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Molecular, genetic and biochemical responses of rice genotypes (*Oryza Sativa* L.) under salinity conditions

Garima¹, D.K. Dwivedi², N.A. Khan³, Hemant Kumar Yadav⁴, Archana Devi⁵, Saurabh Dixit⁶, Preeti Kumari⁷, Noah Nawaz Khan⁸ and Mubeen⁹

^{1,2,3,4} Department of Molecular Biology & Biotechnology, Acharya Narendra Deva University of Agriculture and Technology Kumarganj, Ayodhya-224229, Uttar Pradesh, India

⁵ KVK, Amethi, Uttar Pradesh, India

⁶ CRS, Masodha, Ayodhya, Uttar Pradesh, India

⁷ Department of Genetics and Plant Breeding, Acharya Narendra Deva University of Agriculture and Technology Kumarganj, Ayodhya-224229, Uttar Pradesh, India

⁸ Department of Entomology, Acharya Narendra Deva University of Agriculture and Technology Kumarganj, Ayodhya-224229, Uttar Pradesh, India

⁹ Mohammad Ali Jauhar University, Rampur-244901, Uttar Pradesh, India

*Corresponding Author:- ddwivedi2000@nduat.org

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Abstract

A field experiment was conducted during the kharif season of 2023 to study the molecular, genetic, and biochemical responses of 20 rice genotypes (*Oryza Sativa* L.) under saline conditions. The experiment, conducted at Genetics & Plant Breeding Farm, Acharya Narendra Deva University of Agriculture and Technology, Kumar Ganj, Ayodhya (U.P.), utilized a Randomized Block Design. Analysis of variance indicated significant variability among genotypes for eleven traits. Phenotypic coefficient of variation (PCV) was highest for grain yield, harvest index, spikelet number, biological yield per plant, and grains per panicle. High heritability estimates were observed for grain yield, spikelet number, biological yield per plant, grains per panicle, and harvest index. Medium heritability estimates were noted for panicle bearing tillers per plant, test weight, spikelet fertility, plant height, panicle length, and days to 50% flowering.

Keywords: -Genetic Variability, Heritability, PCV, Biochemical Responses.

1. Introduction

Rice (*Oryza sativa*) is a self-pollinated monocotyledonous angiosperm belonging to the genus *Oryza*, in the family Poaceae (Graminae). It is primarily cultivated in hot, humid tropical climates but can also thrive in subtropical conditions. The plant typically requires a mean

temperature ranging from 21°C to 37°C throughout its growth cycle and rainfall exceeding 100 cm. There are two main cultivated species: Asian rice (*O. sativa*) and African rice (*O. glaberrima*), both of which are diploid with a chromosome count of $2n=24$. Additionally, there are 22 wild species of rice, some of which are tetraploid ($2n=48$). Rice is predominantly a Kharif crop (monsoon crop) grown annually in tropical regions. Global rice production, as estimated by the United States Department of Agriculture for 2023-24, is projected to reach 523.9 million metric tonnes. In India, approximately 46 million hectares of land are dedicated to rice cultivation, producing around 135 million metric tonnes during the 2022-23 season (Ministry of Agriculture & Farmer's Welfare).

In India, rice cultivation spans across 43.78 million hectares (mha), with irrigated areas accounting for 52.6%, rainfed areas for 32.4%, upland areas for 12%, and deepwater ecosystems for 3%. This distribution corresponds to specific areas of 23.02 mha, 14.8 mha, 5.24 mha, and 1.31 mha respectively. Rice is the single most important crop of the world as half of the world population eats rice every day.

Rice production globally faces significant challenges from both biotic and abiotic stresses. Among these, salinity stress poses a particularly severe threat, especially in arid and semi-arid regions. Salinity affects rice at various growth stages, disrupting physiological processes such as water and nutrient uptake, photosynthesis, and ultimately reducing yield potential. The impact of salinity on rice varies across growth stages, with seedling and reproductive stages often being the most vulnerable. Efforts to mitigate these effects include breeding salt-tolerant varieties, improving irrigation practices, and adopting agronomic practices that minimize salt accumulation in the soil. Addressing salinity stress is crucial for ensuring sustainable rice production and food security in affected regions.

2. Material and Methods

The present investigation, titled 'Molecular, genetic, and biochemical responses of rice (*Oryza sativa* L.) under salinity,' was conducted at the Genetics and Plant Breeding Farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.), Indiaduring the Kharif season of 2023. Using a Randomized Block Design, seeds of all 20 rice genotypes were sown on June 25, 2023, in a nursery bed with a spacing of 20×15 cm between rows and 20×15 cm between plants. After 35 days, a single seedling was transplanted per hill.

At the maturity stage of the experiment, observations were meticulously recorded from randomly selected plants within each variety and replication. Key parameters such as the plants were individually harvested and threshed, and various parameters were recorded, including Days to 50% flowering, Plant height (cm), Number of panicle-bearing tillers per plant, Panicle length (cm), Number of spikelet's per panicle, Number of grains per panicle, Spikelet fertility (%), Test weight (g), Biological yield per plant (g), Harvest index (%), and Grain yield per plant (g). By integrating these strategies systematically, you can enhance the likelihood of developing improved rice crop lines that are well-adapted to saline conditions and exhibit higher yields.

the experiments involved aggregating data on ten traits across selected plants or grains. These data were then analyzed using established methodologies. Analysis of Variance (ANOVA) was used to assess the experimental design, while the coefficient of variation was estimated using Burton and de Vane's method. Heritability and genetic advance were estimated using Kempthorne and Curnow's and Johnson et al.'s methods. Correlation coefficients were calculated to understand their interrelationships. Path-coefficient analysis was used to identify direct and indirect contributions of traits to overall performance. Genetic divergence (D₂) analysis was used to categorize genotypes based on their distinct characteristics.

Salinity stress is a major environmental factor limiting rice (*Oryza sativa* L.) productivity worldwide. Under saline conditions, the physiological and biochemical responses of rice plants are crucial for understanding their tolerance mechanisms. Enzymatic activities, such as those of Nitrate reductase (NR) and Nitrite reductase (NiR), play vital roles in nitrogen metabolism, which is critical for plant growth and development. Additionally, the accumulation of Osmo protectants like proline and total soluble sugars helps plants mitigate the detrimental effects of salt stress. **NR** is an enzyme involved in the reduction of nitrate to nitrite, a key step in nitrogen assimilation. Its activity is often affected by salinity, influencing overall nitrogen metabolism and plant health. **NiR** further reduces nitrite to ammonia, integrating nitrogen into organic compounds. Like NR, its activity is sensitive to saline conditions, affecting the nitrogen use efficiency of plants. **Proline** is an amino acid that accumulates in response to osmotic stress, acting as an Osmo protectant and stabilizing cellular structures **Total Soluble Sugars** play a similar role, contributing to osmotic adjustment and providing an energy source under stress conditions

3. Result & discussion

In the study evaluating the response of 20 rice genotypes under saline conditions, significant variations were observed across 11 key traits, as indicated by the mean sum of squares estimation. Conducted using a Random Block Design with three replications, the analysis of variance highlighted distinct impacts of salinized conditions on these traits. Table 3.1 summarizes the mean sum of squares contributions from replication, treatment, and error, reflecting the diverse responses of the genotypes under salt stress. This comprehensive assessment underscores the importance of genotype-specific responses in breeding resilient rice varieties capable of thriving in saline environments.

These parameters provide crucial insights into the genetic and phenotypic variations observed across different rice genotypes under saline stress. The findings contribute to understanding the adaptability and potential for improvement of these genotypes in saline environments, aiding future breeding programs aimed at enhancing rice productivity under challenging conditions. Table 3.2 presents comprehensive estimates for 11 key traits in rice genotypes subjected to saline conditions.

The study evaluated the grand mean, genotypic variability, phenotypic variability, heritability in broad sense (h^2_b), genetic advance, genetic advance as a percentage of the mean, genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), and percent contribution for each trait.

The grain yield in rice demonstrated a highly significant and positive correlation with harvest index (0.640), grains per panicle (0.626), panicle-bearing tillers per plant (0.509), and biological yield per plant (0.491). The harvest index also showed a highly significant and positive correlation with panicle-bearing tillers per plant (0.391) and spikelet number (0.346) while displaying a significant negative correlation with biological yield per plant (-0.347). Furthermore, biological yield per plant exhibited a strong positive correlation with grains per panicle (0.558). Test weight was positively correlated with panicle length (0.415) but negatively correlated with panicle-bearing tillers per plant (-0.329). Additionally, grains per panicle showed a significant positive correlation with panicle-bearing tillers per plant (0.291). Conversely, panicle-bearing tillers per plant had a highly significant negative correlation (-0.397), while

panicle length exhibited a positive correlation with plant height (0.305). Similar findings were reported by Fiyaz et al. (2011), Rahman et al. (2013), and Chamar et al. (2021). Table 3.3 summarizes Phenotypic correlation under saline condition.

Table 3.4 presents the direct and indirect effects of various characters on grain yield per plant in rice genotypes under saline conditions, evaluated at the phenotypic level. The analysis highlights how different traits contribute to grain yield, either through their direct influence or by indirectly affecting other traits. Understanding these relationships is crucial for breeding programs aimed at improving rice yield in saline environments. The table reveals which traits have the most significant impact, providing insights into potential targets for genetic improvement to enhance salinity tolerance and overall yield performance.

Nitrate reductase (NR) is a key enzyme in the nitrogen assimilation pathway, catalyzing the reduction of nitrate (NO_3^-) to nitrite (NO_2^-), which is further reduced to ammonia (NH_3) and incorporated into amino acids and other nitrogen-containing compounds. This enzyme is crucial for plant growth and development, particularly in nitrogen-limited environments. Nitrate reductase activity was assayed from rice leaves of 20 different genotypes is presented in Figure 1.

Nitrite reductase (NiR) is an essential enzyme in the nitrogen metabolism of plants, playing a pivotal role in the reduction of nitrite to ammonia within the nitrogen assimilation pathway. In rice (*Oryza sativa* L.), NiR activity is crucial for maintaining nitrogen homeostasis and ensuring optimal growth and development. Under salinity stress, such as exposure to NaCl, plants undergo significant physiological and biochemical changes, impacting various metabolic processes, including nitrogen assimilation. The estimation of nitrite reductase (NiR) activity was done by rice leaves, from 20 different rice genotypes is Presented in Figure 2.

Proline is an amino acid that plays a crucial role in plant stress responses, particularly under salinity stress. In rice (*Oryza sativa* L.), the accumulation of proline is a common physiological response to salinity, serving as an osmoprotectant that helps in maintaining cell turgor, stabilizing proteins, and protecting cellular structures from the detrimental effects of high salt concentrations. Proline was measured in both root and shoot in vegetative stage and results are presented in Figure 3.

Total soluble sugar (TSS) content in rice (*Oryza sativa* L.) is an essential biochemical trait that significantly influences the plant's ability to cope with various abiotic stresses, including salinity. Soluble sugars serve as vital osmoprotectants, contributing to osmotic adjustment, maintaining cellular turgor, and stabilizing proteins and membranes under stress conditions. The evaluation of TSS levels in different rice genotypes can provide insights into their stress tolerance mechanisms, aiding in the selection and breeding of more resilient varieties. The total soluble carbohydrate was estimated in leaf and grain by phenol sulphuric acid method. The amount of total soluble sugars was greater in grain as compared to leaves is Presented in Figure 4.

The estimation of genetic distance using Mahalanobis distance under saline conditions, as shown in Figure 5, is vital for understanding the adaptability and diversity of rice genotypes. This method, which incorporates multiple trait correlations, offers a robust measure of genetic variability, aiding in the selection of salinity-tolerant genotypes for breeding programs. Protein profiling using SDS-PAGE, presented in Figure 6, analyzes the differential expression of proteins in 20 rice genotypes under salinity stress, revealing specific protein bands associated with salt tolerance. This technique helps identify stress-responsive proteins and potential biomarkers. Additionally, a dendrogram in Figure 7 illustrates the clustering of these genotypes based on leaf protein content, highlighting genetic diversity and varying tolerance levels. This visual tool is essential for identifying and breeding salinity-tolerant rice varieties.

DNA isolation from the 20 rice genotypes was conducted successfully. The extracted DNA was of high quality, suitable for downstream molecular analyses. The integrity of the DNA samples was confirmed through agarose gel electrophoresis, which is presented below in Figure 8. The clear and distinct bands observed on the gel indicate that the DNA samples are intact and free from significant degradation or contamination, ensuring reliable results for subsequent genetic studies. Figure 8: Agarose gel electrophoresis of isolated DNA from 20 rice genotypes.

Table 3.1 Analysis of variance for different characters of 20 rice genotypes under saline condition

Characters	Sources of variation			Total
	Replication	Treatment	Error	
d . f .	2	19	38	59
Daysto 50% flowering	7.32	12.03**	3.98	6.69
Plant height(cm)	52.59	79.25**	14.66	36.75
Panicle bearing tillers/ plant	0.01	1.35**	0.11	0.51
Panicle length(cm)	0.19	5.19**	1.23	2.47
Number of spikelet's	3.41	743.72**	27.00	257.01
Grains/panicle	31.56	273.31**	14.38	98.35
Spikelet fertility(%)	3.83	95.08**	11.69	38.28
Test weight(g)	0.34	10.44**	1.07	4.07
Biological Yield(g)	8.14	69.32**	2.67	24.32
Harvest Index	7.69	138.50**	9.25	50.82
Grain yield(g)	0.28	27.70**	0.61	9.32

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

Table.3.2 Estimates of grand mean, genotypic variability, phenotypic variability, heritability in broad sense (h^2_b), genetic advance, genetic advance in percent of mean, genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV) and percent contribution for 11 characters in 20 rice genotypes under saline condition

Characters	Grand Mean	Min	Max	var(g)	var(p)	Heritability (%)	Genetic advance	Genetic advance in percent of mean	GCV (%)	PCV (%)	Percent contribution
Daysto 50%flowering	93.08	89.33	95.33	2.01	5.99	33.56	1.69	1.82	1.52	2.63	13.10
PlantHeight	116.93	108.83	124.90	16.14	30.81	52.41	5.99	5.12	3.44	4.75	11.91
PanicalLength	23.82	20.29	25.96	0.98	2.22	44.56	1.37	5.74	4.18	6.25	11.99
ProductiveTillers	8.59	7.47	10.47	0.311	0.42	73.91	0.99	11.50	6.49	7.55	10.81
TotalSpikelets	124.80	99.47	170.33	179.18	206.18	86.90	25.71	20.60	10.73	11.51	8.48
Noofgrain/panical	94.26	78.67	115.07	64.73	79.11	81.82	14.99	15.90	8.54	9.44	9.21
%fertility	80.79	67.20	88.79	20.84	32.54	64.07	7.53	9.32	5.65	7.06	9.48
TestWeight(g)	24.47	21.30	27.59	2.34	3.41	68.53	2.61	10.66	6.25	7.55	8.88
BiologicalYieldPerplant(g)	38.96	32.00	49.20	16.66	19.33	86.20	7.81	20.04	10.48	11.29	5.25
HarvestIndex(%)	50.95	35.11	62.34	32.31	41.56	77.74	10.32	20.27	11.16	12.65	4.63
GrainYield(g)	19.73	14.62	25.52	6.77	7.38	91.70	5.13	26.02	13.19	13.78	6.27

Note:*min-Minimum,*max-Maximum,var(g)*-Genotypicvariability,var(p)*-Phenotypicvariability.

Table-3.3Phenotypiccorrelationundersalinecondition

Characters	Daysto50%flowering	Plant Height (cm)	Panical length(cm)	Panical bearing tillers/plant	Spikelets No.	Grains perpanical	Spikelet fertility %	TestWeight(g)	BiologicalYield/plant (g)	Harvest Index(%)	GrainYield/plant(g)
Daysto 50%flowering	1.000	-0.102	0.217	-0.077	-0.051	0.012	-0.062	0.138	0.261*	0.000	0.196
PlantHeight (cm)		1.000	0.305*	-0.397**	-0.002	0.070	0.035	0.138	-0.019	-0.143	-0.156
Panicallength(cm)			1.000	-0.079	-0.143	0.216	-0.234	0.415**	0.312*	0.034	0.286*
Panicalbearingtillers/plant				1.000	0.007	0.291*	0.105	-0.329*	0.142	0.391**	0.509**
Spikelets No.					1.000	0.243	-0.187	-0.184	-0.080	0.346**	0.220
GrainsPerpanical						1.000	0.209	0.246	0.558**	0.197	0.626**
Spikeletfertility%							1.000	0.095	0.259*	-0.190	0.056
TestWeight(g)								1.000	0.307*	-0.061	0.213
BiologicalYield/Plant(g)									1.000	-0.347**	0.491**
HarvestIndex(%)										1.000	0.640**
GrainYield/ Plant(g)											1.000

*,** significantat5%and 1%level, respectively.

Table 3.4 Direct and indirect effect for different characters on grain yield per plant under saline condition at phenotypic level in rice genotypes

Characters	Daysto 50% flowering	PlantHeight(cm)	Panical length(cm)	Panical bearing tillers/plant	Spikelets No.	Grains per panical	Spikelet fertility %	TestWeight(g)	BiologicalYield/plant(g)	Harvest Index(%)	Grain Yield/plant(g)
Daysto 50% flowering	-0.012	-0.001	0	-0.005	0	0	-0.001	0.007	0.208	0	0.196
PlantHeight (cm)	0.001	0.012	-0.001	-0.028	0	-0.002	0	0.007	-0.015	-0.130	-0.156
Panical length(cm)	-0.002	0.003	-0.003	-0.005	0.001	-0.007	-0.002	0.022	0.249	0.030	0.286*
Panical bearing tillers/plant	0.001	-0.004	0	0.070	0	-0.009	0.001	-0.017	0.113	0.355	0.509**
Spikelets No.	0.001	0	0.001	0.001	-0.011	-0.007	-0.002	-0.009	-0.064	0.314	0.220
Grains Per panical	0	0.001	-0.001	0.020	-0.002	-0.032	0.002	0.013	0.446	0.179	0.626**
Spikelet fertility%	0.001	0	0	0.007	0.002	-0.006	0.011	0.005	0.206	-0.172	0.056
TestWeight(g)	-0.001	0.001	-0.001	-0.023	0.002	-0.007	0.001	0.053	0.245	-0.055	0.213
BiologicalYield/Plant(g)	-0.003	0	-0.001	0.010	0	-0.018	0.002	0.016	0.799	-0.315	0.491**
HarvestIndex(%)	0	-0.001	0	0.027	-0.004	-0.006	-0.002	-0.003	-0.277	0.908	0.640**

Residual effect = 0.0060

Bold values show direct and normal values show indirect effect.

*, ** significant at 5% and 1% level, respectively.

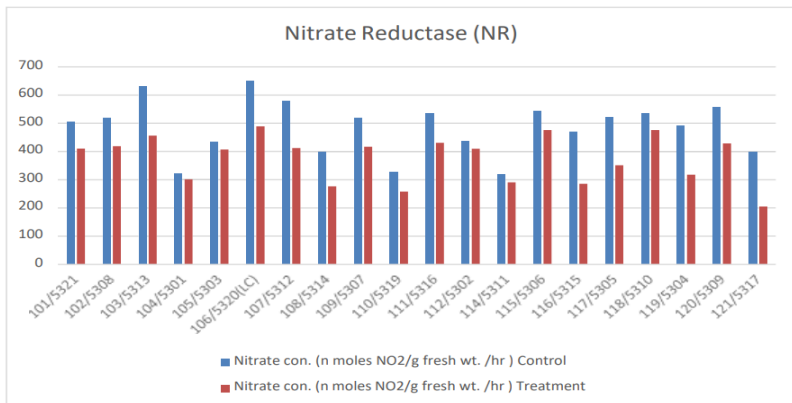


Figure 1. Graph of Nitrate reductase activity in Rice leaves

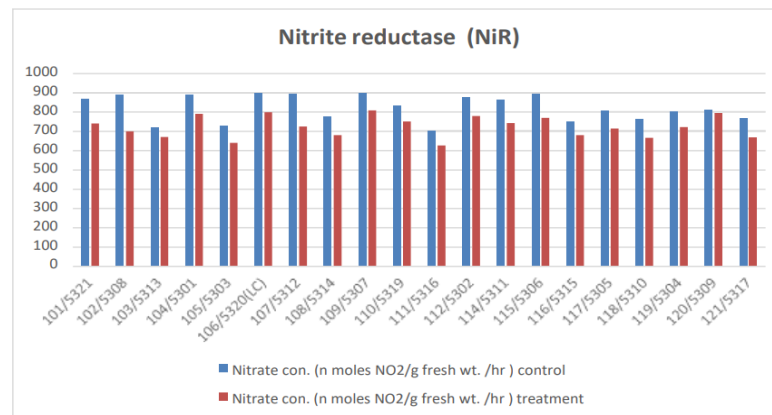


Figure 2. Graph of Nitrite reductase activity in Rice leaves

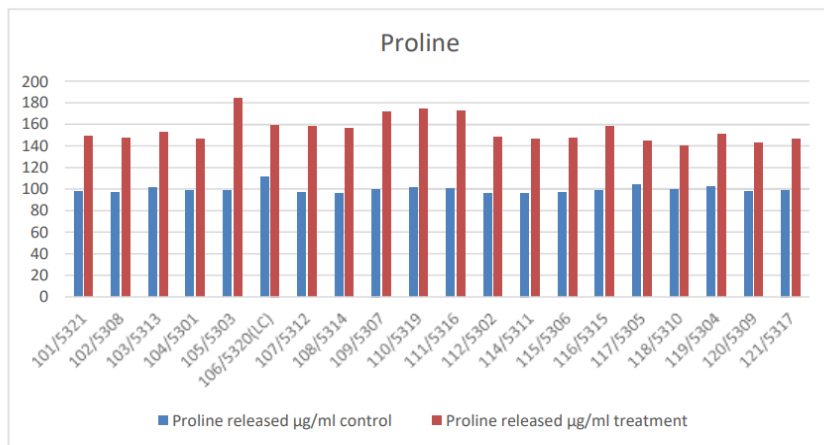


Figure 3. Graph of proline activity in Rice leaves

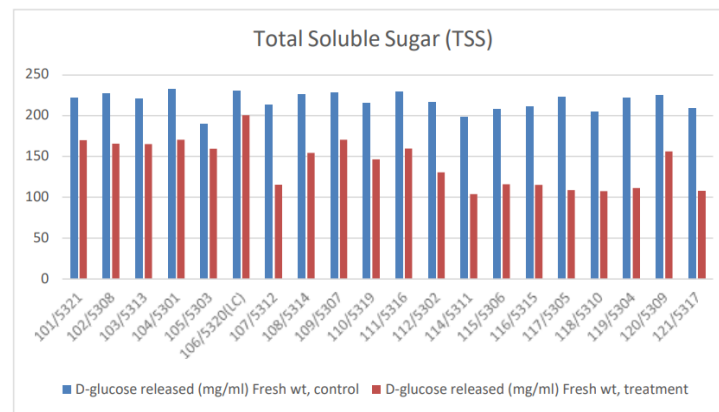


Figure 4. Graph of total soluble sugar estimation in rice leaves

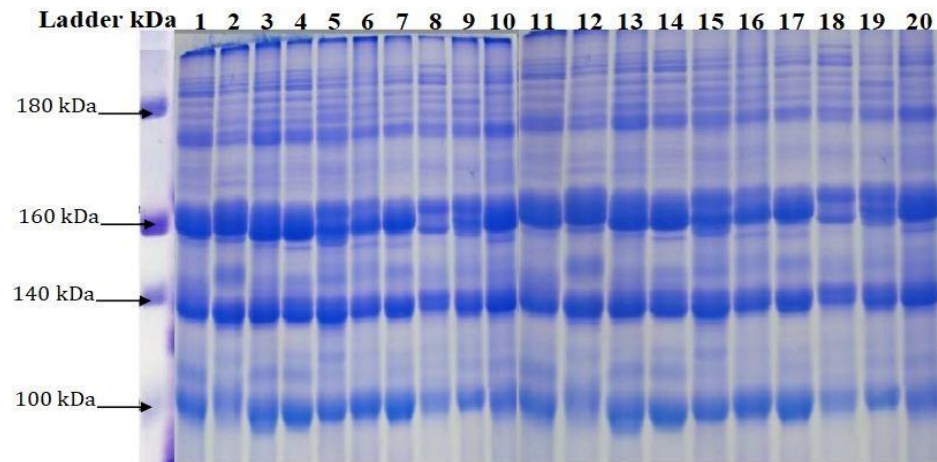
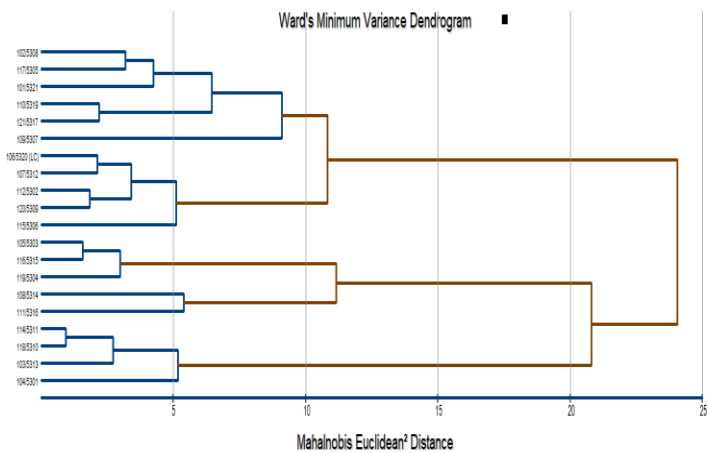


Figure -5 mahalanobis under saline condition Figure-6 SDS PAGE Protein band of rice leaves

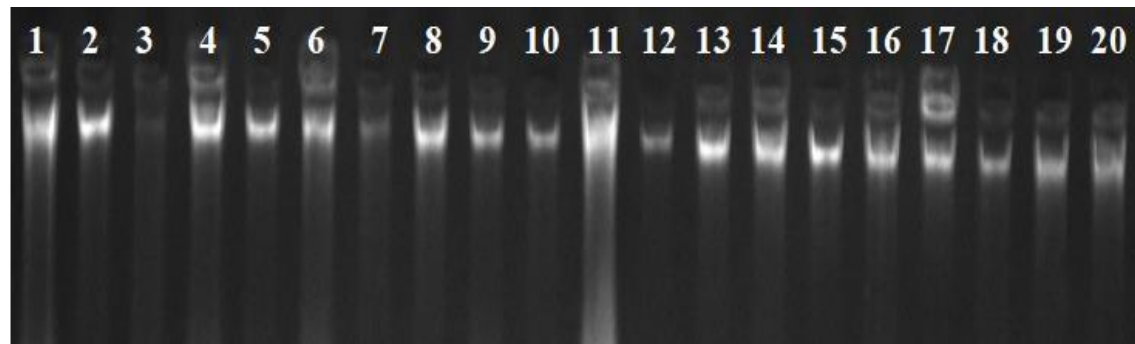
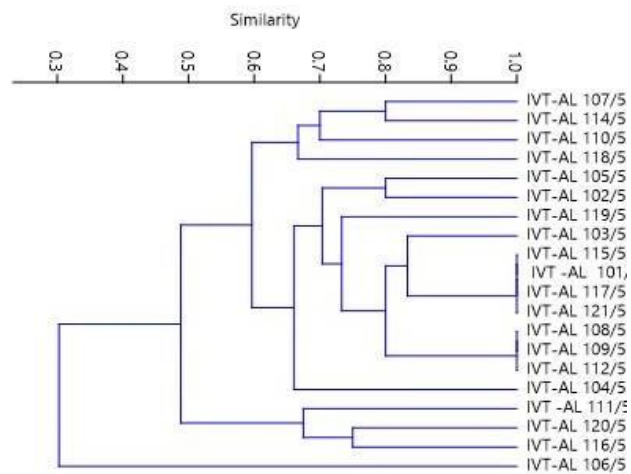


Figure-7 Dendrogram of rice leaves protein Figure-8 Gel electrophoresis of DNA isolation

Conclusion

The statistical analysis revealed significant variability among the 20 rice genotypes under saline conditions. The phenotypic coefficient of variation was higher than the genotypic coefficient for all eleven characters, with notable variability in grain yield, harvest index, spikelet's number, biological yield per plant, and grains per panicle. High heritability was observed for grain yield, spikelet's number, biological yield per plant, grains per panicle, and harvest index, while medium heritability was found for panicle bearing tillers per plant, test weight, spikelet fertility, plant height, panicle length, and days to 50% flowering. The harvest index had the highest positive direct effect on grain yield, followed by grains per panicle and panicle bearing tillers per plant, with grains per panicle also showing a significant positive indirect effect via biological yield per plant. The genotypes were grouped into four clusters, with days to 50% flowering, spikelet fertility, harvest index, grain yield, and spikelet's number contributing the most to genetic divergence.

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