	50.90	55.41	56.05	29.48	28.53
3	NDRK- CS-3	93	97	99.37	95.16	9	7	48.49	40.1	51.97	42.94	25.2	17.22
4	NDRK- CS-4	95	98	103.25	100.44	10	6	47.56	38.49	46.59	36.43	22.16	14.02
5	NDRK- CS-5	96	99	105.28	101.63	11	5	45.36	37.51	54.14	46.6	24.56	17.48
6	NDRK- CS-6	88	92	101.2	97.49	8	7	52.08	48.26	53.48	36.16	27.85	17.45
7	NDRK- CS-7	90	94	99.34	96.37	9	9	53.47	42.31	52.98	42.59	28.33	18.02
8	NDRK- CS-8	89	93	98.46	95.78	10	8	52.03	45.16	58.22	33.79	30.29	15.26
9	NDRK- CS-9	91	95	100.59	97.84	8	7	51.2	44.1	53.63	36.96	27.46	16.3
10	NDRK- CS-10	93	97	104.3	100.96	9	8	48.69	39.64	51.67	37.56	25.16	14.89
11	NDRK- CS-11	92	95	102.38	98.48	11	9	47.89	45.31	51.41	30.96	24.62	14.03
12	NDRK- CS-12	98	101	101.37	101.37	10	7	53.62	49.38	55.54	39.45	29.78	19.48
13	NDRK- CS-13	97	100	105.98	101.47	7	6	51.42	40.3	55.04	46.5	28.3	18.74
14	NDRK- CS-14	99	103	100.28	96.37	8	5	50.39	41.96	53.96	41.68	27.19	17.49
15	NDRK- CS-15	90	95	97.48	95.48	9	7	48.67	42.98	58.54	32.71	28.49	14.06
16	NDRK- CS- 16	96	100	99.35	96.74	10	9	49.5	37.48	51.43	42.56	25.46	15.95
17	NDRK- CS- 17	94	97	100.29	98.74	11	7	51.49	40.21	47.83	36.11	24.63	14.52
18	NDRK- CS-18	93	96	102.09	98.37	8	6	52.3	45.66	49.73	35.87	26.01	16.38
19	NDRK- CS-19	92	96	104.63	101.28	9	5	53.78	40.13	54.82	49.44	29.48	19.84
20	NDRK- CS-20	94	98	102.29	99.38	7	7	53.01	43.55	51.16	40.09	27.12	17.46
21	NDRK- CS-21	97	101	101.02	98.75	9	8	53.09	38.48	55.72	39.22	29.58	15.09
22	NDRK- CS-22	96	100	105.64	101.63	10	6	55.49	39.65	56.01	27.82	31.08	11.03
23	NDRK- CS-23	93	97	103.05	100.22	7	6	53.08	40.14	54.05	45.96	28.69	18.45
24	NDRK- CS-24	92	96	102.69	98.78	8	7	52.47	41.96	57.5	31.12	30.17	13.06
25	NDRK- CS-25	91	95	101.27	98.69	9	8	51.89	42.85	50.16	37.43	26.03	16.04
	Mean	93.41	97.04	101.58	98.47	8.89	6.89	51.19	41.89	53.53	39.06	27.42	16.31
	Std	3	2.81	2.35	2.15	1.25	1.22	2.39	3.11	3	5.55	2.28	2.17
	SE	0.58	0.54	0.45	0.41	0.24	0.23	0.46	0.60	0.58	1.07	0.44	0.42
	CV	3.21	2.90	2.31	2.18	14.06	17.71	4.67	7.42	5.60	14.21	8.32	13.30
	CD 5%	3.56	3.80	4.55	4.97	8.54	8.75	4.47	3.43	3.56	1.92	4.68	4.92
	CD 1%	4.81	5.14	6.14	6.72	11.55	11.83	6.04	4.64	4.81	2.60	6.33	6.65

SN	Characters	Protein(mg/g)		Chlorophyll (nmol/cm)		Amylase (%)		SOD Units/Gfw		Proline	
		N	S	N	S	N	S	N	S	N	S
P1	CSR-10 Tolerant	13.26	10.69	30.54	27.45	24.03	21.67	79.68	81.54	15.36	16.89
P2	Sarjoo-52 Susceptible	11.25	10.32	24.69	21.03	23.21	20.25	83.64	85.3	13.52	11.02
1	NDRK- CS- 1	9.62	6.98	26.22	22.71	22.75	20.85	76.28	78.68	12.96	14.09
2	NDRK- CS-2	8.54	7.48	18.36	17.02	20.73	19.02	79.38	80.37	16.44	16.98
3	NDRK- CS-3	7.5	5.69	29.3	25.36	23.78	21.34	76.41	77.16	14.77	14.55
4	NDRK- CS-4	8.69	6.38	36.47	33.65	22.23	20.46	78.73	79.31	10.45	11.02
5	NDRK- CS-5	8.45	6.01	25.16	22.41	18.63	13.74	74.79	75.48	17.6	19.67
6	NDRK- CS-6	6.34	5.13	29.69	27.66	20.73	18.96	77.6	78.96	10.22	10.99
7	NDRK- CS-7	9.08	8.99	30.89	28.02	15.39	14.78	78.49	79.24	16.37	16.34
8	NDRK- CS-8	8.42	6.34	13.2	12.45	21.58	20.41	76.29	78.32	11.2	12.47
9	NDRK- CS-9	7.15	6.03	25.45	22.55	16.84	15.96	76.76	78.01	9.48	9.3
10	NDRK- CS-10	9.07	7.02	15.47	12.37	23.46	21.48	75.29	76.24	15.3	14.89
11	NDRK- CS-11	7.38	6.31	19.35	18.67	26.32	24.12	76.97	75.96	16.58	15.47
12	NDRK- CS-12	8.24	7.09	30.47	28.78	21.07	20.3	72.24	73.06	10.47	11.63
13	NDRK- CS-13	6.06	4.96	26.96	24.3	27.83	25.69	69.36	70.21	16.37	16.47
14	NDRK- CS-14	5.02	4.03	20.89	18.69	21.44	20.04	66.16	67.96	11.99	11.96
15	NDRK- CS-15	9.1	8.32	22.45	20.1	25.33	23.41	67.67	69.02	13.04	13.27
16	NDRK- CS- 16	11.24	10.3	26.99	25.39	25.76	23.96	79.09	80.14	10.99	11.06
17	NDRK- CS- 17	12.04	10.04	35.19	33.47	27.06	25.74	80.19	82.1	14.23	15.47
18	NDRK- CS-18	8.2	7.89	26.87	22.12	29.44	27.89	80.7	81.66	11.04	11.64
19	NDRK- CS-19	10.47	10.01	40.12	37.46	19.27	18.3	65.82	66.36	12.04	12.78
20	NDRK- CS-20	9.29	8.98	25.46	22.2	20.64	19.37	64.26	65.49	12.51	13.95
21	NDRK- CS-21	10.42	10.21	37.33	35.96	21.88	20.18	67.1	68.96	13.81	15.48
22	NDRK- CS-22	10.52	8.36	39.28	37.58	26.17	25.34	67.5	68.45	15.14	16.34
23	NDRK- CS-23	11.49	9.64	38.33	36.51	24.45	22.39	71.84	73.09	14.74	15.69
24	NDRK- CS-24	9.19	7.33	46.2	43.66	21.39	20.48	73.75	75.3	9.65	10.2
25	NDRK- CS-25	9.4	8.32	38.2	35.78	20.46	18.79	75.3	76.98	9.14	11.04
	Mean	9.28	8.17	28.97	26.59	22.59	21.04	73.92	75.06	13.06	13.71
	Std	2.43	2.61	8.35	8.28	3.4	3.2	5.05	5.03	2.57	2.57
	SE	0.49	0.52	1.67	1.66	0.68	0.64	1.01	1.01	0.51	0.51
	CV	23.22	27.94	28.83	31.12	15.07	15.2	6.83	6.7	19.71	18.72
	CD 5%	4.24	3.5	1.24	1.25	3.03	3.23	2.04	2.05	4.01	4.02
	CD 1%	5.75	5.02	1.67	1.69	4.12	4.37	2.77	2.78	5.43	5.44

Note- DFF- days to 50 % flowering, PH- plant height, NOT- number of tillers per plant, BYP- biological yield per plant, HI- harvest index, GYP- grain yield per plant, SOD- superoxide dismutase, N- normal, S- salinity.

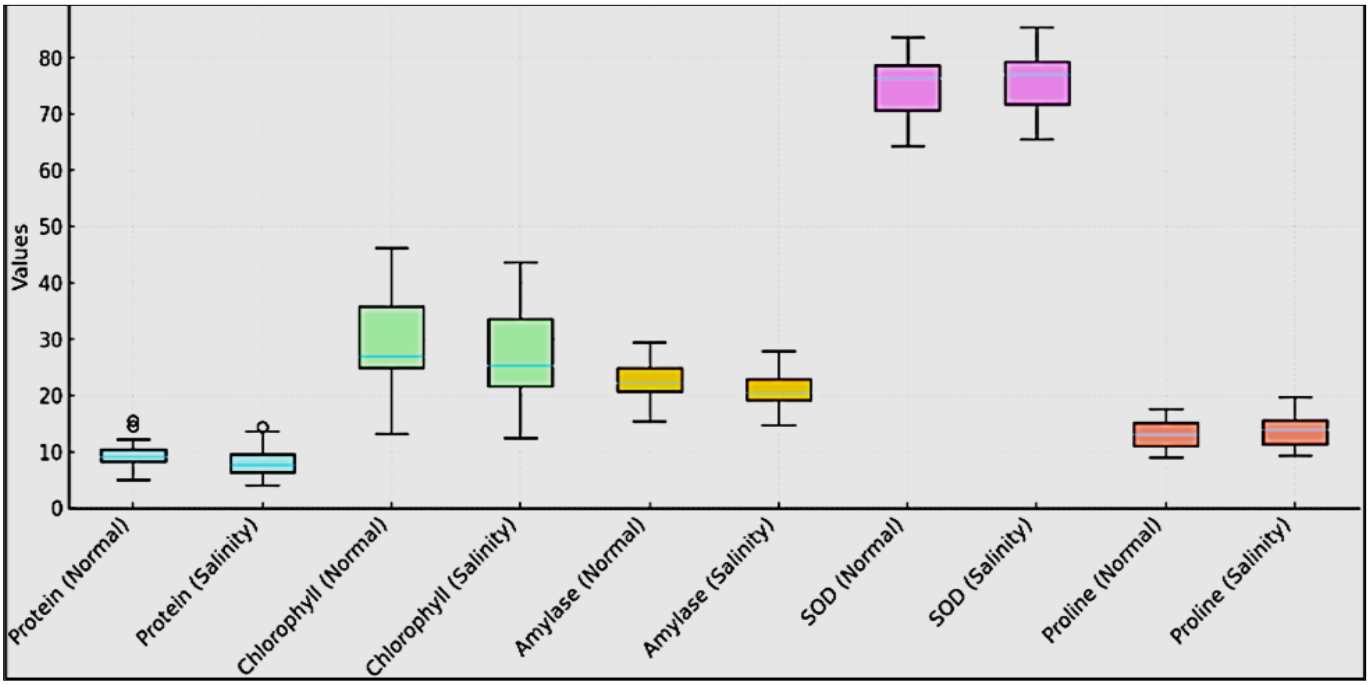

Fig. 1a. Comparison of biochemical mean, range and variance for all 11 characters under both normal and stressed condition by box plot graph.

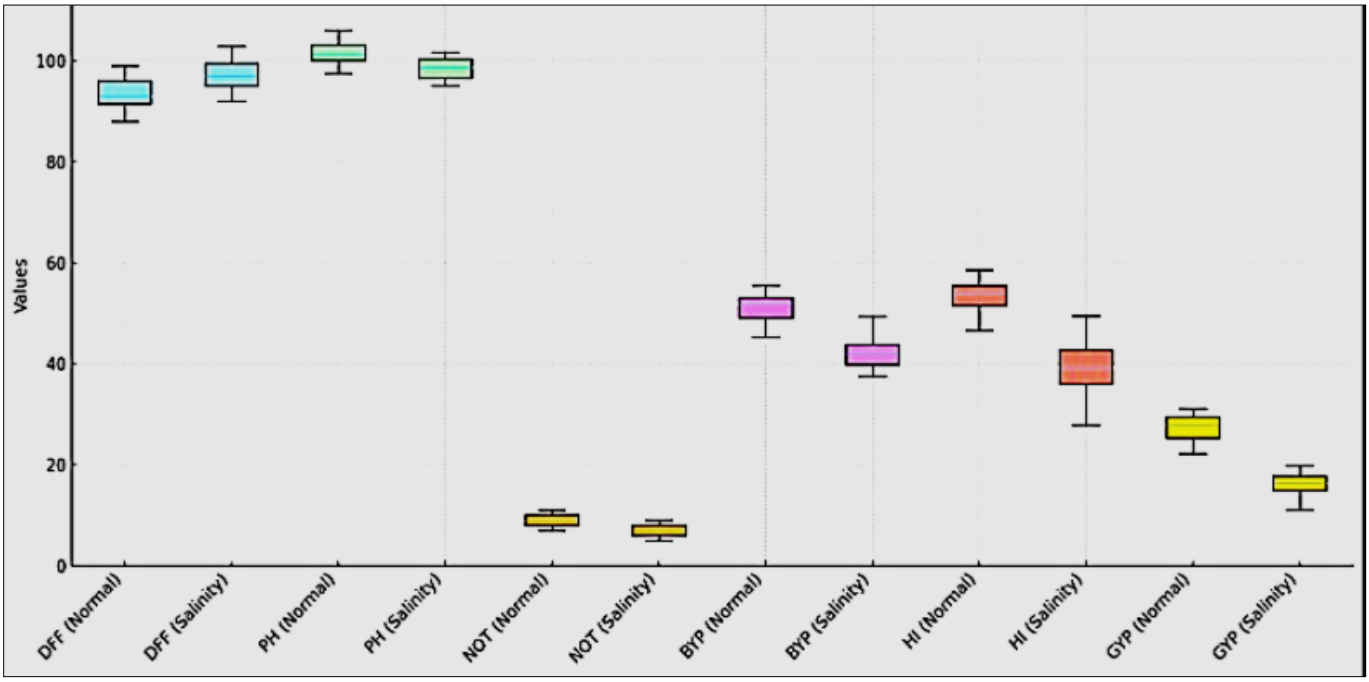

Fig. 1b. Comparison of morphological mean, range and variance for all 11 characters under both normal and stressed condition by box plot graph.

(BYP) had a negative correlation with NOT (-0.43*), SOD (-0.32) and proline concentration (-0.18). Additionally, there was a strong positive association between protein concentration and chlorophyll (0.39*), suggesting a possible connection between protein biosynthesis and photosynthetic activity. The effective partitioning of biomass to grains becomes even more crucial under stress, as seen by the fact that GYP only maintained a substantial positive connection with HI (0.86**) under salinity stress, (Fig. 2). Additionally, HI and Proline had a positive correlation (0.34), suggesting that biomass continues to enhance yield through better partitioning even in saline environments. Significantly, SOD activity demonstrated a significant negative correlation with days to flowering (-0.38*) and plant height (-0.41*) while chlorophyll content showed a weak but significant positive correlation with plant height (0.40*). These findings underscore the role of oxidative stress responses in early developmental stages under salinity. These findings imply that physiological and biochemical features become more important under salt stress, but vegetative traits affect yield under normal circumstances. A similar finding was also reported in a previous study (23).

Principal component analysis (PCA)

PCA was performed on 11 traits by using the Singular Value Decomposition (SVD) approach. A total of 11 principal components (PCs) were computed for both normal and saline conditions. Based on Kaiser's rule (eigenvalues > 1), five PCs were retained under normal conditions and four under saline conditions for further analysis.

PC1 explained the highest variation (21.56 %) with an eigen value of 2.4. The subsequent components PC2, PC3, PC4 and PC5 explained 18.22 %, 15.95 %, 12.07 % and 10.01 % of the total variance, respectively in normal condition while in saline condition PC1 accounted for the highest variation (23.46 %) with

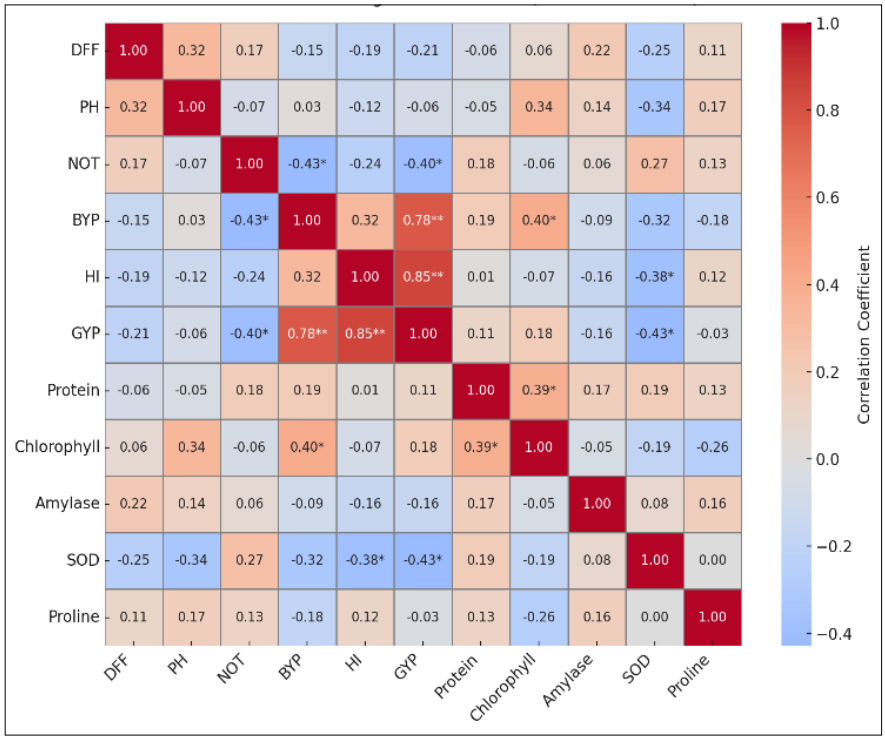

Fig. 2a. Phenotypic correlation of 11 characters under normal condition.

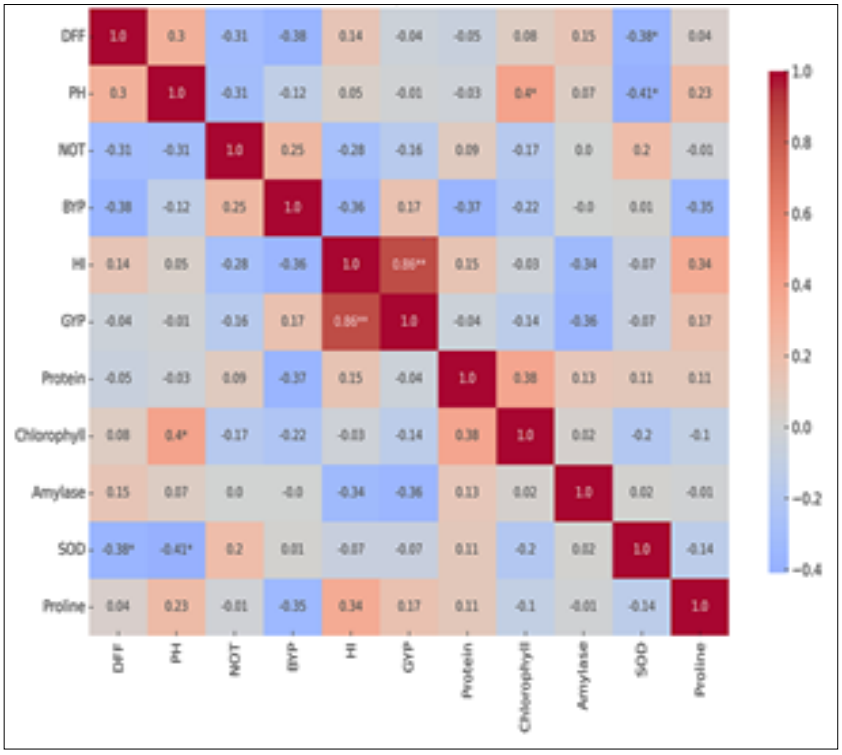

Fig. 2b. Phenotypic correlation of 11 characters under salinity condition.

Note- DFF- days to 50 % flowering, PH- plant height, NOT- number of tillers per plant, BYP- biological yield per plant, HI- harvest index, GYP- grain yield per plant, SOD- superoxide dismutase.

an eigenvalue of 2.61 followed by PC2, PC3 and PC4 which explains 21.29 %, 15.21 % and 11.49 % of the total variance, respectively (Table 3).

A PCA biplot is a graphical representation that simultaneously displays the PCs of a dataset and the contribution of each original variable (24). The genotypes (represent in black dot) NDRK-CS-6, NDRK-CS-11, NDRK-CS-15 and Sarjoo-52 is close to each other showing they have similar observation in normal condition (Fig. 3a). While in saline condition the genotypes NDRK-CS-1, NDRK-CS-4, NDRK-CS-15 and NDRK-CS-18 shows similar values (Fig. 3b). The length and angle of different vector indicate the magnitudes and types of correlation. The BYP and SOD are negatively correlated in normal condition while amylase, proline and SOD are positively correlated in saline condition suggesting a strong relation between them under saline condition (25).

A scree plot in PCA is a 2D line graph plotting the eigenvalues (variance explained by each PC) on the y-axis against the principal component number on the x-axis (Fig. 4). Under normal conditions, PCA-1 to PCA-6 account for the

Table 3. Eigen value, proportion of variance and cumulative variation of principal component axes under both normal and saline conditions

Principal component	Eigen value		Proportion of variance %		Cumulative variation %	
	Normal	Saline	Normal	Saline	Normal	Saline
PC1	2.4	2.6	21.562	23.464	21.562	23.464
PC2	2.0	2.3	18.219	21.288	39.782	44.752
PC3	1.8	1.7	15.945	15.211	55.727	59.963
PC4	1.3	1.3	12.071	11.491	67.798	71.454
PC5	1.1	0.9	10.009	8.249	77.806	79.703
PC6	1.0	0.7	8.944	6.254	86.75	85.957
PC7	0.7	0.6	6.109	5.581	92.859	91.538
PC8	0.3	0.4	2.896	3.882	95.756	95.419
PC9	0.2	0.3	2.239	2.884	97.994	98.304
PC10	0.2	0.2	1.991	1.687	99.986	99.991
PC11	0.0	0.0	0.014	0.009	100	100

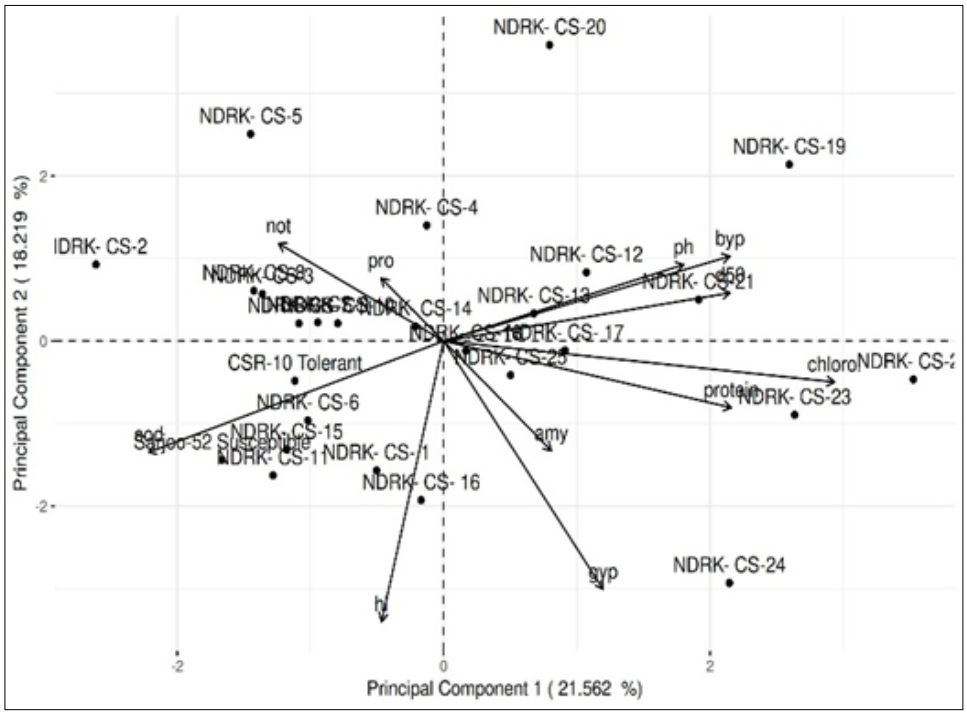

Fig. 3a. Biplot distribution of 27 rice lines and studied traits depending on principal component axes PC1 and PC2 in normal conditions.

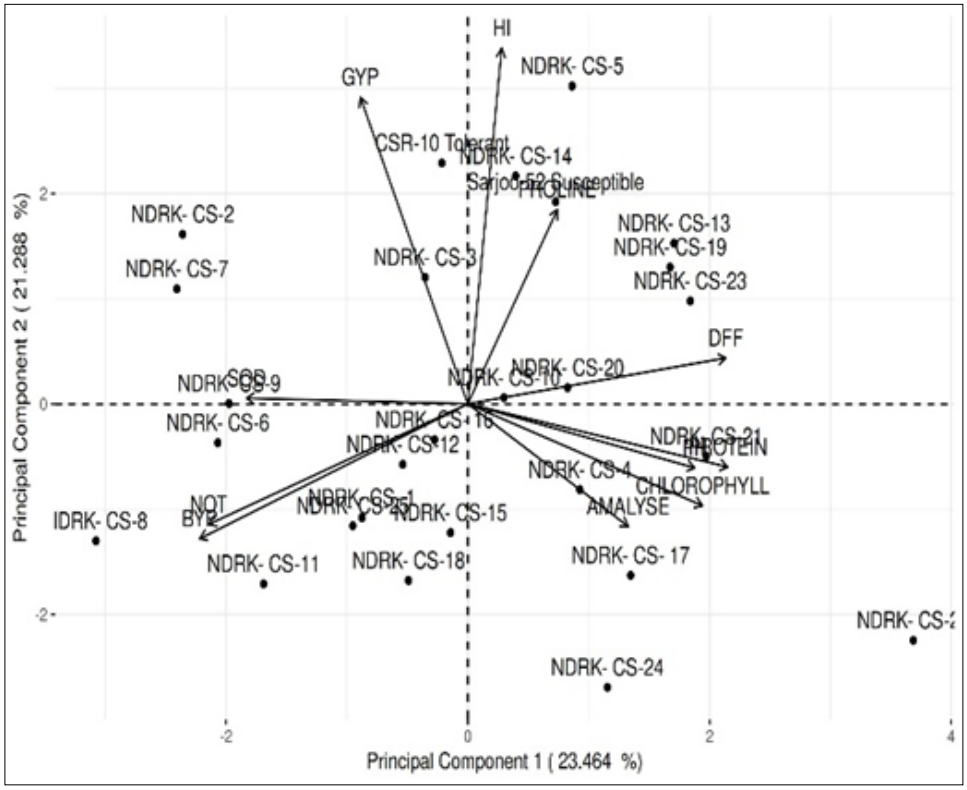

Fig. 3b. Biplot distribution of 27 rice lines and studied traits depending on principal component axes PC1 and PC2 in salinity conditions.

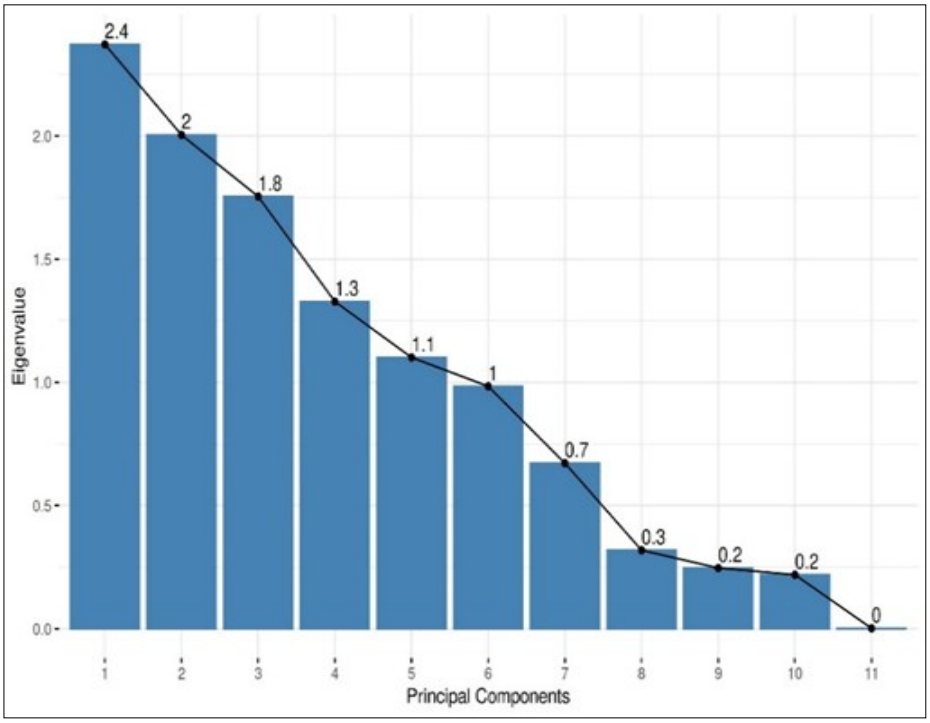

Fig. 4a. Scree plot diagram of Eigen values showing variation for eleven traits under normal condition.

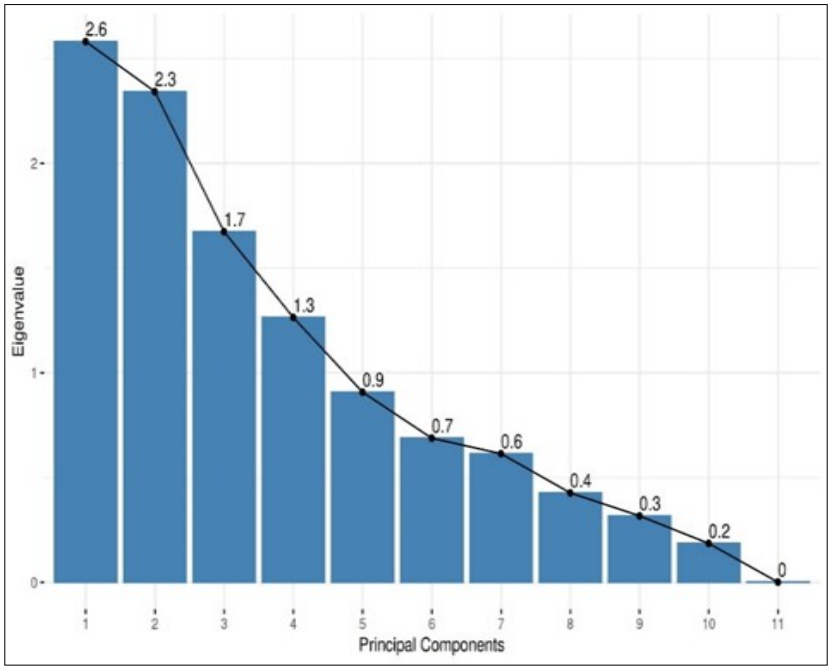

Fig. 4b. Scree plot diagram of Eigen values showing variation for eleven traits under saline condition.

majority of variation among genotypes, whereas under saline stress conditions, only PCA-1 to PCA-4 capture the maximum variation. This suggests that selection and breeding for saline tolerance should focus on the traits loading heavily on PCA-1 to PCA -4 under stress conditions as these likely reflect the most critical adaptive traits (26). These results indicate a substantial amount of total variability captured by the first few PCs in both environmental conditions, validating their importance for further trait-based interpretation (27).

Conclusion

The study was evaluated for various morphological and biochemical parameters in different rice lines under normal and salinity conditions. Results from the study revealed that the highest grain yield was found in NDRK-CS-22 (31.08 g) in normal conditions while in salinity conditions the highest grain yield was found in NDRK-CS-19 (19.84 g). In the tested rice lines under salinity stress condition, decreased in protein and chlorophyll content was observed. In contrast, increased levels of SOD and proline levels were observed interestingly under salinity stress condition. Traditional cultivars like CSR-10 (13.26, 10.69) showed high protein under both normal and salinity conditions. While genotypes such as NDRK-CS-24, NDRK-CS-19 and NDRK-CS-22 maintained higher chlorophyll under salinity suggesting better photosynthetic capacity. Amylase activity tended to decrease with salinity with NDRK-CS-18, NDRK-CS-13 and NDRK -CS-17 showing the highest activity. On the other hand, Sarjoo-52 exhibited high SOD and NDRK-CS-5/11 accumulated proline indicating stress response mechanisms. Phenotypic correlation

analysis revealed that GYP was strongly and positively associated with BYP (0.78) and chlorophyll content (0.40) under normal conditions, but these relationships turned negative under salinity. Notably, proline (0.17) and SOD (0.07) showed positive correlations with grain yield under saline conditions indicating their role in stress tolerance and yield stability. PCA identified five principal components under normal conditions explaining a cumulative variance of 77.81 %, with PC1 contributing 21.56 %. Under saline conditions, four components accounted for 71.45 % of the total variance with PC1 explaining 23.46 % indicating significant trait variability in both environments. The PCA biplot showed clustering of NDRK-CS-6, NDRK-CS-11, NDRK-CS-15 and Sarjoo-52 under normal conditions while NDRK-CS-1, NDRK-CS-4, NDRK-CS-15 and NDRK-CS-18 grouped under salinity. Biological yield and SOD were negatively correlated while amylase, proline and SOD were positively related. The scree plot identified PCA-1 to PCA-4 as key for selecting salinity-tolerant traits. Overall, the genotype NDRK-CS-2 emerged as the most promising line, along with NDRK-CS-19, NDRK-CS-22 and CSR-10 showing superior performance under saline conditions. These top-performing genotypes should be further validated across diverse saline environments using genomic approaches to develop resilient, high-yielding rice cultivars. Their high proline content and enhanced SOD activity, combined with better yield performance, highlight their potential as selection criteria for future salt-tolerant rice breeding programs.

Future scope

Future work should focus on validating these promising lines (especially NDRKCS- 2, 19, 22 and traditional CSR10) across diverse saline environments to confirm stability. Integrating genomic and transcriptomic approaches could unravel the genetic basis of observed biochemical traits (e.g. proline, SOD accumulation). Breeding strategies should target combining high yield, chlorophyll retention and stress-enzymatic profiles to develop robust, salt tolerant rice cultivars.

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Authors' contributions

RKM and SK designed the study, data collection, data analysis, interpretation of data and generated the first draft. DKD and NAK, designed the study related to biochemical parameters. SPS provided experimental materials. HKY and AK designed molecular work related to this research. AKS, JS, CT and AC have provided data analysis with results and discussion. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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