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Genetic Diversity Study on Low Land Rice (Oryza Sativa L.) Genotypes Based on Morphological Traits in Teppi and Fogera, Ethiopia

Mequannit Aklilu
EIAR, Tepi Agricultural Research Center, P.O. Box, 34, Teppi, SNNPs, Ethiopia.
Corresponding author email id: Mequannit@gmail.com

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***Abstract* – The extent of genetic diversity among genotypes and arranging into similar groups have great importance in plant breeding issue. The objective of this study was to estimate the genetic diversity of 36 low land rice genotypes based on quantitative morphological traits. The experiment was taken during 2017 in two locations Teppi and Fogera using 6*6 simple lattice design. Cluster analysis, distance analysis and principal component analysis were done using the SAS software version 9.2. The first five principal components with eigenvalues greater than one explained 80 % of the total variation and the first two principal components contributed 53% for the total variance in rain fed low land rice genotypes. The cluster analysis grouped the genotypes into four major groups consisting of one to 18 genotypes. Cluster and distance analysis of quantitative characters based on multivariate analysis pointed out the existence of four divergent groups. The maximum squared distance was found between cluster three and four ($D^2 = 4601.87$) followed by cluster two and four ($D^2 = 3075.59$), while the minimum was obtained between cluster two and three ($D^2 = 1647.9$). The present study indicated sufficient amount of genetic variability for the characters studied in rain fed low land rice genotype, which will create opportunity for future improvement. Further study with the help of molecular techniques is recommended.**

***Keywords* – Diversity, Genetic, Genotype, Morphological Trait.**

I. INTRODUCTION

Rice is central to the lives of billions of people around the world. It is source of income and principal activity for 100 million households in Asia and Africa [1]. It is the most widely consumed staple food for a large part of the world's human population, especially in Asia. About 3.5 billion people depend on rice as a daily food staple for 20 % of their calories [9]. It is the agricultural commodity with the third-highest worldwide production, after sugarcane and maize [3].

Rice is one of the most important cereal crops globally with annual production of 483.3 million metric tons milled basis, in 2016 [15]. Of the 25 major rice producing nations are in south, south east or East Asia. In 2015/2016, China, India and Indonesia are the top three rice producing countries with 28.4%, 21.2% and 9.6% percentage of the world total production. In Africa, rice production has reached 26.6 million tons and about 20 million people of the content depends on rice production, there by more than 40 contries of the continent cultivate and consume rice [2].

Genetic variability studies was conducted on low land rice by Tefera *et* al., [13] and reported the presence of significant variation among genotypes for all of the studied traits. Low land rice germplasm suited for production are imported every year from African rice, International Rice Research institute to evaluate adaptability and performance in our country, Ethiopia. Hence there is little information on the genetic diversity of low land rice genotypes. Therefore, the objective of this study was to determine the extent of genetic diversity among low land rice genotypes using multivariate statistical techniques.

II. MATERIALS AND METHODS

Description of Experimental Sites

The experiment was conducted at Tepi Agricultural Research Centre and Fogera National Rice Research and Training Center. Tepi Agricultural Research Centre is located 611 km from Addis Ababa in the South - western part of Ethiopia. The experimental site is situated at an altitude of 1200 meter above sea level, latitude of 7° 3' N and longitude of 35° 18' E and the center receives average annual rainfall 1678 mm and the mean monthly minimum and maximum temperatures of 15.4^{0}C and 29.5^{0}C, respectively. The soil type is clay with fine textured 30 to 80 % with pH of 6.9-8 (neutral to moderately alkaline [4]. Fogera National Rice Research and Training Center is located 607 km from Addis Ababa (capital of Ethiopia) in the North-western part of Ethiopia. The site is located at 11°58' N latitude, 37° 41'E longitude and at an elevation of 1810 m above sea level. Based on ten years' average meteorological data, the annual rainfall, and mean annual minimum and maximum temperatures are 1300 mm, 11.5 C and 27.9°C, respectively. The soil type is black (Vertisol) with pH of 5.90 [12].

Experimental Materials

The experimental materials consisted of 36 lowland rice genotypes obtained from Fogera National Rice Research and Training Center, formerly introduce from International Rice Research Institute (IRRI) and Africa Rice Center (WARDA). The list, origin, seed source and genotypes used in the experiment were given below in table 1.

Table 1. Description of experimental materials (lowland rice genotypes).

S/NO	Genotype	Seed Source	Origin
1	Aromatic	2016/17 PVT	Africa Rice
2	Edirne	2016/17 PVT	Africa Rice
3	Halilbey	2016/17 PVT	Africa Rice
4	Osmancik-97	2016/17 PVT	Africa Rice
5	Trakya	2016/17 PVT	Africa Rice
6	Tunca	2016/17 PVT	Africa Rice
7	Suitou Chuukanbohon Nou 11	2016/17 PVT	Africa Rice
8	Condai	2016/17 PVT	Africa Rice
9	Pepita	2016/17 PVT	Africa Rice
10	Saegyejinmi	2016/17 PVT	Africa Rice
11	Lunyuki	2016/17 PVT	Africa Rice
12	Hangamchal	2016/17 PVT	Africa Rice
13	Hawaghaelo-2	2016/17 PVT	Africa Rice
14	Namcheobyeo	2016/17 PVT	Africa Rice
15	Samgangbyeo	2016/17 PVT	Africa Rice

16	SCRID091-10-1-3-2-5	2016/17 PVT	Africa Rice
17	SCRID091-15-2-2-1-1	2016/17 PVT	Africa Rice
18	SCRID091-18-1-5-4-4	2016/17 PVT	Africa Rice
19	SCRID091-20-2-2-4-4	2016/17 PVT	Africa Rice
20	SCRID091-24-3-2-2-3	2016/17 PVT	Africa Rice
21	SCRID091-38-3-1-3-1	2016/17 PVT	Africa Rice
22	SCRID090-60-1-1-2-4	2016/17 PVT	Africa Rice
23	SCRID090-72-3-1-3-5	2016/17 PVT	Africa Rice
24	SCRID090-164-2-1-2-1	2016/17 PVT	Africa Rice
25	SCRID090-177-2-4-3-4	2016/17 PVT	Africa Rice
26	SCRID090-18-1-2-2-1	2016/17 PVT	Africa Rice
27	SCRID091-20-3-1-3-4	2016/17 PVT	Africa Rice
28	SCRID122-5-2-1-1-3	2016/17 PVT	Africa Rice
29	SCRID122-13-1-1-4-3	2016/17 PVT	Africa Rice
30	SCRID186-72-1-1-2	2016/17 PVT	Africa Rice
31	SCRID198-73-5-1-3	2016/17 PVT	Africa Rice
32	GSR IR1-17-Y16-Y3-Y2	2016/17NVT	IRRI
33	GSR IR1-15-D4-D1-Y1	2016/17 NVT	IRRI
34	Ediget (Check1)	Breeder Seed	Released
35	X-Jigna (Check2)	Breeder Seed	Local
36	Hiber	2016/17 NVT	WARDA

IRRI = International Rice Research Institutes, WARDA = West Africa Rice Development Association PVT = Preliminary Variety Trial, NVT = National Variety Trail.

Data Collected

Data were collected on both plant (average of 5 randomly taken plants) and plot basis from the central five rows (4x1.25m = 5m^2) of each plot following the Standard Evaluation System for Rice (SES) [5].

Data Collected on Plant Basis

Plant Height (PH, cm):

Height of the plant in centimeter from the base of the main stem to the tip of the panicle was recorded as the average of five randomly taken plants in the middle five rows of each plot.

Panicle Length (PL, cm):

Length of the panicle in centimeter was measured from the node, where the first panicle branch starts, to the tip of the panicle as the average of five randomly taken plants in the middle five rows of each plot.

Culm Length (CL, cm):

The culm length was measured in centimeters from ground level to the base of the panicle or neck node (panicle base node) from five randomly taken plants in the middle five rows of each plot.

Number of Fertile Grains per Panicle (FGPP, No.):

Taken by counting the number of fertile grains from the main panicle at harvest maturity from five randomly taken plants and averaged.

Number of Infertile Grains per Panicle (IGPP, No.):

Taken by counting the number of infertile grains from the main panicle at harvest maturity from five randomly taken plants and averaged to find the character.

Number of Fertile Tillers per Plant (FTTP, No.):

The average number of fertile tillers from five randomly taken sample plants in the middle five rows of each plot was recorded.

Number of Total Tillers per Plant (TTPP, No.):

Number of total tillers per plant was recorded from five randomly taken plants in the middle five rows of each plot.

Number of Unfertile Tillers per Plant (UTPP, No.):

Number of non-fertile tillers per plant was recorded from five randomly taken plants in the middle five rows of each plot.

Number of Primary Branches per Panicle (PBPP, No.):

Taken by counting the number of all primary branches produced on main panicle axis at harvest maturity from five randomly taken panicles and averaged.

Data Collected on Plot Basis

Days to Heading (HD, days):

Number of days from days to sowing to the date when the tips of the panicles first emerged from the main shoots on 50% of the plant in a plot.

Days to Maturity (MD, days):

Number of days from the date of sowing to the date when 85% of grain on panicle are matured.

Harvest Index (HI, %):

The ratio of grain yield per plot to biological yield per plot was expressed in percent at harvest maturity.

Thousand Grain Weight (TGW, g):

The weight of 1000 grains in gram from bulked grains, which were collected from five central rows of each plot were measured and adjusted at 14% moisture content.

Biological Yield (BY, g):

At harvest maturity, an area of 0.25m^2 (0.5 m x 0.5m) biomass within each plot was harvested, and oven-dried for 72 hours at 70 ℃ and weighed, then converted into biological yield per plot.

Grain Yield per Hectare (GY, kg):

Grain yield in gram obtained from each plot of the middle five rows at physiological maturity was converted into kilogram per hectare, after cleaned and adjusted to 14% moisture content level.

Statistical Analysis

Cluster and Genetic Distance Analysis:

In order to examine the grouping pattern of the 36 lowland rice genotypes, average linkage method was used. The numbers of clusters were determined based on pseudo-F and t^2 values. The point where local peaks of psedo-t^2 F-statistics join with small value of the pseudo-t^2 followed by a large pseudo-t^2 for the next cluster combination was used to determine the number of cluster by using SAS statistical package [11]. The dendrogram was constructed on the basis of average linkage using SAS statistical package version 9.3 Mahalanobis's statistics [7] was used to examine the genetic distance between populations. The generalized genetic distance between any two populations is defined as: $D^2ij = (xi - xj)\ cov\text{-}1(xi - xj)$.

Where, ij = the distance between cases i and j; and j; xi and xj = vectors of the values of the variables for cases i and j; and cov-1 = the pooled within groups variance-covariance matrix.

The significance level of genetic distance between clusters was tested at both 1% and 5% level of probability using Chi-square test. The D^2 values obtained for pairs of cluster using SAS statistical package was considered as the calculated values of the Chi-square(x^2) and tested for P degree of freedom, where P is the number of characters considered (Singh and Chaudhary, 1985).

Principal component analysis (PCA) Principal component analysis was performed using correlation matrix's of SAS version 9.2. From this analysis, only PCs with eigenvalues greater than one was retained. Generally, the goal of PCA is to extract the most important information from the table, compress the size of the data set by keeping only this important information, simplify the description of the data set and analyze the structure of the observations.

III. Results and Discussion

Principal Component Analysis (PCA)

The principal component analysis showed that the first five principal components with Eigen values greater than one explain 80% of the total variation among the 36 rice genotypes evaluated for 15 traits. The result is in agreement with the findings of by Nachimuthu *et al*. [8]. Which identified five the most chief contributors in 192 rice germplasm for 12 agro- morphological traits that accounted for 80 % of the total variation. The first principal component explained 33% of the total variation originated chiefly from all traits except days to 50% heading, days to 85% maturity and harvest index. Similarly, the high contribution of panicle length for separating genotypes in the first principal was reported by Tuhina-Khatun *et al*. [14]. It has high negative weight for fertile grain per panicle, fertile tiller per plant, unfertile tiller per plant and thousand grain weights. The second principal

component explained 53% of the total variation. Higher variation had obtained from 85% maturity days, plant height, grain yield, infertile grain per panicle, unfertile tiller per plant, fertile tiller per plant, total tiller per plant and harvest index and the third principal component accounted for about 64% of the total variation and it was chiefly contributed by panicle length, primary branches per panicle, unfertile tiller per plant, total tiller per plant and culm length.

The fourth principal component accounted 72% of variation and was indicated with thousand grain weight, grain yield, primary branches per panicle and days to maturity. Biological yield and days to 50% heading expressed highest negative loads in principal component four (PC4). The major contributing traits for the variation in the five principal components (PC5) were chiefly obtained from variation of days to 50% heading and fertile grain per panicle. The above mentioned characters with high positive or negative loads contributed more to the diversity and they were the ones that most differentiated the clusters. Characters with relatively greater positive weight of eigenvectors in pc1 include culm length, panicle length, total tiller per plant and primary branches per panicle. Selection based on these characters may be effective because of the higher comparative variability. Plant height and days to 85% maturity had relatively larger contribution to the second principal component.

Table 2. Relative contributions of characters to the total divergence in lowland rice genotypes.

Traits	PC1	PC2	PC3	PC4	PC5
HD	0.28	0.04	0.29	-0.34	0.72
MD	0.03	0.85	-0.19	0.29	0.11
PH	-0.04	0.88	0.05	0.21	0.15
PL	0.69	-0.22	0.6	0.28	-0.15
CL	0.78	0.16	0.36	-0.01	0.17
PBPP	0.66	-0.25	0.6	0.3	-0.18
TTPP	0.69	0.41	-0.31	-0.02	-0.21
FTPP	-0.73	0.43	0.46	-0.1	-0.05
UFTPP	-0.66	0.44	0.5	-0.12	0.06
FGPP	-0.74	0.09	0.16	-0.02	-0.48
INFGP	0.71	0.49	-0.19	-0.05	-0.27
TGW	-0.55	-0.14	-0.2	0 .43	0.29
HI	0.35	-0.4	-0.28	0.29	0.2
BY	0.54	0.21	-0.09	-0.68	-0.07
GY	0.32	0.56	0.01	0.3	0.05
Eigen value	4.9	2.9	1.71	1.27	1.13
Proportion (%)	0.33	0.2	0.11	0.08	0.08
Cumulative (%)	0.33	0.53	0.64	0.72	0.8

Different characters also contributed to the variation in the third fourth and five principal components. Generally, the presence of phenotypic diversity among the varieties is also reflected in the principal component analysis which revealed that the entire variation could be in consideration for crop improvement.

Table 3. Distribution of genotypes in to 4 clusters based on D^2 analysis for rice genotypes.

Clusters	Number of genotypes	Proportion (%)	Name of genotypes
Cluster I	18	50	Aromatic-1, Edirne, Halilbey, Trakya, Tunca, Pepita, Samgangbyeo, SCRID091-15-2-2-1-1, SCRID091-18-1-5-4-4, SCRID091-38-3-1-3-1, SCRID091-24-3-2-2-3, SCRID090-72-3-1-3-5, SCRID090-177-2-4-3-4, SCRID122-5-2-1-1-3, SCRID122-13-1-1-4-3, SCRID198-73-5-1-3, X-Jigna, Hiber
Cluster II	17	47.2	Osmancik-97, Suitou Chuukanbohon Nou 11, Condai, Saegyejinmi, Lunyuki, Hangamchal, Hawaghaelo-2, Namcheobyeo, SCRID091-20-2-2-4-4, SCRID090-60-1-1-2-4, SCRID090-164-2-1-2-1, SCRID090-18-1-2-2-1, SCRID091-20-3-1-3-4, SCRID186-72-1-1-2, GSRIR1-17-Y16-Y3-Y2,GSR IR1-15-D4-D1-Y1, Ediget.
Cluster III	2	5.55	Saegyejinmi, Lunyuki
Cluster IV	1	2.7	SCRID091-10-1-3-2-5

Cluster Analysis

Cluster analysis showed that 36 genotypes were classified in to four clusters based on their similarity (table 11) which makes them moderately divergent. The first clusters CI, n = 18) had the largest number of genotypes obtained from Africa rice and one local checks(X-jigna) and one from WARDA (Hiber). Cluster II consist of 17 genotypes, two of them originated from IRRI and one check (Ediget). Cluster III consists of 2 genotypes originated from Africa rice and the fourth cluster only one obtained from Africa rice.

Cluster Mean Analysis

The pooled mean values of each quantitative trait in each cluster is presented in table 4. Genotypes in cluster I were the earliest to heading but those genotypes in cluster III were relatively late in heading. Genotypes in cluster IV were early maturing whereas in cluster III Late maturing. The highest mean for plant height was recorded by genotypes in cluster II, while lowest mean for plant height was recorded in genotypes grouped under cluster IV. The highest mean for panicle length and culm length was recorded by genotypes in cluster II, while lowest mean in panicle length obtained in cluster I. But in culm length lowest mean found in cluster IV. The highest mean value for number of primary branches per panicle was recorded by genotypes in cluster II whereas lowest recorded obtained in cluster III. The largest mean value for total tiller per plant and fertile tiller per plant was recorded in cluster III, while the lowest mean was recorded in cluster IV. The highest unfertile tiller per plant was recorded in genotypes found in Custer I. The highest mean for fertile grain per panicle was recorded in genotypes which were grouped under cluster II, while lowest mean was obtained in cluster I. Infertile grain per panicle had higher mean value in cluster I, while lower value found in cluster IV.

Table 4. Mean values of 15 characters for the 4 clusters of 36 rice genotypes.

Traits	Cluster I	Cluster II	Cluster III	Cluster IV
HD	89.29	92.95	101.13	93
MD	127	129.77	141.88	126
PH	90.07	91.67	86.2	80.00
PL	17.63	18.35	18.15	18.2
CL	72.9	73.96	69.15	61.80
PBPP	8.76	9.82	8.57	9.60
TTPP	7.92	7.28	8.13	4.80
FTPP	7.49	6.97	7.78	4.80
UFTPP	0.50	0.30	0.42	0.00
FGPP	73.94	88.87	80.4	75.6
INFGP	6.63	4.16	5.85	3.8
TGW	26.06	26.11	26.55	28.00
HI	0.38	0.44	0.32	0.49
BY	4635.14	5981.61	7510.50	2920.00
GY	2368.02	3523.17	2908.57	3231.16

The genotypes in cluster IV showed the highest harvest index. But cluster I had lower mean value for harvest index. Thousand grain weights had greater mean value in cluster IV, and the lowest mean value in cluster I. The highest mean value for biological yield obtained in cluster III, while lowest mean value for biological yield found in cluster IV. The largest mean cluster value for grain yield was recorded by cluster III, while the lowest mean for grain yield was recorded by genotype in cluster I.

Distance among Clusters (Genetic Divergence Analysis)

The D^2 statistics is a tool for estimating the distance between clusters, which is the basis in choosing parents for hybridization in a successful crop improvement and breeding program. The extent of diversity present between genotypes determines the extent of improvement gained through selection and hybridization. Progenies derived from diverse cross are expected to show the broad spectrum of genetic variability providing greater scope for isolating high yielding segregates in the succeeding generations. The standardized Mahalanobis's D^2 statistics showed 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 three and four ($D^2 = 4601.87$) followed by cluster two and four ($D^2 = 3075.59$) and cluster one and three ($D^2 = 2925.81$). The minimum squared distance was found between cluster two and three ($D^2= 1647.9$). Generally; this study revealed that genotypes included in this study are divergent (table 5).

Table 5. Pair wise generalized square distance (D^2) among 36 genotypes in four clusters.

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I		1774.15**	2925.81**	1920.15**

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster II			1647.9**	3075.59**
Cluster III				4601.87**
Cluster IV				

** = significant, X^2 = 21.06 and 13.3 at 1% and 5% probability level, respectively.

Minimum inter cluster distance was observed between two and three (D^2 = 1647.9), 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 three and four followed by cluster two and four and cluster one and three. According to Khodadi *et al*. [6] and Rahim *et al.* [10] the cross between genotypes with maximum genetic distance would bring maximum heterosis.

IV. Conclusion

The principal component analysis identified five important principal components PC1 to PC5 with eigenvalues greater than one and explains about 80% of the total variation. The presence of phenotypic diversity among the varieties is also reflected in the principal component analysis which revealed that the entire variation cannot be explained in terms of few PCs.

Clustering and divergence analysis of quantitative characters based on multivariate analysis, indicated the existence of 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 three and four closely followed by cluster two and four; and then cluster one and four, respectively.

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AUTHOR'S PROFILE

First Author
Mequannit Aklilu Gebre, EIAR (Ethiopian Institute of Agricultural Research), Tepi Agricultural Research Center, P.O. Box, 34, Teppi, SNNPs, Ethiopia.
email id: mequannit@gmail.com