



Genetic Divergence and Cluster Analysis for Yield and Yield Contributing Traits in Lowland Rice (*Oryza sativa* L.) Genotypes at Fogera, Northwestern Ethiopia

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Abstract

The history of rice production and research in Ethiopia is very short. Hence, genetic improvement is of paramount importance to increase the production and productivity of the crop, which requires understanding of genetic divergence and cluster distance between the genotypes. The study was conducted during 2017/2018 main cropping season at Fogera, Ethiopia, to determine the magnitude of genetic distance among 30 lowland rice genotypes through cluster analysis, which helps to identify parental lines for hybridization programs. The experiment was laid out using randomized block design with three replications. Data were collected for 17 agronomic characters and analysis of variance revealed significant differences among the genotypes for all characters. Grain yield ranged from 2766.7 to 7062 kg ha^{-1} with a mean of 4736.13kg ha^{-1} . Genotypes G26 (7062kg), G14 (6900kg), G8 (6583.1kg), G27 (6486.9kg), G29 (6400.6kg) and G30 (6343.1kg) were found to be high yielding. Cluster analysis showed the existence of five divergent groups and the maximum inter-cluster distance was between clusters II and III ($D^2=6758$); while the minimum inter-cluster distance was between clusters III and V ($D^2=2432$). It is suggested to cross genotypes from cluster II and III, I and III to get genotypes/varieties with high grain yield and early maturing genotypes. For future breeding program that employ hybridization, parental material selection should be carried out between clusters rather than within clusters.

Keywords: Cluster distance, Genetic divergence, *Oryza sativa* (rice)

Introduction

Rice is one of the most significant food crops of the world. It belongs to the family Poaceae and genus *Oryza* (Wang *et al.*, 2014). The genus *Oryza* is known to consist of two cultivated species i.e.; Asian rice (*O. sativa*, $2n=24=AA$) and African rice (*O. glaberrima*, $2n=24=AA$) and 22 wild species ($2n=24, 48$) (Singh *et al.*, 2015). The river valleys of Yangtze, Mekon River area in China could be the primary center of origin of *Oryza sativa* (Zhao 2011; Gross and Zhao, 2014). *Oryza glaberrima* is indigenous to the upper valley of

the Niger River and it is cultivated in western tropical Africa (Ansari *et al.*, 2015). Cultivated rice (*O. sativa*) is predominantly self-pollinating and has lower out crossing ability than *O. rufipogon*. According to Messeguer *et al.* (2001), the cross-pollination rates of *O. sativa* are less than 1%. However, the estimated out crossing rates among wild rice populations ranges from 4.3% to 55.9% (Oka, 1988). Rice is a highly diverse crop species with wide geographic dispersal from sea level up to 3000 m.a.s.l. in both temperate and tropical climate (Oka, 1988; Mickel *et al.*, 1990).

Rice is the second most-produced cereal in the world after wheat and represents a staple food source for more than half of the world's population (Luz *et al.*, 2016). Most of the world's rice is cultivated and consumed in Asia (Chakravarthi and Naravaneni, 2006). Although Asia is the main place of rice cultivation, rice is also produced in other continents like Latin America, Europe, USA and some parts of Africa (Zibae, 2013). From the total production, Asia accounts the largest production totaling to about 144.25 million tons whereas Africa produces approximately 11.58 million tons (FAO, 2015).

Rice was introduced in to Ethiopia during the 1970s and fast distribution of the crop within the country has been achieved (Beakal *et al.*, 2016). Presence of potential land under irrigation (3.7 million ha) and rain fed (25 million ha), existence of diverse ecosystems such as the uplands, rain fed low lands and flash flood prone areas, long shelf life and acceptance of rice amongst rural population due to the possibility of using rice to a range of traditional food recipes, relatively higher productivity as compared to other main staple crops and the by-products from rice such as straws and husks that shall be fed to livestock and/or used as alternate source, are the main attracting factors for rapid increase in rice production in the country (MOARD, 2010).

In Ethiopia, rice covered about 48,418.09 hectares of land and 136, 0007. 26 tons of grain was produced per annum in 2016/17 with average productivity of 2.81 tons per hectare (CSA, 2017). As the demand of rice production is increasing in alarming rate, the area of production almost doubled from 18000 ha in 2006 to 48,418.09 ha in 2016/2017 (Assefa *et al.*,2011; CSA, 2017). In Ethiopia, rice offers a variety of uses. It is used in the preparation of local foods (*injera, dabbo, genffo, kinchie,shorba*) and local beverages (*tella* and *katikalla/Areki*) either alone or mixed with other cereal grains (Heluf and Mulugeta, 2006).

However, the average rice productivity in Ethiopia is estimated at 2.81 t ha⁻¹ (CSA, 2017), which is much lower than the world's average of 4.6t ha⁻¹ (FAO, 2015). Despite the fact that rice has been recognized by Ethiopian government as “the new millennium crop of Ethiopia” to attain food security, lack of improved varieties, lack of recommended crop management, lack of pre and postharvest management coupled with biotic and abiotic stresses limit the production and productivity of the crop in the country (Tsfaye *et al.*, 2005; MoARD, 2010; EIAR/ FRG II, 2011). Among

these problems, lack of improved varieties for different agro ecologies of the country is the most serious concern (MoARD, 2009; EIAR/ FRG II, 2011). In many countries, rice is a long established crop and cultivars have been selected that are well adapted to local conditions and the local market. It is estimated that about 120,000 varieties of rice exist in the world (Sasaki and Moore, 1997). But in Ethiopia which has diverse agro-ecologies, there are no more than elven lowland rice varieties in the whole country. Farmers of South Gondar, especially those in Libokemikem, Fogera and Dera districts, largely produce lowland rice under rain-fed condition. Due to swampy nature of the study area, crop production was limited before rice adoption. Fogera and surrounding districts are swampy areas which are ideal for lowland rice cultivation. However, one of the major constraints in the area is the absence of high yielding improved lowland rice varieties resistant to diseases and to terminal water deficit (terminal moisture stress). Hence, as rice is a potential crop in study area, increasing its productivity per unit area and its total production will enable farmers get encouraging returns and improves its role in achieving food self-sufficiency. To increase the productivity of rice in the country, research has been conducted mainly at Fogera National Rice Research and Training Center (FNRRTC). The center introduced a bulk of genotypes from International Rice Research Institute (IRRI) and African Rice Center (WARDA), which are sources of variability for future rice improvement in Ethiopia.

The success of plant breeding research depends on the availability of genetic variation. However, full information is lacking on the genetic divergence and cluster distance between recently introduced low land rice genotypes in the study area. Genetic improvement mainly depends on the amount of genetic variability present in the population which is a universal property of all species in nature (Dutta and Burua, 2013). Variability in genotypes for yield and yield component traits forms the basic factor to be considered while making selection (Haydar *et al.*, 2007). The character yield reflects the performance of all plant components and might be considered as the final result of many other traits. i.e. every plant contains an inherent physiological production capacity that operates on energy required for normal plant performance. Not all genotypes have the same inherent physiological capacity to yield (Welsh, 1981).

The knowledge of diversity and genetic distance among groups of genotypes helps to identify parental lines for hybridization programs. Therefore, the present study has been conducted to determine the level of genetic divergence among lowland rice genotypes through cluster analysis.

Materials and Methods

Experimental Site Description

The experiment was conducted in the North-Western part of Ethiopia at FNRRTC during the rainy season (June-December) of 2017/18. FNRRTC is located in

Amhara Regional state, in the North-Western part of Ethiopia, 607 km far from Addis Ababa. The experimental site is found at Woreta and located 11°58' N latitude, 37° 41' E longitude and at an elevation of 1810m above sea level. Based on ten years' average meteorological data, the annual rainfall, and mean annual minimum, maximum and average air temperatures are 1300mm, 11.5°C, 27.9°C and 18.3°C, respectively. The soil type is black *Vertisol* with pH of 5.90 (Dejen, 2020). The main water source for rice production in the study area is rain-fall water. Irrigation water from rivers Rib and Gumara was also used in the off season for production of vegetables as the second crop after rice.

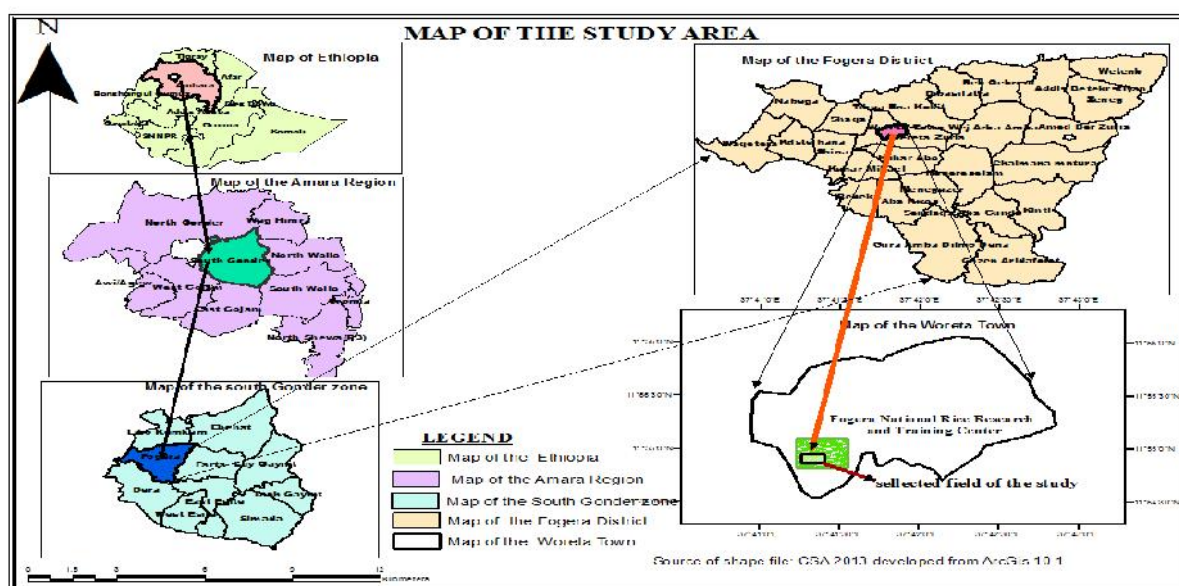


Figure 1. Diagrammatic descriptions of experimental site

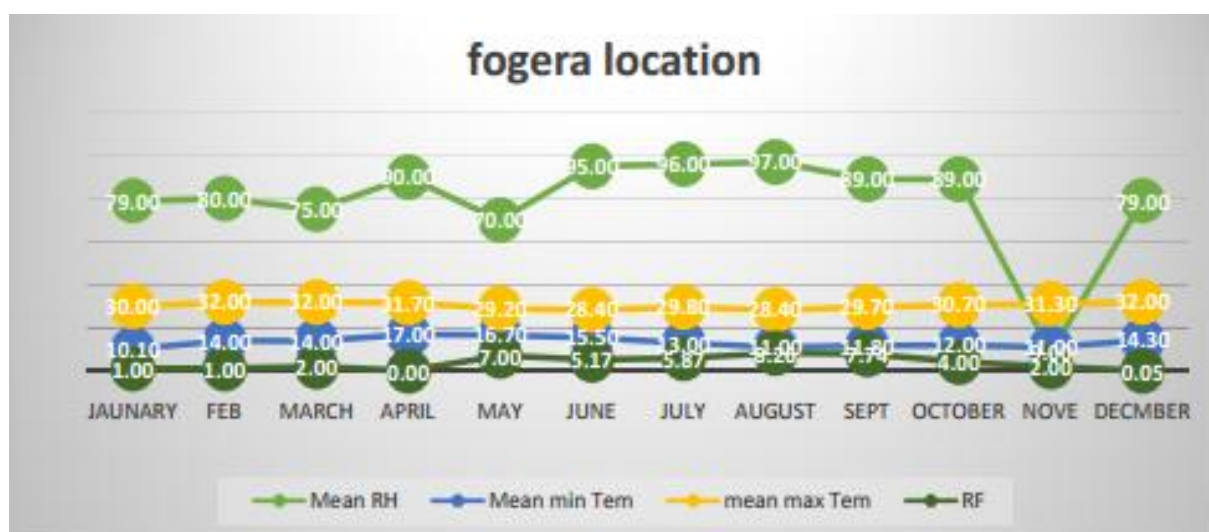


Figure 2. Fogera location environmental descriptions

Experimental Materials and Design

Thirty genotypes consisting of 27 lowland rice genotypes introduced in 2015 from African Rice Center (Formerly called WARDA), two released lowland varieties (Ediget and Hibir) and one locally available genotype (X-Jigna), obtained from FNRRTC, were used for this study (Table 1). The majority (20) of the material had its origin in Nepal. Two genotypes each were from Madagascar and Ruawnda. Three genotypes had IRRI coding.

The experiment was laid out in randomized complete block design with three replications. Each plot had six rows each 4 m long, with a spacing of 25 cm between

rows and 15cm between plants. The plot size was 4 x 1.5m = 6 m². Net plot size was 4 rows x 4m = 4m². The distance between plots and replications was 0.3 m and 1 m, respectively. Three healthy and uniform sized seeds were drilled per hill on date 28 June 2017 and thinning was conducted after germination to ensure single plant per hill.

Fertilizer in the forms of N and P₂O₅ was applied at a rate of 69/23 Kg/ha, Urea and NPS, respectively. All the NPS was applied at sowing. Urea was applied as split three times, 1/3 at sowing, 1/3 at tillering and the remaining at panicle initiation stage. All other agronomic practices were applied as recommended for rice production in the study area.

Table 1. List of lowland rice genotypes used for this study

No.	Genotype	Code	Origin	No.	Genotype	Code	Origin
1	B6144F-MR-6-0-0-0	G1	Madagascar	16	MERING	G16	Nepal
2	CHOMRONG	G2	Nepal	17	NERICA L-19	G17	Nepal
3	DEMIR	G3	Nepal	18	OSMANLIK-97	G18	Nepal
4	DIAMANTE	G4	Nepal	19	PADISASHAL	G19	Nepal
5	DURAGAN	G5	Nepal	20	PARTAO	G20	Nepal
6	(Edgt)WAB189*	G6	Released in 2011	21	SCRID2-1-2-4	G21	Nepal
7	FARO-35	G7	Nepal	22	SILEWAH	G22	Nepal
8	FOFIFA160	G8	Nepal	23	SIM2SUMADEL	G23	Nepal
9	HIBIR*	G9	Released in 2013	24	4181-SOAMOVA	G24	Nepal
10	HS379	G10	Nepal	25	WITA 4	G25	IRRI
11	IR64	G11	IRRI	26	X-243	G26	Nepal
12	KIRKPINAR	G12	IRRI	27	X-265	G27	Nepal
13	MACHAPACHURI	G13	Nepal	28	X-JIGNA	G28	Locally available genotype Ruawnda
14	MAKALOIKA34	G14	Nepal	29	YUN-KENG	G29	Ruawnda
15	4182—MANJAOVE	G15	Madagascar	30	ZONG-ENG	G30	Ruawnda

IRRI= International rice research institute, *=released varieties.

Data Collection

Based on the standard evaluation system developed by International Rice Research Institute (IRRI, 2002), seventeen quantitative traits such as days to heading, days to 50% flowering, days to maturity, thousand-grain weight, biomass yield, grain yield, harvest index, number of tillers per plant, number of panicles per plant, culm length, panicle length, plant height, flag-leaf length, flag-leaf width, number of filled grains per panicle, number of unfilled grains per panicle and panicle weight were recorded at appropriate growth stage on plot and plant basis. These data were recorded from pre-tagged ten randomly sampled plants in the four central harvestable rows of each experimental unit/plot. However, yield per plot and phenological traits were taken on plot basis.

Statistical Analysis

A measure of a group distance based on multiple characters was given by generalized Mahalanobis D^2 statistics (Mahalanobis, 1936) for 17 quantitative characters and was analyzed using the procedure Procdiscrim of SAS Software. Squared distance (D^2) for each pair of genotype combinations was computed using the following formula:

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

Testing the significance of the squared distance values obtained for a pair of clusters was taken as the calculated value of χ^2 (chi-square) and tested against the tabulated χ^2 values at $p-1$ degree of freedom at 5% and 1% probability level, where p = number of traits used for clustering the genotypes.

The average inter cluster distances were calculated by the formula given by Singh and Chaudhary (2005)

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

Where, D^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 .

Cluster analysis based on Average's method was performed using MINITAB 17 statistical packages

(Minitab, 1998) to cluster the genotypes based on their agronomic traits.

Results and Discussion

Genetic Divergence Analysis

Clustering of genotypes

The 30 lowland rice genotypes exhibited significant differences for 17 characters. The presence of significant differences among genotypes for all the characters justified further calculation of D^2 (Sharma, 1998). The dendrogram obtained from the cluster analysis grouped 30 lowland rice genotypes into four clusters based on the averages clustering method (Table 2). The D^2 values were based on the mean of genotypes; cluster II was the largest cluster which consisted of 12 genotypes (40%) followed by Cluster I which comprised of 10 genotypes (33.33%), and Cluster III had 5 (16.67%) genotypes, while Cluster IV had the lowest number of genotypes that comprises only three genotypes (10%). Two genotypes from Madagascar and two genotypes from Ruanda were in Cluster I. All three checks were in cluster II. One genotype with IRRI code was assigned to Cluster I, One to Cluster II and the other to Cluster IV. The result of cluster analysis indicated, some genotypes were not manifested the area of their origin.

Different authors reported the presence of diversity among rice genotypes classifying in different number of distinct clusters. Baloch *et al.* (2016) classified 20 irrigated lowland rice genotypes with 11 morphological characters into four clusters and showed wide genetic diversity among the tested genotypes. Using 17 morpho-agronomic traits, Worede *et al.* (2014) grouped 24 upland rice genotypes into two clusters. Chakma *et al.* (2012) had grouped 39 irrigated rice genotypes in to six distinct clusters. Sarker *et al.* (2013) classified 32 early maturing rice genotypes in to three clusters. Ravikumar *et al.* (2015) classified 24 irrigated rice accessions in to five clusters. Alamir (2018) classified 36 low land rice genotypes with 12 morphological characters into seven clusters and showed the existence of genetic diversity among the tested genotypes.

Table 2: Grouping of 30 lowland rice genotypes into five clusters by Average's method

Cluster No.	No. of genotypes	Percentage	Name of genotypes
I	10	33.33%	G(1,7,8,11,13,15,17,24,29&30)
II	12	40%	G(2,3,4,5,6,9,10,12,16,18,21 &28)
III	5	16.67%	G(14,26,27,20 &25)
IV	3	10%	G(19,22&23)

G= Genotype

Cluster mean analysis

The mean value of genotypes in each cluster was computed and cluster means are presented in Table 3. There was considerable difference among the clusters for different characters.

Cluster I had the largest PP (9.5), FGPP (121.6), PW (3.3g) and HI (50.7%). It had the second highest BY (11458.4kg), and GY (5727.8 kg ha⁻¹) next to cluster III. All 10 genotypes in this cluster except G7 (4753.9 kg ha⁻¹) gave more than 5000 kg ha⁻¹ (5014 to 6583 kg ha⁻¹). Majority of the genotypes in this cluster showed moderate performance in most of the yield and yield related traits as compared to the remaining clusters. It had relatively moderate culm length, plant height, flag leaf length and thousand grain weight with mean values of 72.2cm, 94.7cm, 29.7cm and 27.8g, respectively. However, this cluster had the second earliest heading, flowering, and maturing genotypes (97.7, 101.8 and 138.7days) after Cluster II.

Cluster II consisted of the largest number of genotypes (40%) and was the earliest heading, early flowering and maturing genotypes (85, 89 and 127 days, respectively). Majority of the genotypes in this cluster showed least performance in most of the yield and yield related traits. It had least culm length (61.04cm), panicle length (17.34cm), plant height(78.38cm), flag leaf length(20.32cm), flag leaf width(1.08cm), number of filled grain panicle⁻¹(93.7), number of unfilled grain panicle⁻¹(8.98), panicle weight(2.04g) and biomass yield (7980kg) but the highest number of tillers plant⁻¹(9.8) and thousand grain weight (30.75g). It had the second lowest grain yield per ha (3863.13kg ha⁻¹) next to cluster IV (3067.9 kg ha⁻¹).

Cluster III contained four genotypes from Nepal (G14, 20, G26 and G27) and one from IRRI (G25) and characterized by the largest FL (31.5cm), UGPP

(13.0), BY (14157.2kg) and GY (5848.9kg) with the lowest thousand grain weight (25.8g). Except G25(3728.7 kg ha⁻¹) grain yield of this cluster ranged from 5066.7 kg ha⁻¹ (G20) to 7062 kg ha⁻¹ (G26). The cluster had the second largest mean in TP, PP, CL, PH, PW and FGPP. Moreover, it had the second latest genotypes for days to heading, flowering and maturity (108.8, 113.0 and 148.5 days) after genotypes in Cluster IV. It also had moderate flag leaf width (1.2cm). Hence, genotypes from this cluster can be used in lowland rice breeding program for grain yield improvement.

Cluster IV contained three genotypes characterized by late genotypes in days to 50% heading, 50% flowering and 85% maturity (109.9, 113.7 &150.2days). It had the highest culm length, panicle length, plant height and flag leaf width (89.3, 24.3, 113.6, and 1.4cm, respectively). The genotypes in this cluster had also relatively moderate number of filled grains per panicle (104.4), number of unfilled grains per panicle (11.7) and panicle weight (2.6g). This cluster had characteristics of the lowest number of tillers per plant (7.8), panicles per plant (7.8), grain yield (3067.9kg ha⁻¹) and harvest index (28.7%). It had also relatively lowest biomass yield (10861.3kg) next to cluster II (7980kg).

This might have resulted due to late maturity of the genotypes in the clustered which might have exposed them to terminal moisture stress. Thus, genotypes in this cluster started flowering after three months (113.7 days) from date of emergence at the time of much decreasing of rain fall and relative humidity with correspondence increasing of mean temperature in October (Fig2). These genotypes face challenges of moisture stress and diseases occurrence at grain filling period. Therefore, supplementary irrigation should be required to finish their physiological activities.

The results of mean and inter cluster distance analysis suggested that parental lines selected from these clusters could be used in hybridization programs, since crossing between divergent parents is likely to produce wide variability and transgressive segregations with high heterotic effects. To get genotypes/ varieties with high grain yield and early maturing genotypes, it is

possible to cross genotypes from cluster II and III, I and III. Sarker *et al.* (2013), Ahmed *et al.* (2014) and Mohammad *et al.* (2017) had also reported that selection of parents for hybridization should be done from two clusters having wider inter-cluster distances to get maximum variability in segregating generations.

Table 3. Cluster means for 17 characters of 30 low rice genotypes

Traits	I	II	III	IV
DH	97.7	85	108.8	109.9
DF	101.8	89.11	113.0	113.7
DM	138.7	127	148.5	150.2
TP	9.8	9.83	9.6	7.8
PP	9.5	8.98	9.4	7.8
CL	72.2	61.04	74.3	89.3
PL	22.5	17.34	22.3	24.3
PH	94.7	78.38	96.6	113.6
FL	29.7	20.32	31.5	29.8
FW	1.2	1.08	1.2	1.4
FGPP	121.6	93.7	119.8	104.4
UGPP	10	8.98	13.0	11.7
PW	3.3	2.04	2.8	2.6
TGW	27.8	30.75	25.8	26.7
BY	11458.4	7980	14157.2	10861.3
GY	5727.8	3863.13	5848.9	3067.9
HI	50.7	48.57	41.5	28.7

DH= days to heading , DF= days to Flowering, DM=days to maturity, TP=tillers plant⁻¹ , PP=panicles plant⁻¹, CL=culm length, PL= panicle length, PH=plant height, FL= flag leaf length ,FW= flag leaf width , FGPP= filled grain panicle⁻¹,UGPP=unfilled grain panicle⁻¹ ,PW= panicle weight, TGW= thousand grain weight, BY= biomass yield ha⁻¹ in Kg, HI= harvest index, GY=paddy yield ha⁻¹ in Kg

Estimation of inter cluster square distances (D²)

The distance between clusters were estimated by Mahalanobis distance such that the values calculated between pairs of clusters were considered as chi-square values and tested for significance using p-1 degrees of freedom, where "p" indicates the number of characters used (Singh and Chaudhary, 1985). The results of distance between clusters are presented in Table 4. Accordingly, the χ^2 -test for the four clusters, there was highly significant difference among the clusters.

The highest inter-cluster distance was exhibited by cluster II and III (D² =6488.76) followed by cluster III

and IV (D² = 4312.45) and cluster I and II (D² =3946.90), which implied these clusters were genetically more divergent from each other than any other pairs of cluster. The smallest inter-cluster distance was observed between Cluster I and III (D² = 2701.60) succeeded by cluster I and IV (D²= 2726.41). The genotypes belonging to these clusters were relatively close to each other, in comparison to genotypes grouped in other clusters. According to Rama (1992) crossing of genotypes from those clusters might not give higher heterotic value in F₁ and narrow range of variability in the segregating F₂ population. Such analysis was meant to avoid selection of parents from genetically homogeneous clusters to maintain a relatively broad genetic base.

Accordingly, it is well recognized that the greater the distance between clusters, the wider the genetic diversity would be between the genotypes. Therefore, highly divergent genotypes would produce a broad spectrum of variability in the subsequent generation enabling further selection and improvement and it is important for rice breeding program.

Generally, this divergence analysis showed presence of high genetic divergence among the tested thirty lowland rice genotypes evaluated at Fogera plain. Therefore, maximum recombination and segregation of progenies is expected from crosses involving parents selected from cluster two and three, followed by cluster three and four and cluster one and two. However, the breeder must specify his objectives in order to make best use of the characters where the characters are divergent.

Table 4. Average inter-cluster squared distance (D^2) between clusters based on 17 characters of 30 lowland rice genotypes tested in 2017/18 at Fogera, Ethiopia

Cluster	I	II	III	IV
I	-	3946.90**	2701.60**	2726.41**
II		-	6488.76**	2989.80**
III			-	4312.45**
IV				-

* and **, significant ($\alpha = 32\%$) and highly significant ($\alpha = 34.27\%$) at 5 and 1% probability levels, respectively

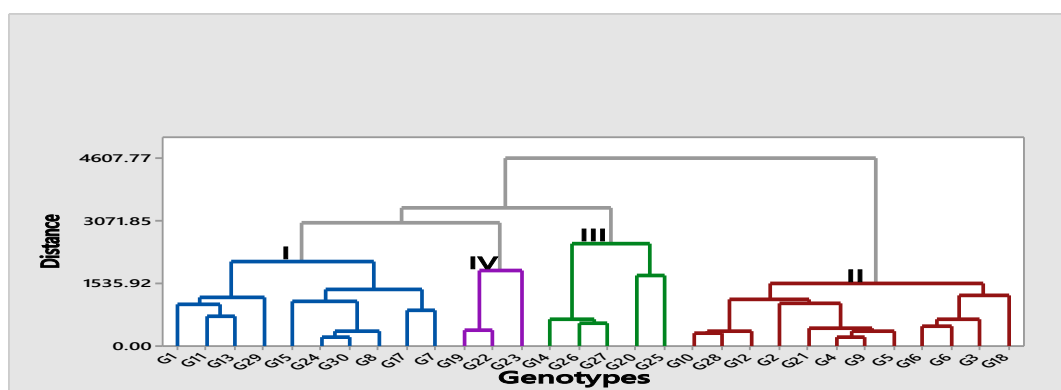


Figure 3. Dendrogram generated by average’s cluster analysis method for 30 lowland rice genotypes for 17 characters evaluated at Fogera in, 2017/18

Conclusion

Rice has relatively short history of production and research in Ethiopia that variety development mainly dependent by the introduction of materials in which understanding of the genetic variability of genotypes is the critical step. The present study was an attempt to know the magnitude of genetic distance between recently introduced lowland rice genotypes for future utilization in the breeding program. To generate this information a total of 27 lowland rice genotypes with two standard checks and one locally available genotype were evaluated using randomized complete

block design with three replications during the 2017/18 main cropping season at FNRRTC. The analysis of variance showed highly significant differences among the tested genotypes for all 17 studied traits, which indicates presence of considerable genetic variability between the genotypes.

Paddy grain yield ranged from 2766.7 kg ha⁻¹ for G12 to 7062.0 kg ha⁻¹ for G26 with a mean of 4736.13kg ha⁻¹. About 46.7% and 90% of the genotypes had higher mean grain yield than the standard check G6 (Ediget) and the local check G28 (X-Jigna), respectively.

The maximum cluster distance was found between cluster two and three while the minimum was found between cluster one and three. Based on the present investigation results, it can be concluded that there is adequate genetic variability for most of quantitative characters evaluated, that the genotypes with high grain yield should be selected from different clusters and crossed so as to improve grain yield. The study also identified the best performing genotype for further evaluation and/or recommended for release for possible commercialization.

For future breeding programs that employ hybridization, parental material selection should be carried out between clusters rather than within clusters. It is recommended to repeat the study at more seasons and locations with more number of genotypes to predict genotypic performance across seasons and locations which helps to validate the obtained current results. Moreover, the future rice research should be supplemented by molecular characterization to further confirm the outcome of current study findings.

Conflict of Interests

The authors have not declared any conflict of interests.

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