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Genetic Diversity Study of Rainfed Lowland Rice (*Oryza Sativa* L.) Genotypes Based on Cluster and Principal Component Analysis at South Western Ethiopia

Abayneh Kacharo Kampe*

Department of Agricultural Research, Bonga Agricultural Research Center, Bonga, Ethiopia

***Corresponding author:** Abayneh Kacharo Kampe, Department of Agricultural Research, Bonga Agricultural Research Center, Bonga, Ethiopia; E-mail:abaynehkacharo@gmail.com

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Abstract

A field experiment was conducted with the objective of determining the magnitude of genetic diversity among rained lowland rice genotypes. Twenty-five rainfed lowland rice genotypes were evaluated during the 2016 main cropping season at two rainfed lowland agro-ecologies of Southwestern Ethiopia. The experiment was laid out in a simple lattice design and data on 14 yield and yield component traits were collected and subjected to various statistical analyses. Cluster and distance analysis of quantitative characters based on multivariate analysis pointed out the existence of four divergent groups. The maximum inter cluster distance was observed between cluster one and four (D2=258.7) followed by cluster one and three (D2=191.5), while the minimum was obtained between cluster one and two (D2=45.06). Maximum recombination and segregation of progenies were expected from crosses involving parents selected from these divergent groups. Principal component analysis retained the first five principal components that accounted for 76.5% of the total variation in lowland rice genotypes and the first two principal axes contributed 43.7% for the total variation in lowland rice genotypes. Number of fertile tillers plant, thousand seed weight, grain yield per plant and harvest index in different principal components as the most prominent traits for differentiation of the total variation in lowland rice genotypes. The present study indicated sufficient amount of genetic diversity for the majority of the characters studied in rain-fed lowland rice genotype for future exploitation and, could be kept into consideration during hybridization and conservation programs.

Keywords: Lowland rice; Genetic diversity; Cluster analysis; Principal component analysis; Hybridization; Segregation

Introduction

Rice (*Orzo sativa* L.) is the fastest growing food security and self-sufficiency crop in an increasing number of low-income food deficit countries in Africa. In Ethiopia, the cultivation of rice is of a recent history, however, its use as food crop, income source, employment opportunity and animal feed has been well recognized. The government of Ethiopia considered rice as the most strategic food security crop that has received special attention in promotion of agricultural production and as such it is named as the "millennium crop" expected to contribute in ensuring food security in Ethiopia. The country expected to find export market in near future for other African countries and thus, the crop is one of the main trust areas of attracting for domestic consumption or international trade purposes [1].

Despite the country has immense potential for growing this crop, production, productivity and expansion of the rice has been challenged by lack of improved varieties, lack of recommended crop management practices for different rice ecosystems, lack of pre and post-harvest management technologies and lack of awareness on its utilization. As a result, rice yield remains progressively low with average national productivity of 2.8 tons/ha which is very much lower than the average yield of rice in the world that accounts 4.54 tons per hectare [2]. Among the production constraints, lack of improved varieties is one of the most pressing constraint in rice production and productivity in the country.

Among the available options to tackle the low rice production and productivity in order to meet the fastest growing demand, exploiting genetic diversity which exists among rice genotypes for grain yield and yield components receives due emphasis. Diversity in Plant Genetic Resources (PGR) provides opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits and breeders preferred traits. One of the important approaches to rice breeding is hybridization and subsequent selection. Parents' choice is the first step in plant breeding program through hybridization. In order to benefit transgressive segregation, genetic distance between parents is necessary. The higher genetic distance between parents, the higher heterocyst in progeny can be observed. Estimation of genetic distance is one of appropriate tools for parental selection in rice hybridization programs. Appropriate selection of the parents is essential to be used in crossing nurseries to enhance the genetic recombination for potential yield increase. Some appropriate methods, cluster analysis and PCA for genetic diversity identification, parental selection, tracing the pathway to evolution of crops, center of origin and diversity, and study interaction between the environments are currently available. Principal component analysis helps researchers distinguish significant relationship between traits [3].

This is a multivariate analysis method that aims to explain the correlation between a large set of variables in terms of a small number of underlying independent factors [4-6]. The cluster analysis is also an appropriate method for determining family relationships but the main advantage of using PCA over cluster analysis is that each genotype can be assigned to one group only. The main objective of this study is to assess the genetic diversity among rained lowland rice genotypes by using cluster and principal component analysis based methods for selection of parents in hybridization programmer to obtain desirable sergeants in advanced generation [7].

Materials and Methods

Description of the experimental site

The experiment was carried out at Tepi Agricultural Research Center in Yeki Worde, Sheka zone and Shomba Kichib Keble in Gimbo Woreda, Kaffa zone, which are located at distance of 611 km and 402 km from south west of Addis Ababa, respectively. Tepi Agricultural Research Center situated at an altitude of 1200 meter above sea level, latitude of 7°3'0″ N and longitude of 35 °18'0″ E and the center receives average annual rain fall of 1678 mm and the mean monthly minimum and maximum temperature of 15.4 °C and 5.92 °C, respectively. Shomba Kichib experimental field located at an altitude of 1235 meter above sea level, latitude of 7°15′0″N and longitude of 36°0′0″E and receives an annual rainfall of 1710 mm and mean monthly minimum and maximum temperature of 16.7 °C and 0.42 °C, respectively. The soil type of experimental site was generally classified as verticals or light black clay soils[8-10].

Description of the experimental materials

The experimental materials consisted of 25 rained lowland rice genotypes (Advanced lines) obtained that from Fog era National Rice Research and Training Center (FNRRTC) and Bongo Agricultural Research Center, formerly introduced from International Rice Research Institute (IRRI) and African Rice Center (WARDA).

No	Genotypes/ pedigree	Origin	Seed source	Ecotype
1	WAB189-B-B-B-HB	WARDA	2014 LRNVT-FNRRTC	Rainfed lowland
2	IAC-164	WARDA	2014 LRNVT-FNRRTC	Rainfed lowland
3	DEMOZE	-	2014 LRNVT-FNRRTC	Rainfed lowland
4	ROJOMENA2 71/10	-	2014 LRNVT-FNRRTC	Rainfed lowland
5	IRGA370-38-1-1F-B1-1	IRRI	2014 LRNVT-FNRRTC	Rainfed lowland
6	scrid113-3-5-3-5-4	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
7	scrid037-4-2-2-5-2	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
8	scrid017-1-4-4-4-1	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
9	scrid014-1-1-1-1	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
10	scrid006-3-2-3-2	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
11	WAB95-B-B-40-HB (Hiber)	WARDA	2014 LRNVT-FNRRTC	Rainfed lowland
12	RPBIO4919-117	India	2015 LRRVT-BARC	Rainfed lowland
13	IR82912-B-B-5	IRRI	2015 LRRVT –BARC	Rainfed lowland
14	IR88628-B-B-30	IRRI	2015 LRRVT-BARC	Rainfed lowland
15	IR83106-B-B-6	IRRI	2015 LRRVT –BARC	Rainfed lowland
16	scrid006-2-4-3-4-5	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
17	WAB-4507	WARDA	2014 LRNVT-FNRRTC	Rainfed lowland
18	FOFIFA172	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
19	FOFIFA171	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
20	FOFIFA161	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
21	WAB502-8-5-1	WARDA	2014 LRNVT-FNRRTC	Rainfed lowland
22	WABC165	WARDA	2014 LRNVT-FNRRTC	Rainfed lowland
23	scrid079-1-5-4-2	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland
24	NERICA-18	WARDA	2014 LRNVT-FNRRTC	Rainfed lowland
25	FOFIFA 165	Madagascar	2014 LRNVT-FNRRTC	Rainfed lowland

Table 1: Description of the experimental materials.

Source: Fogera National Rice Research and Training Center (FNRRTC) and Bonga Agricultural Research Center (BARC)

Where, WARDA: West Africa Rice Development Association, IRRI: International Rice Research Institute., LRNVT: Lowland Rice National Variety Trial, LRRVT: Lowland Rice Regional Variety Trial

Experimental design and trial management

The experiment was laid out in a 5 × 5 simple lattice design and five genotypes were assigned into each incomplete block. The spacing between replication, incomplete block and plot were 1 m, 50 cm and 25 cm respectively and rice seeds were drilled in rows with seed rate of 60 kg per hectare and row spacing maintained at 25 cm apart. The gross and net harvestable plot size of the experiment was 7 m^2 and 5 m^2, respectively and five inner most central rows were used in data collection. Fertilizer was applied at a rate of 100 kg DAP and 100 kg Urea ha^{-1} as per national recommendation. All DAP was applied during planting while urea was applied in three splits at planting tillering and at panicle initiation stages [11]. Weeding done four times

manually during the whole experimental period since weeds were vigorous in south western Ethiopia[12-16].

Data collected

Standard evaluation system developed by IRRI, 2013 was followed in order to collect fourteen yields and yield component data. Days to 50% heading and days to 85% maturity, biomass yield, harvest index, thousand seed weight and grain yield per hectare were computed on plot basis. Five representative plants for each genotype in each replication were randomly taken to record observations on plant height (cm), panicle length (cm), total tillers per plant, fertile or productive tillers per plant, filled grains per panicle, unfilled grains per panicle, primary branches per panicle grain yield per plant. Grain yield obtained on plot base was converted into Kg ha^{-1} and adjusted to 14% grain moisture content. For each of 14 traits, the standardized means of 25 varieties were used in all succeeding multivariate statistical analysis, including cluster analysis, distance analysis and principal component analysis[17].

Cluster analysis

Clustering was performed using the cluster procedure of SAS version 9.00 by employing the method of average linkage clustering strategy of the observation. The number of cluster was determined by following the approach suggested by Copper and Milligan by looking into three statistics namely Pseudo F, Pseudo t2 and cubic clustering criteria. The points where local peaks of the CCC and pseudo F-statistic join with small values of the pseudo-t2 statistic followed by a larger pseudo-t2 for the next cluster combination was used to determine the number of clusters by using SAS statistical package. The dendrogram was constructed based on the average linkage and Euclidean distance used as a measure of dissimilarity (the distance) technique[18-21].

The inter cluster distances were calculated by the formula described:

Square of the inter cluster distance = $\Sigma D^2_i/n_i n_j$

Where, ΣD^2_i is the sum of distances between all possible combinations ($n_i n_j$) of the genotypes included in the clusters under study, n_i is number of genotypes in cluster i and n_j is number of genotypes in cluster j.

Genetic divergence

Genetic divergence between clusters was calculated using the generalized Mahalanobis's D2 statistics using the equation:

$D^2_p=((X_i-X_j)\ S^{-1}(X_i-X_j)$.

Where, D^2_p=The squared distance between any two genotypes i and j;

X_i and X_j=The p mean vectors of genotypes i and j, respectively.

S^{-1}=The inverse of the pooled covariance matrix.

The D2 values obtained for pairs of clusters were considered as the calculated values of Chi-square (X2) and tested for significance both at 1% and 5% probability levels against the tabulated value of X2 for 'P' degree of freedom, where P is the number of characters considered.

Principal component analysis

Principal component analysis was performed using correlation matrix of SAS version 9.00 in order to examine the relationships among the quantitative characters that are correlated among each other by converting into uncorrelated characters called principal components. Below is the general formula to compute scores on the first component extracted (created) in a principal component analysis;

$C_1=b_{11}(X_1)+b_{12}+\ldots b_1p\ (X_p)$

Where, C_1=The subject's score on principal component 1 (the first component extracted);

b1p=The regression coefficient (or weight) for observed variable p, as used in creating principal component 1; Xp=the subject's score on observed variable p.

Results

Cluster analysis grouped the genotypes into four distinct groups based on their similarity (Figure 1). Genotypes obtained from Madagascar and WARDA were almost distributed in all clusters, indicating the existence of more genetic diversity in these origins and genotypes from the same origin might have different genetic background. The first cluster (C_1, n=13) had the largest number of genotypes obtained from IRRI and Madagascar but, dominated by genotypes originated from Madagascar. The second cluster comprised eight genotypes, five of them were originated from Madagascar and the rest three were from WARDA. The third cluster consisted of three lowland rice genotypes, two of them were originated from WARDA and one from Madagascar and the fourth cluster only included one solitary genotype obtained from WARDA. The result showed that in most cases, genotypes tend to form their own distinct groups rather than forming their own hierarchical sub-groups based on their original background indicating seldom association between clustering pattern and eco-geographical distribution of genotypes, i.e., genotypes, collected from the same geographic area were placed into different cluster groups and those obtained from different geographic regions were placed into the same cluster (Figure 1).

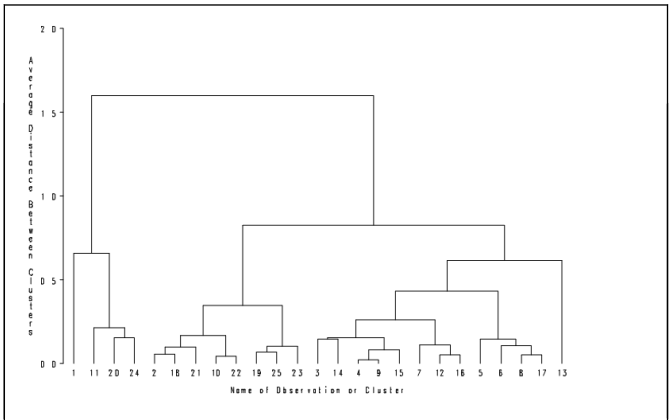

Figure 1: Dendrogram indicating the genetic relationship of 25 rainfed lowland ricegenotypes evaluated across two locations at south western Ethiopia in 2016/17 cropping season.

Numbers and corresponding genotypes are: 1). WAB189-B-B-B-HB, 2). IAC-164, 3.) DEMOZE, 4.) ROJOMENA271/10, 5.)

IRGA370-38-1-1F-B1-1, 6) Scrid113-3-5-3-5-4, 7) Scrid037-4-2-2-5-2, 2, 8) Scrid017-1-4-4-4-1, 9) Scrid014-1-1-1-1, 10) Scrid006-3-2-3-2, 11 Hibirre, 12) RPBIO4919-117, 13) IR82912-B-B-5, 14) IR88628-B-B-3 0, 15) IR83106-B-B-6, 16) Scrid006-2-4-3-4-5, 17) WAB-4507, 18) F FOFIFA172, 19) FOFIFA171, 20) FOFIFA161, 21) WWAB502-8-5-1, WABC165, 23) Scrid079-1-5-4-2, 24) NERICA-18, 25) FOFIFA 165.

Comparison of genotype performances among clusters

Cluster I was characterized by the highest cluster mean estimate for days to 50% heading, days to 85% maturity, plant height, number of filled grains per panicle and harvest index and it produced the lowest cluster mean estimate for grain yield hectare. Cluster II was characterized by having higher cluster mean values for number of total tiller per plant, number of fertile tiller per plant and biomass yield and the lowest cluster mean value for grain yield plant [22,23].Eventhogh cluster III displayed the second largest cluster mean estimates for several attributes *viz*., days to 50% heading, days to 85%maturity, number of primary branches per panicle, grain yield per plant, thousand seed weight and grain yield per hectare. It had the lowest cluster mean estimates for some of the traits evaluated *viz.*, plant height, panicle length, number of total tiller per plant, fertile tiller number per plant, filled grains per panicle and unfilled grains per panicle. Cluster IV contained only one per grain on solitary genotype, i.e., WAB189-B-B-B-HB and it produced the highest cluster mean values for most of the traits i.e., panicle length, number of unfilled grains per panicle, number of primary branches per panicle, grain yield per plant, thousand seed weight and grain yield hectare and the lowest cluster mean values for days to 50% heading, days to 85% maturity and harvest index (Table 1), indicating that this genotype is exceptionally the best genotype for performance under rainfed lowland condition (Tables 1 and 2).

Traits	Cluster I	Cluster II	Cluster III	Cluster IV
DH	9.62	9.46	9.54	9.35
DM	127.33	124.78	125.33	124
PH	80.07	79.01	69.93	77.1
PL	20.02	19.95	19.72	22.25
TTPP	3.09	3.21	2.59	2.62
FTPP	7.06	7.66	5.7	6.2
FGPP	8.14	7.48	7.33	7.55
UGPP	4.98	5.01	4.44	5.59
PBPP	9.27	9.48	10.03	10.5
GYPP	3.1	3.09	3.17	3.26
BY	4460	4810.6	4608.4	3680
HI	0.39	0.39	0.38	0.36
TSW	23.19	23.09	24.75	28.25
GY	2317.86	2882.55	3535.82	4015.27

Note: Weight, GY: Grain Yield Per Hectare DH: Days to Heading, DM: Days to Maturity; PH: Plant Height; PL: Panicle Length, TTPP: Number of Total Tiller Per Plant; FTPP: Number of Fertile Tiller Number Per Plant, FGPP: Number of Filled Grains Per Panicle; and UGPP: Number of Unfilled Grains Per Panicle; PBPP: Number of Primary Branches Per Panicle; GYPP: Grain yield per plant, BY: Biological Yield; HI: Harvest Index; TSW: Thousand Seed.

Table 1: Cluster mean on 25 rainfed lowland rice genotypes for each of the evaluated traits.

Clusters	Number of genotypes	Proportion	Name of genotypes
Cluster I	13	52	ROJOMENA271/10,scrid014-1-1-1-1, RPBIO4919-117, scrid006-2-4-3-4-5, scrid017-1-4-4-4-1, WAB-4507, IR83106-B-B-6, scrid113-3-5-3-5-4, scrid037-4-2-2-5-2, DEMOZE, IR82912-B-B-5,IR88628-B-B-30 and IRGA370-38-1-1F-B1-1
Cluster II	8	32	scrid006-3-2-3-2, WABC165, IAC-164, FOFIFA172, FOFIFA171, FOFIFA 165, WAB502-8-5-1 and scrid079-1-5-4-2,
Cluster III	3	12	FOFIFA161, NERICA-18 and Hibirre
Cluster IV	1	4	WAB189-B-B-B-HB

Table 2: Distribution of the 25 rainfed lowland rice genotypes in different clusters.

Distance among clusters (genetic divergence analysis)

The standardized Mahalanobis D2 statistics revealed the existence of high genetic distance among the four clusters and showed highly significant variation at $P<0.01$.

The maximum squared distance was found between cluster one and four (D2=258.7) followed by cluster one and three (D2=191.5) and cluster two and four (D2=187.3). The minimum squared distance was found between cluster one and two (D2=45.06) followed by cluster three and four (D2=65.37) and cluster two and three (D2=124.6). Generally this study revealed that genotypes included in this study are highly divergent [24].

Minimum inter cluster distance was observed between cluster one and two (D2=45.06), indicating that genotypes in these clusters were not genetically diverse or there was little genetic diversity between these clusters. This signifies that crossing of genotypes from these two clusters might not give higher heterotic value in F1 and narrow range of variability in the segregating F2 population.

Maximum genetic recombination is expected from the parents selected from divergent clusters groups. Therefore, maximum recombination and segregation of progenies is expected from crosses involving parents selected from

cluster one and four followed by cluster one and three and cluster two and four (Table 3).

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	0	45.06**	191.5**	258.7**
Cluster II		0	124.6**	187.3**
Cluster III			0	65.37**
Cluster IV				0

Table 3: Pair-wise generalized square distance (D2) between four clusters constructed from 25 rainfed lowland rice genotypes.

Principal component analysis

Principal component analysis revealed that the first five components (PC1 to PC5) with eigenvalues greater than one explained 76.5% of the total variation among traits in rainfed lowland rice genotypes suggesting these principal component scores might be used to summarize the original 14 variables in any further analysis of the data Out of the total principal components retained, PC1, PC2 and PC3 with values of 26.7%, 17.0% and 14.3%, respectively contributed more to the total variation. The result is in agreement with the findings of by Robin which identified five the most chief contributors in 192 rice germplasms for 12 agro-morphological traits that accounted for to 80% of the total variation. Mahendran also identified the first 5 components that accounted for 77.38% of the total variation in rice (Table 4).

Traits	PCA 1	PCA 2	PCA 3	PCA 4	PCA 5
DH	-0.32	0.14	-0.67	0.47	0.02
DM	0.42	-0.51	-0.1	-0.43	0.07
PH	-0.09	-0.45	0.014	-0.24	-0.36
PL	0.65	0.48	0.35	-0.17	-0.05
TTPP	0.31	0.61	0.4	-0.14	-0.24
FTPP	0.86	0.12	0.001	0.1	-0.3
FGPP	0.74	-0.02	0.25	0.42	-0.14
UGPP	0.27	-0.18	0.39	-0.03	0.81
PBPP	-0.14	0.62	-0.1	-0.47	0.33
GYPP	-0.32	0.09	0.73	0.16	-0.08
BY	-0.8	0.03	0.43	0.09	-0.12
HI	0.13	0.58	-0.14	0.54	0.22
TSW	0.26	-0.7	0.37	0.4	0.14
GY	-0.85	0.11	0.38	0.05	-0.05
Eigen value	3.74	2.37	2.003	1.425	1.16
Total variance explained (%)	26.7	17	14.3	10.2	8.3
Cumulative total variance explained (%)	26.7	43.7	58	68.2	76.5

Note: DH: Days to Heading; DM: Days to Maturity, PH: Plant Height, PL: Panicle Length; TTPP: Total Tiller Number Per Plant; FGPP: Filled Grains Per Panicle; and UGPP: Unfilled Grains Per Panicle; PBPP: Number of Primary Branches Per Panicle; GYPP: Grain Yield Per Plant; BY: Biological Yield; HI: Harvest Index; TSW: Thousand Seed Weight; GY: Grain Yield Per Hectare; FTNPP: Fertile Tiller Number Per Plant;

Table 4: Estimates on principal component analysis for 14 traits in 25 rainfed lowland rice genotypes.

The first principal component had high positive loading for 8 characters out of 14 *viz.* days to 85% maturity, panicle length, and number of total tiller per plant, number of fertile tiller per plant, filled grains per panicle, unfilled grains panicle, harvest index and thousand seed weight which contributed more to the variation. It has high negative weights for days to heading, biomass yield and grain yield per hectare. The major contributing traits for the variation in the second Principal Components (PC2) were chiefly obtained from variations of number of primary branches per panicle, number of total tiller per plant and harvest index and for the Principal Component three (PC3), grain yield per plant, biomass yield and number of total tiller per plant that contributed more to the variation. Days to 85% maturity and thousand seed weight expressed highest negative loads in Principal Component two (PCA2)

Positive and negative weight shows the presence of positive and negative correlation trends between the components and the variables. They therefore, the above mentioned characters with high diversity in a positive or negative loads contributed more to the diversity and they were the ones that most differentiated the clusters. A PC biplot in Figure 2 showed that variables and genotypes are superimposed on the plot as vectors. The distance of each variable with respect to PC1 and PC2 showed the contribution of these variables in the variation of genotypes used (Figure 2).

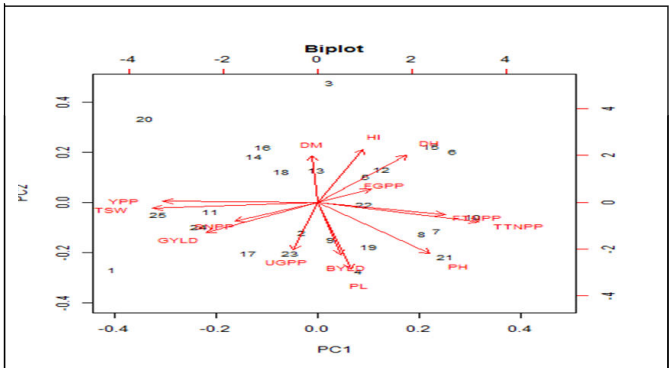

Figure 2: Graphical representation (biplot) of the contribution of the first two principal components to the total variation in rainfed lowland rice genotypes.

Discussion

Characters with largest absolute value closer to unity within the first principal component influence the clustering more than those with lower absolute value closer to zero. Thus, the prominent characters coming together in different principal components and contributing towards explaining the variability have the tendency to remain together. Hence, for the first group weight, number of fertile tiller per plant is best choice, which had the largest loading from component ones, thousand seed weight for the second, grain yield per plant for the third group and harvest index for the fourth group as the most chief contributors for the total variations in rainfed lowland rice genotypes. Therefore, in the present study, grouping of the genotypes into different clusters were because of relatively high contribution of these traits rather than small contribution from other traits. These results indicated the presence of genetic diversity and could be kept into consideration during hybridization and conservation programs.

Conclusion

Clustering and divergence analysis of quantitative characters based on multivariate analysis, indicated the existence four distinct groups and showed wide genetic diversity between different clusters. Thus, maximum recombination and segregation of progenies are expected from crosses involving parents selected from cluster one and four closely followed by cluster one and three and cluster two and four, respectively. Principal component analysis retained the first five chief contributors that played prominent role in explaining the total variation existing in the lowland rice genotypes. The analysis identified number of fertile tillers plant, thousand seed weight, grain yield per plant and harvest index in different principal components as the most prominent traits for differentiation of the total variation in low land rice genotypes. Therefore, differentiation of genotypes into different groups was relatively because of larger contribution of these traits and might be considered in hybridization for evolving high yielding rainfed lowland rice genotypes. The present study indicated that there is adequate genetic diversity for most of yield and yield component traits evaluated in rainfed lowland rice genotypes for future exploitation and hence, could be kept into consideration during hybridization and conservation programs.

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