Original Research Article

Genetic diversity analysis of *Oryza glaberrima* derived introgression linesunder direct seeded condition

Comment [NNG1]: Corrected

ABSTRACT

Comment [NNG2]: Corrected

The increasing water scarcity and labour wages have led to the search for alternative crop establishment in rice, such as direct seeded rice (DSR). DSR can reduce unproductive water flow, reduce labour requirements, and mitigate greenhouse gas emissions. Genetic diversity was assessed for hundred and three rice introgression lines using Mahalanobis D^2 analysis and Tocher's clustering method. Cluster I had the largest number of genotypes (73), tailed by Cluster II with 15 genotypes. Cluster III and IV had the maximum intra-cluster distances, with Cluster IV having a significant number for desirable characters. The results disclosedthat days to 50% flowering, tailed by plant height contributed much to diversity. The most significant inter-cluster distances were found in Clusters invloving III and IV, II and III, I and IV and II and IV. These introgression lines can be utilised in crosses, resulting in fruitful recombination during the selection process.

Keywords:Direct seeded rice (DSR), Diversity, Introgression lines, Oryza glaberrima.

Comment [NNG3]: Corrected

1. INTRODUCTION

Comment [NNG4]: Added section number

Rice (*Oryza sativa* L.) is a vital crop crucial for food security worldwide, serving as a staple food for in excess of half of the globe's population. With Asia being the primary rice supplier, providing over 90% of the world's production and contributing 20% for dietary energy Cheng [1], it is evident that Asia dominates both production and consumption of rice.

The increasing demand for rice requires higher yields rather than horizontal expansion of rice, as stated by Pathak [2]. Consequently, there are critical concerns viewing the sustainability of the rice ecosystem and the ability to greet the demand for rice production in parallel with population growth. These concerns arise from depleting natural resources, a declining water table, rising labour charges, energy scarcity, increased input prices, and changing climatic conditions. In traditional rice cultivation methods, 40% of the global irrigation water is utilized for production and also factors such as labour shortages and decreasing arable land compound the need for new ideas and innovations in rice cultivation to ensure food security and meet the rising demand.

In addressing the challenges associated with transplanted rice production, direct seeded rice (DSR) emerges as a potential solution. DSR involves sowing of rice seeds directly into the field, presenting one of the most efficient, sustainable and economically viable rice production systems available today. Comparing DSR with the conventional puddled transplanted rice (PTR) method commonly practiced in Asia, DSR offers advantages such as faster planting and maturation, conservation of valuable resources like water and labour, increased potential for mechanization, and reduced greenhouse gas emissions contributing to climate change.

In this study, the genetic diversity of one hundred three rice introgression lines was assessed using Mahalanobis' D^{2}statistics. The outcomes of breeding programs heavily rely on the selection of suitable parents for hybridization. Diverse parents play a crucial role in ensuring the successful development of varieties. By opting for genetically divergent parents, breeders can create desirable combinations in subsequent generations. The exploration of genome diversity through diversity studies offers valuable insights into the kind and scale of genetic divergence. This information aids plant breeders in making cognizant decisions when opting the most appropriate parents for their breeding programs Vivekananda and Subramaniam [3].

Genetic diversity assessment between and within groups or clusters is very important for the proper choice of parents for the better search of heterosis Murty and Arunachalam [4]. Multivariate analysis with D^2 technique measures the degree of diversity in a population pertainingto several characters and assesses relative impact of variousfactors to the over-all divergence Zahan [5]. The intent of this study is to explore the genetic diversity of rice introgression linesin order to augment the utilization of rice genetic resources.

2. MATERIAL AND METHODS

Comment [NNG5]: Added section number

To ascertain the diversity for yield attributes under direct seeded rice, one hundred three introgression lines were raised at the Indian Institute of Rice Research during *Kharif*, 2022. The investigate was set up using an augmented block design with five checks. Recommended agronomic practices were adopted, with a spacing of 20 x 15 cm was employed for sowing. Nine attributes pertaining to direct seeded rice *viz.*, panicle length, days to 50% flowering (DFF), number of tillers, plant height, spikelets for each panicle,number of productive tillers, test weight, grain-length/breath ratio (L/B ratio), and individual plant grain yield, were measured. Mahalanobis D^2 analysis [6] was tapped to calculate the total genetic distance amongst the introgression lines, and the Tochers method, as described by Rao [7], was exercised to cluster the varieties.

3. RESULTS AND DISCUSSION

Comment [NNG6]: Added section number

Analysis of variance (ANOVA)disclosed a substantial variation across the introgression linesfor all the characteristics considered.

3.1 Assembling of introgression lines into various clusters

Comment [NNG7]: Corrected

Hundred and three lines were diverted into four clusters with respect to the obtained D^2 values using the Tocher method. The D^2 values of the introgression linesfitting to the similar cluster were lower than those of introgression linespertaining to separate clusters. Table 1 portrays the distribution of introgression linesinto multiple clusters (Fig.1). Out of the four clusters, cluster I had the utmost introgression lineswith 73, tailed by clusters II and III with fifteen and nine accessions, respectively. Seven introgression linesin the cluster IV indicate a significant level of heterogeneity. Biswash [8] observed a similar outcome after classifying the accessionsinto four groups in their study.

3.2 Average intra cluster and inter cluster distances

Comment [NNG8]: Corrected

Table 2 represents the cluster diagram, which has been utilized to calculate clusters' intra and inter D^2 distances across one hundred three accessions.

The range of D^2 values within the clusters varied from 222.72 to 451.98. Cluster III (451.98) and Cluster IV (311.93) exhibited the highest intra-cluster distances, indicating significant divergence amongst the accessions. This divergence holds potential for effectual utilization in yield improvement through recombination breeding. The peak divergence was observed between cluster III and IV (1473.63), trailed by cluster II and III (1600.08), cluster I and IV (1151.1), cluster II and IV (957.79), and cluster I and III (630.47), while the lowermost divergence was detected between cluster I and II (604.16), as determined by the inter-cluster D^2 values assessed for all four clusters. The inter-cluster distances were greater than the intra-cluster distances, highlighting the substantial genetic variation present in the parental genome composition Lakshmi [9]. Crossbreeding between parents from divergent clusters is strongly encouraged as it would lead to a significant degree of heterosis. The larger the distance between two clusters, the greater the genetic diversity between the accessions, emphasizing the importance of hybridization between introgression linesfromdivergent clusters.

Table 1. Grouping of one hundred three introgression lines into different clusters based on yield and yield attributing traits by D^2 analysis

Comment [NNG9]: Corrected

Clusters	No. of Entries	Introgression lines
I	72	IL - 10, 44, 21, 101, 92, 76, 27, 45, 13, 6, 87, 14, 91, 93, 65, 31, 97, 72, 29, 56, 28, 103, 39, 48, 84, 1, 58, 94, 96, 7, 8, 50, 37, 78, 15, 5, 20, 55, 26, 81, 52, 63, 60, 88, 16, 24, 61, 66, 36, 62, 47, 41, 46, 99, 34, 82, 75, 67, 80, 35, 79, 73, 43, 77, 54, 40, 33, 38, 42, 69, 89, 53
II	15	IL - 68, 90, 74, 95, 51, 22, 12, 11, 17, 49, 32, 30, 64, 98, 59
III	9	IL - 9, 71, 83, 23, 18, 19, 70, 86, 102
IV	7	IL - 25, 100, 2, 57, 85, 3, 4

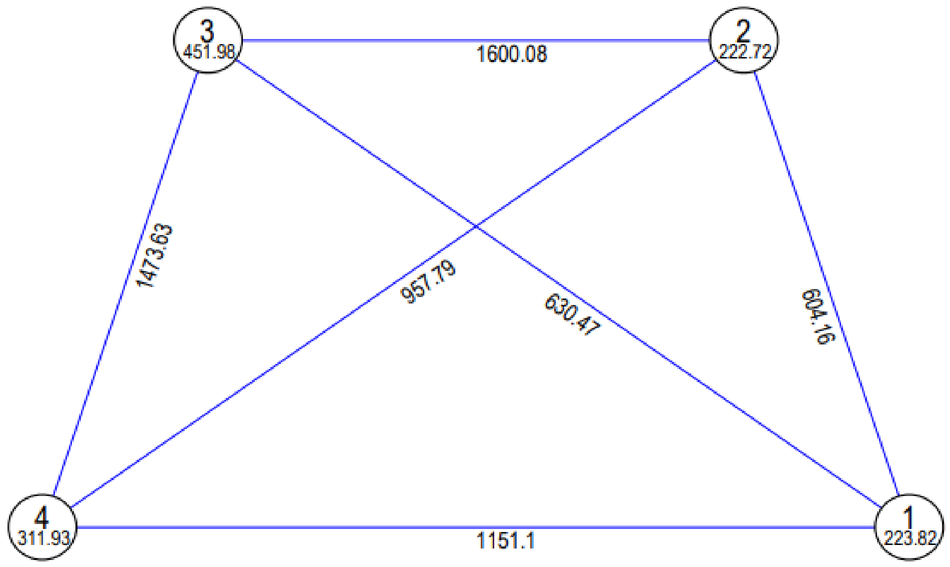

Fig. 1. Cluster diagram of one hundred three introgression lines based on D2 values by Tocher method

Comment [NNG10]: Corrected

Table 2.Average Inter and intra cluster distances of one hundred three introgression lines obtained by D^2 analysis using nine yield and yield attributing traits

Comment [NNG11]: Corrected

	Cluster I	**Cluster II**	**Cluster III**	**Cluster IV**
Cluster I	**223.82**	604.16	630.47	1151.1
Cluster II		**222.72**	1600.08	957.79
Cluster III			**451.98**	1473.63
Cluster IV				**311.93**

*Diagonal values designate intra cluster distance

*Above diagonal values designate inter cluster distances

3.3 Cluster means of the characters

Comment [NNG12]: Corrected

Table 3 displays the cluster means for thenine characteristics considered. The data demonstrated that cluster III had the maximumaverage for DFF (117.78) and cluster II had the lowermost mean (82.67). Plant height was high in cluster IV (86.95 cm) and found lowest in cluster I (60.12 cm). Cluster I reported the least panicle length (15.95 cm), while Cluster IV had the longest (20.07 cm). In relation to tillers per plant, cluster IV had the most number (6.26) while cluster III had the lowermost number (6.02). The most productive tillers per plant were found in cluster IV (4.76), whereas the fewest were found in cluster III (4.15).Cluster III had most lines forspikelet number (8.24), while cluster II had the lowermost for spikelet number per panicle (8.24). Cluster IV recorded the highest 1000 grain weight (15.96 g), while cluster II noted the lowest (13.8 g). Cluster IV had the highest grain production per plant (3.21 g), while cluster III had the lowest yield (2.05 g). Cluster IV had the greatest grain L/B ratio (3.52) and cluster II had the least (3.34). The outcomes suggested that selection of introgression linesfrom various clusters for hybridization and selection in order to enhancegrain output would be possible provided they had high estimations for desirable features. It has been noteworthy that Cluster IV possessed a significant number of many characters' desirable means. Thus, the introgression lines viz.IL - 25, 100, 2, 57, 85, 3, 4 of cluster IV may be used directly as parents in future hybridization initiatives to combine desirable characteristics.

Table 3. Cluster means for nine yield and yield attributing traits in one hundred three introgression lines by D^2 analysis

Comment [NNG13]: Corrected

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Days to 50 % flowering	102.35	82.67	117.78	89.43
Plant height(cm)	60.12	61.3	69.05	86.95
Panicle length (cm)	15.95	16.77	16.57	20.07
Total tillers per plant	6.25	6.14	6.02	6.26
Productive tillers per plant	4.55	4.65	4.15	4.76
Spikelets per panicle	8.37	8.24	8.93	8.86
Test weight (g)	14.45	13.81	14.95	15.96
L/B ratio	3.47	3.34	3.47	3.52
Yield/Plant (g)	2.42	2.56	2.05	3.21

3.4 Contribution of characteristics towards genetic divergence

Comment [NNG14]: Corrected

Table 4 illustrates the frequency of occurrence and the corresponding percentage contribution of each of the nine traits that obtained the highest rank in terms of divergence.

The observations revealed that role of DFF was maximum towards divergence (53.76 %) by 2824 times ranking first, plant height (40.53%) by 2129 times, test weight (4.63%) by 243 times, panicle length (0.63%) by thirty three times, spikelets/panicle (0.27%) by 14 times, number of tillers per each plant (0.15%) by eight times, yield per plant and L/B ratio (0.02%) by one time respectively contributed to the genetic divergence. Characters DFF and plant height have contributed 94.29% to the overall divergence. For height and DFF, similar findings in rice were previously reported by Kaw [10]; for DFF, by Kuchanur [11], Bhati [12], and Rathan [13]. Therefore, by selecting plants with early duration and more productive tillers, these characteristics should be given priority during hybridization and selection in segregating populations.

Table 4. Relative contribution of different characters to genetic diversity

Comment [NNG15]: Corrected

S.No	Character	Times ranked First	Contribution %
1	**Days to 50 % flowering**	2824	53.76
2	**Plant height(cm)**	2129	40.53
3	**Panicle length (cm)**	33	0.63
4	**Total tillers per plant**	8	0.15
5	**Productive tillers per plant**	0	0.00
6	**Spikelets per panicle**	14	0.27
7	**Test weight (g)**	243	4.63
8	**L/B ratio**	1	0.02

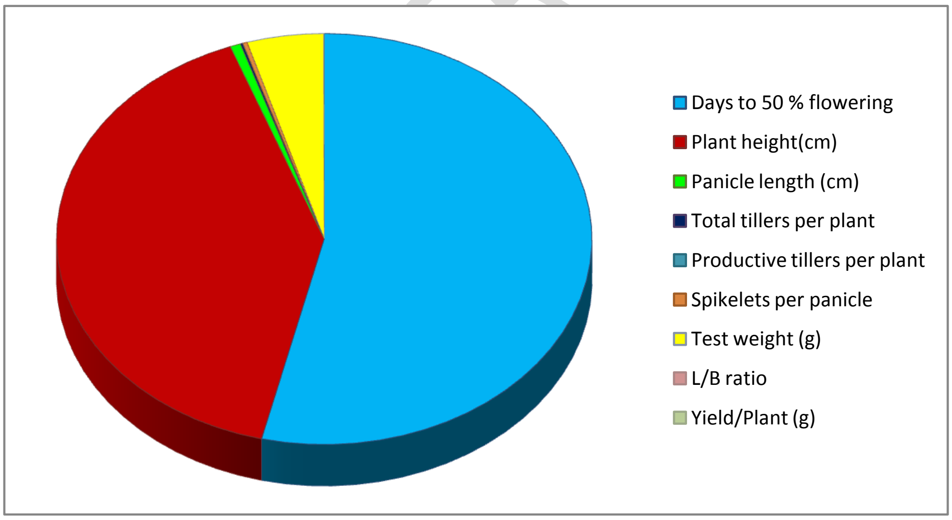

Fig. 2. Per cent contribution of yield and its component characters towards diversity

Comment [NNG16]: Corrected

4. CONCLUSION

Comment [NNG17]: Corrected

According to the findings, considerable variation exists among the introgression linesconsidered for the current study. The emergence of different yield attribute clusters implies the possibility of divergence among the lines used. Recombination breeding for

Cluster III introgression linescould increase the yield potential as they have the peak intra-cluster distance. Clusters III and IV, II and III, I and IV, and II and IV had the most significant inter-cluster distance. The introgression linesin these clusters can be employed in crosses, which will be fruitful due to favourable recombination throughout the selection process.

COMPETING INTERESTS

Authors have declared that no competing interest exists.

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Comment [NNG20]: Corrected

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