Mysore J. Agric. Sci., 57 (3) : 88-96 (2023)

Estimation of Genetic Diversity for Grain Yield and its Component Traits in Rice (*Oryza sativa* L.) under Coastal Saline Condition

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Received : May 2023
Accepted : July 2023

Abstract

Mahalanobis D^2 statistics were used to determine the nature and extent of genetic divergence in 48 rice genotypes while taking 11 quantitative traits into account. ANOVA divulges the presence of the significant amount of variability. Effective conservation and utilization of rice genetic resources depend on a thorough under standing of genetic diversity. Forty-eight rice genotypes were grouped into eight clusters. Maximum genotypes were included in Cluster II (19) followed by cluster III (11) and Cluster I (8) and the rest of the clusters contain two genotypes each. The maximum inter-cluster distance was obtained between clusters IV and VIII (218.39) and the minimum inter-cluster distance was observed between Cluster V and VII (63.541). The intra-cluster range was from 23.84 to 116.68. High intra-cluster distances were shown by cluster VIII (116.68) followed by cluster II (99.38) and cluster I (84.997). The hybridization among the genotypes having high inter-cluster distances with high cluster mean values for grain yield (clusters I and II), number of productive tillers plant^{-1} (cluster I, VII, IV, III), number of grains per panicle (cluster IV, I), 1000 grain weight (cluster V and VI) and grain length (cluster VIII and II) would give high heterotic F_1's and better segregants in the F_2 generation. This indicated that the genotypes in these clusters have a broad spectrum of genetic diversity and could very well be used in the hybridization programme to develop high-yielding varieties. The genotypes from cluster VIII (FL478 and pokkali) having high inter-cluster distance with remaining clusters except cluster II may be used as donors in future breeding programmes for their potential tolerance to salinity in the development of high-yielding rice varieties under saline conditions.

Keywords : Rice, Salinity, Mahalanobis D^2 analysis, Clustering of genotypes, Genetic divergence

Rice (*Oryza sativa* L.) belongs to the family Poaceae (Graminae) and is one of the most important food crops in the world, providing food for more than half of the world's population (Baroudy *et al*., 2020). Globally rice is cultivated in an area of 166.25 million hectares with a production of 515.08 million metric tonnes. In India, rice is cultivated in an area of 47 million hectares producing 130.29 million tonnes with an average productivity of 2629.25 kg ha^{-1}. Asia produces and consumes 90 per cent of the world's rice (Singh *et al*., 2015). India is second only to China in terms of production with 117.94 million metric tonnes (Anonymous, 2020). Owing to a burgeoning population, it is estimated that the demand for rice will be 121.2 million metric tonnes by the year 2030, 129.6 million metric tonnes by the year 2040 and 137.3 million metric tonnes by the year 2050. If the cultivated area under plough remains at the current level, rice production should increase to 3.4 metric tons per ha from the current 2.4 metric tons per ha (Anonymous, 2013).

Against the backdrop of all these successes, rice farmers and researchers, however, face threats from

climate change, temperature fluctuations, low water availability, poor soil quality and low nutrient availability. These abiotic stresses cause significant problems by reducing crop growth and productivity of rice. Although active suppression of growth is a strategy helpful for maximizing plant survival in a stressed condition, it often negatively impacts crop productivity (Zhang *et al*., 2020). Increasing salinity is a major challenge for continuing rice production. Though rice is affected by salinity at all the developmental stages, it is most sensitive at the early seedling stage. The yield thus depends on how many seedlings can withstand saline water at the stage of transplantation, especially in coastal farms (Tiwari *et al*., 2022). Around 955 Mha of global earth land out of which 58 per cent are in irrigated areas nearly accounts for 20 per cent of cultivable land and half of the total irrigated land is more or less affected by different kinds of salt and associated threats (Basak *et al.,* 2022 and Srinivasan *et al.,* 2022). Millions of hectares in the humid regions of South and Southeast Asia are technically suited for rice production but are left uncultivated or are grown with very low yields because of salinity and abiotic stresses (BojeKlein 1986).

Thus, it is understood that the utilization of less productive lands, including salinized lands is an absolute requirement for the growing demand for food. The use of some management options can ameliorate yield reduction under salinity stress. However, implementation of such practice is often limited because of high cost and less availability of good quality water. The NaCl decreases the seed germination in proportion to the increase in NaCl concentration. Higher concentrations of NaCl results in strong inhibition of germination, root length and shoot length in comparison to lower concentrations. There was a decreased trend in the vigour index when salt concentration increased (Meghana et al., 2015).Therefore, the need for genetic improvement of salt tolerance in rice plants through an integrated approach that employs physiology, biochemical, proteomics and molecular studies for identifying salt tolerance genes for genetic improvement of rice varieties is of utmost concern (Reddy *et al*., 2017) as it is the practical way to meet the ever-growing demand for food for the burgeoning population. Another complication that needs to be considered while breeding for salinity tolerance in rice genotypes with varying levels of salinity tolerance at different growth stages. Salinity tolerance at the seedling stage is independent of salinity tolerance at the flowering/reproductive stage. Due to the variation in sensitivity to salinity during the life cycle, the evaluation of salinity tolerance in rice is complex. Some genotypes have been comparatively tolerant at the flowering stage due to better viability of pollen and higher grain yields (up to 40%) under salinity stress. Several traditional, landraces & wild types of rice like pokkali, CSR types and *Porteresia coaratata* appear as promising materials for donation of requisite salt tolerance genes (Sahil *et al.,* 2006). One of the traditional cultivars, *Pokkali* has been recognized for having higher degree of salt tolerance than tolerant cultivars and thus used as a high potential salt tolerant donor parent for breeding programme (Bonila *et al*., 2002). The FL478 (*Oryza sativa* L. ssp. *indica*) line was developed, which has high levels of seedling-stage salinity tolerance, lacks photoperiod sensitivity and is shorter in height and life cycle than the original salt-tolerant Pokkali landrace (Thomas *et al*., 2010). The genotypes FL-478, CSR-23, CSR-36, KMP-220 and Binadhan-8 displayed high tolerance to salt stress at both germination and initial seedling growth stages for several growth parameters (Sandesh *et al.,* 2022).

Genetic improvement is responsive to crossings between parents who have the greatest genetic divergence. It has been determined that the multivariate approach created by Mahalanobis (1936) is the most effective at quantifying the level of divergence in germplasm. To identify and develop desirable genotypes, the presence of genetic variability for morphological, yield-related and quality traits is of utmost importance. This is because achievement in any trait improvement depends on the degree of genetic variability existing in the experimental material for that trait. In addition to genetic diversity, useful characteristics on which selection efficiency relies are heritability and genetic advancement. Heritability is a measure of how easily a trait is passed

down from one generation to the next and plays a prognostic role in crop breeding programmes. However, estimates of heritability alone fail to indicate the response to selection. As a result, assessments of genetic advance and heritability take into consideration the genetic advancement of related genotypes over the parental population for various attributes. Admitting the importance of genetic diversity and variability in plant breeding experiments present research work was undertaken in rice.

TABLE 1

Particulars of the field

Particulars	Field details
Location	Experimental farm Department of Genetics and Plant Breeding Annamalai Nagar Cuddalore dt.Tamilnadu
Latitude	11.3921° N
Longitude	79.7146° E
Season	Kuruvai, June 2020
Soil type	Clay
Soil Ph	7.9
EC	4.01 ± 0.13 ds/m
N	High
P	Medium
K	High

MATERIAL AND METHODS

The present investigation was conducted during *Kuruvai* (June, 2020) at the Experimental farm, Department of Genetics and Plant Breeding, Annamalai University, Chidambaram, Tamil Nadu, India (Table 1). In the present study, forty-eight genotypes were assessed to study the variability and genetic parameters for yield and its components, quality and nutritional traits (Table 2). Twenty-six days old seedlings were sown in three rows of 6m in length following a spacing of 20cm between the rows and 15cm between the plants in a randomized block design (RBD) with three replications. Standard agronomic practices were performed uniformly for all the experimental units. The crop was raised following recommended package of practices. Phenotypic data on days to 50 per cent flowering was recorded for plots of each genotype. At maturity ten plants from each accession were selected randomly

TABLE 2

List of genotypes

S. No	Genotypes	S. No	Genotypes	S. No	Genotypes
1	ADT 43	17	ADT 55	33	CO 51
2	ADT45	18	ADT 52	34	IR 64
3	ADT47	19	ADT 57	35	IR 20
4	ADT 37	20	ADT 53	36	IR 36
5	ADT 36	21	IVT 4910	37	IR 50
6	ADT 41	22	ADT 54	38	FL478
7	ADT 42	23	TRY 1	39	POKKALI
8	ADT 48	24	TRY 2	40	SWARNA SUB -1
9	ADT 39	25	TRY 3	41	PUSA 44
10	ADT 44	26	CSR 10	42	PKM 3
11	ADT 40	27	CSR 23	43	MDU 5
12	ADT 49	28	CSR 36	44	TKM 13
13	ADT 50	29	CSR 13	45	TKM 11
14	ADT 46	30	CO43	46	TKM 9
15	ADT 38	21	CO 50	47	TKM 14
16	ADT 56	32	CO 49	48	TKM 6

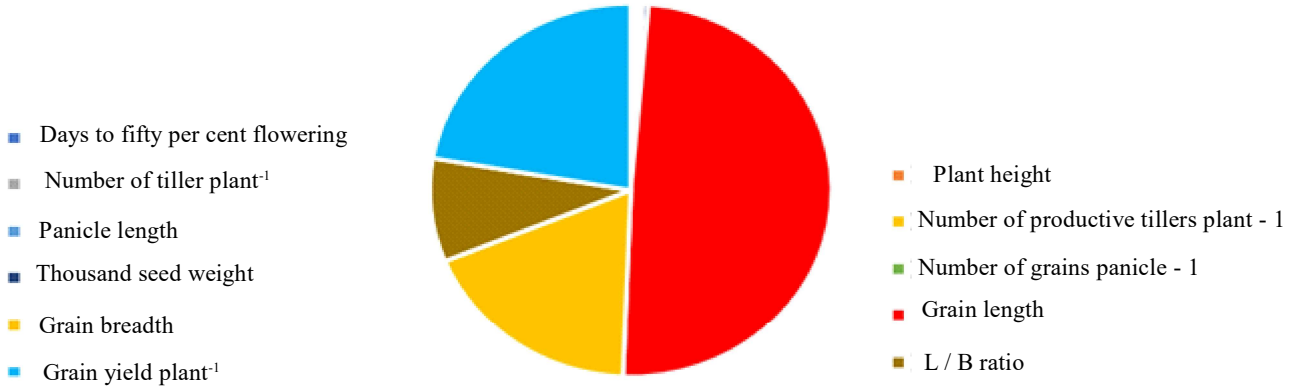

Fig. 1 : Genetic diversity contribution %

for recording data on days to fifty percent flowering, plant height at maturity, number of tillers plant^{-1}, number of productive tillers plant^{-1}, panicle length, number of grains panicle^{-1}, thousand seed weight, grain length, grain width, L/B ratio and grain yield plant^{-1} The treatment means for all the characters were subjected to analysis of variance techniques based on the model proposed by Panse and Sukhatme, 1967. In contrast, observations for test weight and all the quality and nutritional traits studied were obtained from a random grain sample drawn from each plot and replication using standard procedures. The mean performance of the genotypes was calculated and the data were subjected to Mahalanobis D^2 statistics to

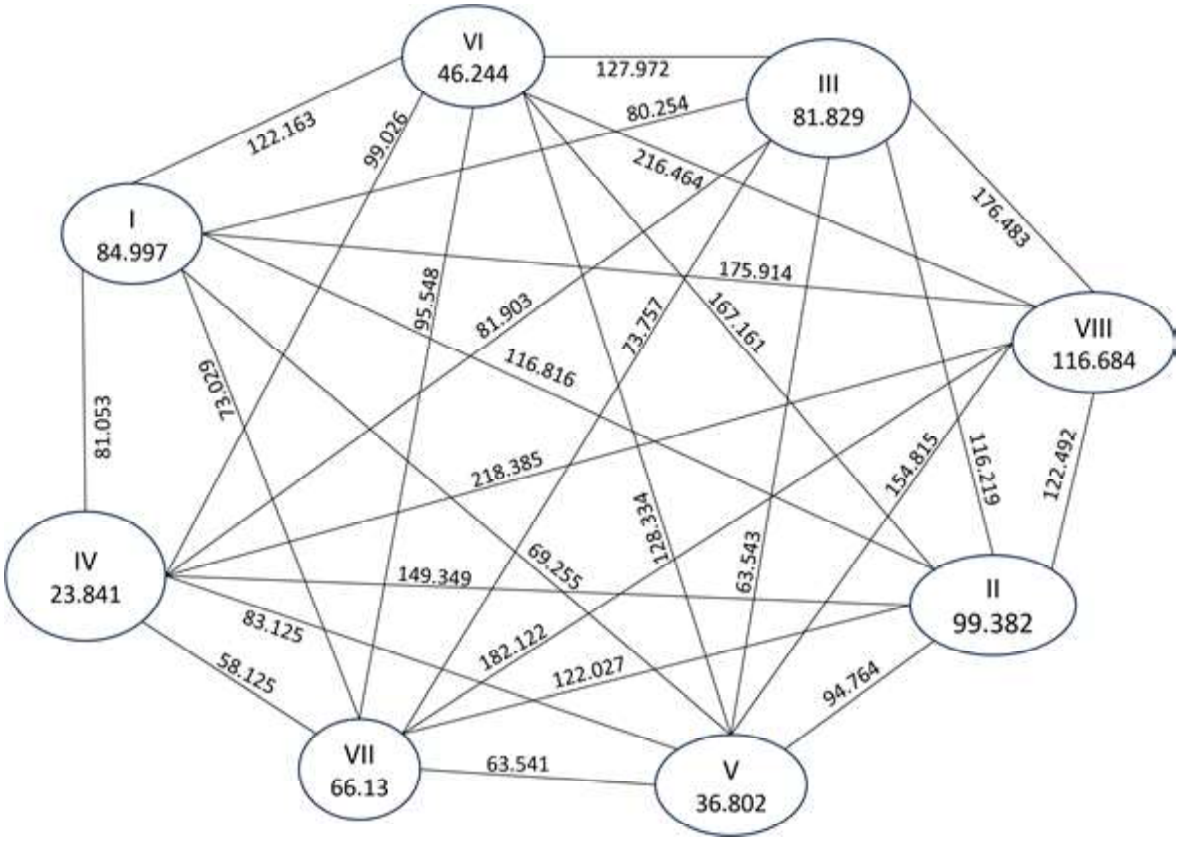

Fig. 2 : Cluster diagram based on Mahalanobis D values

measure the genetic divergence as suggested by Rao (1952). Cluster analysis was employed to determine genotype diversity and the contribution of traits to total divergence, respectively. The collected data was subjected to software analysis TNAU-STAT.

Results and Discussion

The analysis of variance (ANOVA) revealed significant differences among the genotypes for all characters studied (Table 3), demonstrating the presence of the significant amount of variability and intrinsic genetic variation in the genotypes studied which provides the plant breeder with the opportunity to select appropriate genotypes for the further breeding program. Mahalanobis D^2 statistics have been analysed to determine the degree of differentiation among {n(n-1)/2=1128} pairs of n=48 population. The perusal of the results revealed that all 48 genotypes were grouped into eight clusters (Table 4) based on D^2 values. The genotype distribution showed that the geographical and genetic diversities were unrelated to one another, indicating that there were additional forces at work other than just geographic separation.

Table 4

Distribution of the 48 rice genotypes into different clusters

Clusters	No. of Genotypes	Genotypes
I	8	ADT-43, ADT-45, ADT-47, ADT-37, ADT-36, ADT-41, CO-49, CO- 51
II	19	ADT-42, ADT-48, ADT-39, ADT-44, ADT-40, ADT-49, ADT-50, ADT-6, ADT-38, ADT-56, ADT-55, ADT- 52, ADT-57, ADT-53, IVT-4910, ADT-54, TRY-1, TRY-2, TRY-3
III	11	CSR-10, CSR-23, CSR-36, CSR-13, CO-43, CO-50, IR-64, IR-20, IR-36, IR-50, SWARNA SUB-1
IV	2	TKM-13, TKM-9
V	2	MDU-5, TKM-6
VI	2	PUSA-44, PKM-3
VII	2	TKM-11, TKM-14
VIII	2	FL-478, POKKALI

Table 3

Analysis of variance for yield and its components traits in rice (*Oryza sativa* L.)

Sources	df	Mean Sum of Square									
		Days to 50 percent flowering	Plant height at maturity	Number of tillers plant^{-1}	Number of productive tillers plant^{-1}	Panicle length	Number of grains panicle^{-1}	Thousand seed weight	Grain length	Grain width	L/B ratio	Grain yield plant^{-1}
Replication	2	6.15	9.125	2.789	1.00	0.85	31.54	0.91	0.0009	0.0004	0.0001	2.17
Genotype	47	189.92 **	738.88 **	54.15 **	22.94 **	18.70 **	1293.68 **	42.35 **	3.912 **	0.58 **	1.114 **	83.78 **
Error	94	1.83	3.20	0.94	0.33	0.33	10.05	0.34	0.0003	0.0002	0.0005	0.50

df= Degrees of freedom, **= Significant at1% level.

Various factors contributed to the diversity, including genetic drift, exchange of breeding material, natural and artificial selection and environmental variation. Cluster analysis using average linkage methods based on their similarity through quantitative traits grouped 48 rice genotypes into eight clusters. Maximum genotypes were included in Cluster II (19) followed by Cluster III (11) and Cluster I (8) and the rest of the clusters with two genotypes each. Clustering did not follow any particular pattern with respect to the origin. The present study revealed that considerable diversity existed both within and between the clusters and was thereby reliable enough for hybridization and selection.

The discrimination of the germplasm into so many clusters revealed that the material under study contained a significant level of genetic diversity. Significant genetic divergence has also been discovered by earlier researchers in the rice material. Because the germplasm lines tested in the current study had a significant amount of genetic diversity, this material may be used to choose the diverse parents for hybridization programmes intended to isolate superior segregants for yield and other essential traits. Additionally, the choice of parents is influenced heavily by the character's contributions (Devi *et al.,* 2016). The trait grain length (49.20) contributes more to genetic diversity followed by grain yield plant^{-1} (22.25) and grain breadth (18.17) (Table 7). It would be more effective to select suitable distinct parents based on genetic divergence analyses than to do so based solely on geographical distances. This result is consistent with earlier studies that argued there was no correlation between the genetic and geographic diversity of rice. In order to boost the probability of getting good segregants in segregating populations, (Cheema *et al*., 2004) proposed that the number of clusters produced and the number of genotypes present in the cluster reflected the probability of genetic improvement for yield and yield components. Crossing small diversified genotypes from the same cluster has very little chance of producing good segregants.

The estimates of average intra and inter-cluster distance for eight clusters revealed that the genotypes present in a cluster had little genetic divergence from each other with respect to the aggregate effect of 11 characters under study while much more genetic diversity was observed between the genotypes belonging to different clusters. The varying magnitude of intra and inter-cluster distances among the genotypes studied are presented in Table (5). The maximum inter- cluster distance was obtained between clusters IV and VIII (218.39) followed by clusters VI and VIII (216.46); the minimum cluster distance was observed between Clusters V and VII (63.541) followed by clusters III and VIII (63.543). The intra-cluster range was from 23.84 to 116.68; high intra-cluster distances were shown by cluster VIII (116.68) followed by cluster II (99.38) and

TABLE 5

Average D- square distance between 8 clusters

Clusters	I	II	III	IV	V	VI	VII	VIII
I	84.997	116.816	80.254	81.053	69.255	122.163	73.029	175.914
II		99.382	116.219	149.349	94.764	167.161	122.027	122.492
III			81.829	81.903	63.543	127.972	73.757	176.483
IV				23.841	83.125	99.026	58.125	218.385
V					36.802	128.334	63.541	154.815
VI						46.244	95.548	216.464
VII							66.13	182.122
VIII								116.684

TABLE 6

Cluster mean values of 8 clusters for 11 quantitative characters in 48 rice genotypes.

Clusters	days to 50 per cent flowering	plant height at maturity	Number of tillers plant^{-1}	Number of productive tillers plant^{-1}	Panicle length	Number of grains panicle^{-1}	Thousand seed weight	Grain length	Grain width	LB ratio	Grain yield plant^{-1}
I	74.25	78.07	23.00	17.35	23.05	151.86	16.92	6.69	2.29	2.95	37.29
II	77.18	87.21	19.54	15.26	22.43	134.45	20.59	7.89	2.51	3.25	32.71
III	72.86	82.38	20.87	15.73	24.09	140.63	19.85	6.71	2.18	3.10	30.00
IV	75.83	80.78	19.17	15.94	23.69	153.73	14.80	5.79	2.20	2.64	30.28
V	85.61	102.15	13.76	11.41	25.02	135.22	22.25	7.01	2.22	3.16	27.28
VI	83.28	113.33	22.63	15.47	26.24	149.18	22.21	5.64	3.33	1.70	28.09
VII	71.09	110.41	21.98	16.18	23.13	125.41	18.06	6.30	2.50	2.54	29.67
VIII	76.81	107.80	13.64	11.92	23.34	112.54	21.25	8.97	3.10	2.95	26.17
General mean	75.98	87.85	20.15	15.49	23.3	138.93	19.62	7.18	2.43	3.03	31.94

cluster I (84.997). The inter-cluster distances indicated greater divergence between clusters IV and VIII followed by clusters VI and VIII. This indicated that the genotypes in these clusters have a broad spectrum of genetic diversity and could very well be used in the hybridization programme. Similar results were reported by Yadav *et al*. (2011) and Sandhya *et al.* (2015). Hybridization between genetically distant clusters resulted in heterotic expression for yield and its components (Bhanumathy *et al.,* 2010). For a successful crossing programme, the genotypes from highly distinct clusters may produce the best progenies leading to the accumulation of favourable genes into a single genotype. The minimum inter-cluster distance was found between clusters V and VII (63.54) followed by clusters III and V (63.543). Parents should be chosen from two clusters with a larger inter-cluster distance (Chaturvedi and Maurya, 2005).

The cluster means for the different characters are presented in Table 6. Cluster V (85.61) exhibited a maximum mean value for days to 50 per cent flowering which indicates delayed flowering and cluster VII (71.09) followed by cluster III (72.86) shows a lower mean value which indicates early

TABLE 7

Estimation of range and Genetic diversity contribution per cent

Characters	Range	Genetic diversity contribution %
Days to fifty percent flowering	58.29-93.82	0.266
Plant height at maturity	67.80-146.15	0.266
Number of tillers plant^{-1}	8.97-27.92	0.089
Number of productive tillers plant^{-1}	6.72-20.18	0.089
Panicle length	17.30-29.38	0.177
Number of grains panicle^{-1}	81.04-169.70	0.089
Thousand seed weight	10.56-28.02	0.443
Grain length	5.32-9.79	49.202
Grain breadth	1.73-3.40	18.174
L/B ratio	1.60-4.69	8.954
Grain yield plant^{-1}	16.72-42.45	22.252

flowering, Cluster VI (113.33) recorded the highest mean value for plant height, cluster I (23.00) followed by cluster VI (22.63) and VII (21.98) exhibits greater mean value for the number of tillers plant^{-1}, the maximum mean value for the number of productive tillers was present in cluster I (17.35) followed by cluster VII (16.18), IV (15.94) and III (15.73); The cluster VI (26.24) followed by cluster V (25.02) registered the highest mean value for panicle length; the cluster IV (153.73) followed by cluster I (151.86) recorded the maximum mean value for the number of grains panicle^{-1}. The maximum mean value for 1000 grain weight was exhibited in cluster V (22.25) and VI (22.21); cluster VIII (8.97) followed by cluster II (7.89) obtained the maximum mean value for grain length which indicates slender grain type, the highest mean value for grain width was exhibited in clusters VI (3.33) and VIII (3.10) shows the bold grain, Cluster II (3.25), V (3.16), III (3.10) exhibits maximum mean value for L/B ratio; cluster I (37.29) followed by II (32.71) recorded maximum mean value for grain yield per plant which indicates higher yield potential. It could be recommended that, intercrossing the genotypes among these clusters with high mean value results in the improvement of respective traits. Among the eleven characters studied grain length (49.20) contributes more to genetic divergence followed by grain yield plant^{-1} (22.25) and grain width (18.17) (Table 7). The selection and choice of parents are determined solely by the contribution of characters to divergence (Nayak *et al*., 2004), which would increase the yield potential.

The hybridization among the genotypes included in diversified clusters with high cluster mean value for grain yield (cluster I and II), number of productive tillers plant^{-1} (cluster I, VII, IV, III), number of grains per panicle (cluster IV, I), 1000 grain weight (cluster V and VI) and grain length (cluster VIII and II), grain width cluster (cluster III and IV) for slender grain type would give high heterotic F_1's and better segregants in the F_2 generation. The genotypes with high mean values of economic traits from various clusters could be utilized for hybridization programme to exploit better segregants.

Further, the genotypes present in cluster VIII (FL478 and pokkali) having high inter-cluster distance with all the remaining clusters except cluster II may be used as donor parent in breeding programmes as these genotypes having high degree of salt tolerance genes Bonila *et al.*, 2002; Sahil *et al.,* 2006; Thomas *et al.,* 2010 and Sandesh *et al.,* 2022 and their potential tolerance to salinity in the development of high-yielding rice varieties for saline conditions.

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