Cluster, Divergence and Principal Component Analysis of Niger seed (*Guizotia abyssinica* (L. f.) Cass.) Genotypes

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Abstract: *Niger seed is among the most important oilseeds in Ethiopian Agriculture. The genetic divergence of niger seed genotypes collected from diverse ecologies of Ethiopia were evaluated using simple lattice design based on 12 quantitative traits to assess the genetic diversity of Ethiopian niger seed genotypes using multivariate techniques. Cluster analysis grouped the genotypes into five distinct groups. Each group showed admixtures of accessions rather than traceable patterns of geographical origin. The highest inter cluster distance was observed between cluster IV and V (D2=94.75) and the lowest divergence was observed between cluster II and IV (D2=19.80). The first five Principle components explained 81 % of the total variation. The first component strongly influenced by days to flowering (-0.438) and number of head (0.377). Component two highly influenced by yield per plot (-0.542) and oil content (-0.532). Number of seed per head (0.623), number of primary branch and days to maturity (0.651) strongly influence component three, four and five respectively. Therefore, the study indicates the existence of genetic divergence among the tested genotypes and the possibility to identify divergent material with desirable agronomic features for the development of new superior niger seed cultivars through hybridization and selection by crossing accessions from different clusters.*

Keywords: *Cluster, Ethiopia, Genetic divergence, Niger seed, Principal component analysis*

1. INTRODUCTION

Niger seed (*Guizotia abyssinica* (L.f.) Cass.) is the oldest indigenous oil crops with longest history of its cultivation in Ethiopia. Commercial niger seed is grown in Africa, India and Southeast Asia and the seed is imported around the world as a popular type of birdseed. The nutty test and pleasant odor; pale yellow oil of niger seed is the main source of edible oil for local consumption in Ethiopia and India (Adarsh *et al.,* 2014).

The oil is used for cooking, lighting, anointing, painting and cleaning of machinery. Its oil is also a substitute for sesame oil for pharmaceutical purposes and can be used for soap-making. The press cake of niger seed can be used for animal feed, manure and fuel. It is also used in the making of soap and as carrier of scent in perfume industry (Ramadan, 2012). The oil extracted from the seed contains a high content of linoleic acid and essential fatty acid for mono-gastric animals including humans. Substances such as tocopherols, phospholipids and sterols obtained from niger seed provide protection against cardiovascular disorders and cancer (Kandel and Porter, 2002).

In addition to lack of high seed and oil yielding cultivars growing on marginal land with poor adoption of improved agronomic packages are the cause for low productivity of the crop in Ethiopia. The indeterminate growth habit, seed shattering, self-incompatibility, low yielding, lodging, less or low responsiveness to management inputs, disease, insect and parasitic weeds are the major production problems (Teklewold and Wakjira, 2004). The availability of limited genetic information and semi domesticated nature of the crop has been an obstacle for further improvement programs (Dempewolf *et al.,* 2015). Systematic breeding efforts are necessary to evolve high seed and oil yielding niger seed cultivars. To achieve this, information about the nature and magnitude of genetic divergence in a given set of genotypes is essential for selection of diverse parents for hybridization which leads to wide range of gene recombination (Govindaraj *et al.,* 2015).

Genetic divergence can play a key role and the basis for any crop improvement program to get maximum recombination in hybridization programs. The knowledge of genetic variation existing in germplasms are an important and essential aspect for initiating any crop breeding program because

hybrids between lines of diverse origin generally display greater heterosis than those between closely related parents (Ekhlaque *et al*., 2016 and Bhandari *et al*., 2017).

Multivariate analysis such as Mahalanobis's D^2 statistic, cluster and principal component analysis are important techniques for assessing the degree of divergence and the relative contribution of different characters to the total divergence. They are powerful tools in quantifying the degree of divergence in most crops. The D^2 statistics measures the degree of diversification and determines the relative proportion of each component character to the total divergence (Sharam, 1996 and Shing and Chaudhary 1999).

For any successful breeding programs identification and knowledge of differences among genetic materials is critical for selection of appropriate resources adapted to specific environments. This crucial information obtained by evaluation of the available genotypes. However, genotypic characteristic of niger seed is still poorly understood because of the fact that information on this aspect is very limited. This has made the identification of the various genotypes and improvement of the crop very difficult.

Different researchers such as Yadav *et al.,* (2012), Pulate *et al.* (2013), Kumar (2016) Goyal and Bisen, (2017) conducted genetic divergence study on niger seed genotypes. In Ethiopian niger seed genotypes, the information on these aspects is very scarce. Therefore, there is a need to generate information on the genetic divergence and identify traits for total variation among the niger seed genotypes both plant breeders and germplasms curators to optimize the use of the variability available. Even though niger seed provide significant contribution in the livelihood of Ethiopian farmers it is underutilized and considered as orphan oilseed. Hence, the present investigation was undertaken to study the genetic diversity of niger seed genotypes using genetic divergence (D^2), cluster and principal component analysis.

2. MATERIAL AND METHODS

2.1. Experimental Materials and Design

One hundred genotypes of niger seed were evaluated for some morphological traits under field conditions at Holetta Agricultural Research Center (HARC), Ethiopia. The experiment was arranged in a 10 x 10 simple lattice design. Planting was done in a plot of three rows with 2 m length and 0.3 m spacing between rows and 0.6 m distance between plots with 8 kg/ha of seed rate. The distance between the blocks was maintained to 3 m.

2.2. Data Collection

On the basis of ten randomly selected plants, data were recorded for number of primary branches (NPB), number of secondary branches (NSB), number of heads per plant (NH), number of seeds per head (NSH), number of seeds per plant (NSP) and plant height (PH). Days to 50 % flowering (DF), days to 90 % maturity (DM), grain filling period (GF), yield per plot (YPP), thousands seed weight (gm) and oil content were recorded on plot basis.

2.3. Statistical Analysis

Before proceeding to the multivariate analysis; the average mean value of genotypes were standardized by subtracting the mean and dividing by standard deviation. Genetic divergence analysis was computed based on multivariate analysis using Mahalanobis (D^2) statistic (Mahalanobis, 1936) using SAS software v9.3. Clustering of genotypes was performed by average linkage clustering method and the dendrogram was generated using MINITAB v14.

The D^2 values obtained from pairs of clusters were considered as the calculated values of Chi-square (χ^2) and were tested for significance at 1% and 5% probability level against the tabulated values of χ^2 for 'P' degree of freedom, where P is the number of characters considered (P=12).

In order to highlight the resolving power of the ordination and to reduce a large set of variables to a small set and to assess the pattern of variations principal component analysis was performed. Following the criterion established by only PCs with Eigen values greater than one are considered as important.

3. RESULT AND DISCUSSION

3.1. Cluster Analysis

The cluster analysis has grouped the hundred niger seed genotypes into five distinct clusters based on their similarity (Figure 1). Raza *et al.* (2019) observed similar trend in diversity study of rapeseed and

mustard germplasm using cluster analysis. Ahirwar *et al.* (2017) grouped 114 niger seed genotypes in to 8 clusters in which 90 of the genotypes were grouped in one cluster.

The distribution of accessions into different diversity classes was presented in table 1. Members within a single cluster being considered as having more close relationships with each other than they are with those in significantly distant clusters. Each cluster comprises different number of genotypes and has unique characteristics. The current cluster analysis showed that the diversity presented in niger seed genotypes cannot be reduced into a few number of groups as it was done by Petros *et al.* (2007). According to their report 36 accessions of niger seed were grouped in to two major clusters. Conversely Parameshwarappa *et al.* (2009) grouped 52 niger seed accessions in to seven groups which is higher than our result. The divergence analysis by Goyal and Bisen (2017) grouped 71 niger seed genotypes into eleven clusters based on their genetic dissimilarity.

Even though, there are accessions grouped together from similar geographic origins; clustering of germplasms do not group exclusively on the basis of their origin rather genotypes from different origin grouped together due to their morphological differences. The evolutionary forces such as gene flow, genetic drift, mutation, migration, selection and germplasms exchange play roles in assigning the accessions to different clusters. These factors might be separated them into related but different gene pools. The result showed that selection of different traits in different areas might have been occurred. This finding was in agreement with the findings of other researchers Mekonnen and Wakjira, 2014 and Naznin *et al.*, 2015. They found absence of association among different clusters established on agronomic traits and origin of genotypes in Ethiopian mustard and Brassica rape genotypes respectively.

Geographically distant accessions are grouped together; which indicates that accessions from different geographic origin might have similar genetic background. The random and independent clustering pattern and discordance among diversity patterns and geographical distribution of genotypes in this investigation showed that geographical isolation is not the only factor causing genetic diversity in niger seed. Therefore, for niger seed hybridization parental lines should be selected based on genetic diversity rather than the geographical distribution. The conflict of niger seed clustering and geographic origin was previously reported by different authors. Kumar (2016) clustered niger seed accessions from different geographic origins using Torcher clustering method; according to the result accessions from similar geographic origins clustered randomly and independently in to eleven separate clusters. Cluster analysis of forty niger seed genotypes based on their genetic similarity has grouped in to eleven clusters without considering their geographic origin (Pulate *et al.*, 2013).

Table1. *Distribution of 100 niger seed genotypes collected from different Geographic origins grouped into five clusters using 12 quantitative traits*

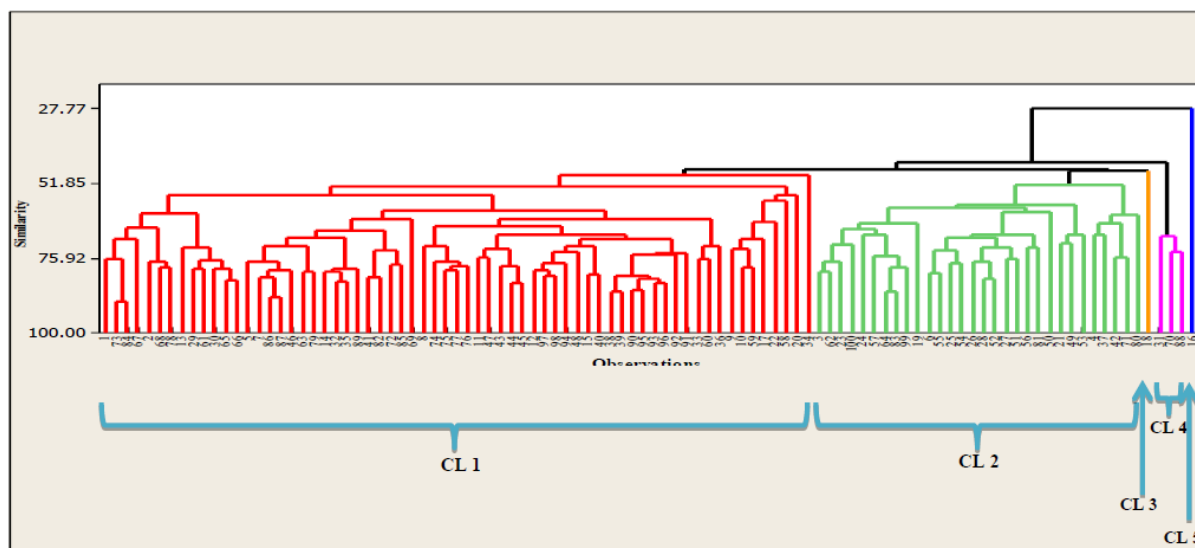| Cluster	Number of accessions	Accessions included in the cluster	Source of genotypes
1	65	15008 15012 15018 15022 15037 15054 15066 15080 15083 15092 15121 15131 15154 15202 15509 15520 15528 15535 15541 15543 15549 15553 15560 15574 15587 15617 15657 15659 15660 15661 15662 15667 15668 15669 15671 15680 15681 15683 15686 15692 15693 15697 15701 15721 15730 15733 15746 15763 15781 200428 202248 203184 203192 203194 203195 208396 208400 208944 208945 212721 212731 225954 225962 225964 225966	Collections
2	30	9978 15020 15056 15079 15167 15765 15770 15780 15787 202242 202245 202254 202255 202258 202264 202266 202269 202270 202271 202448 202454 202461 202472 202473 208686 208830 216875 225955 Ginchi-1 Shambu	Collections and improved varieties
3	1	15779	Collections
4	3	15115 15132 15622	Collections
5	1	214217	Collections

Figure1. *Dendrogram showing relationship among 100 niger seed genotypes based on average linkage and Euclidean distance using the mean of 12 quantitative traits.*

3.2. Comparison of Clusters Mean

The first cluster constitutes 65 accessions which were collected from the whole regions of Ethiopia. Accessions from Wellega and Shewa constituted more than 40% of the group. The whole accessions from Gonder except one were grouped in cluster I. The second cluster has 30 accessions; contains accessions from all populations except Gonder and Illubabor. In this group about 60 % of the members were collected from Wollo and Gojjam. The geographic proximity of the two populations may favor the possible exchange of genetic material by human or natural factors. Improved varieties Ghinchi-1 and Shambu were grouped together in cluster two. The third and fifth clusters are solitary and they contain only single accessions. Similar trained were observed in the previous niger seed clustering studies. Pulate *et al.* (2013) they reported that seven solitary clusters were observed from the total eleven clusters. Kumar (2016) evaluated 71 niger seed accessions grouped into eleven clusters of which two of them are solitary clusters. Divergence analysis based on eleven important yield attributing traits grouped niger seed genotypes in to eleven clusters. Out of eleven clusters, three of them were solitary/monotypic (Goyal and Bisen, 2017).

According to the cluster mean values presented in table 2; the first cluster (C1) was explained by late flowering and late maturity. This cluster was also characterized by lowest mean value for the number of seed per plant and thousand seed weight. The second cluster (C2) was differentiated by early flowering, long grain filling period and short plant height. Good performance was observed for the number of heads and number of secondary branch. The third cluster (C3) which form separate cluster was characterized by shorter grain filling period and lower number of primary branch and lowest mean for number of secondary branch. But the cluster mean was among the top high yielder and top in thousand seed weight.

The fourth cluster (C4) was described by late maturing and long grain filling period. Whereas, this cluster had lowest performance for mean value for number of primary branch, number of heads, yield per plot, thousand seed weight and oil content. The average performance for number of seed per plant was also higher. Compared to the other clusters cluster five (C5) generally showed highest mean performance in most of the desirable traits. The cluster exhibited early maturity and short grain filling period, and highest number of primary branch, number of heads, number of seed per head, yield per plant and oil content. The cluster has also shorter days for flowering, long plant height and higher thousand seed weight. It is important to note that the superiority of a particular niger seed accessions in respect to a given character gets diluted by other genotypes that are related and grouped in the same cluster which are inferior or intermediary for that character in question. Likewise, wide variations in clusters mean have been reported by Ahirwar *et al.* (2017) in niger seed genotypes; Naznin *et al.*, 2015 in Brassica rape; Mekonnen and Wakjira, 2014 in Ethiopian mustard. The occurrence of such

variability among the clusters of great genetic value implies the need of developing suitable breeding strategy for niger seed improvement.

The cluster contributing maximum to the divergence was given emphasis for deciding the type of cluster for the purpose of selection and the choice of parents for hybridization. Therefore, clusters with highest mean value for the desired traits will be considered or selected as parents for hybridization or for other population improvement methods in future niger seed improvement.

Table2. *Cluster mean values among the five clusters of 100 niger seed genotypes for 12 quantitative traits*

Traits	Clusters					Grand Mean
	C1	C2	C3	C4	C5	
DF	101.39	81.78	96.00	88.67	87.00	91.54
DM	155.29	148.97	149.00	156.33	138.00	147.81
GFP	53.90	67.18	53.00	67.67	51.00	56.27
PH	133.93	120.47	132.00	127.33	133.00	129.85
NPB	7.70	7.31	6.60	6.57	8.50	7.53
NH	19.31	26.02	19.80	17.50	40.20	26.33
NSB	10.45	15.23	8.00	8.40	22.30	13.99
NSH	40.31	63.80	66.25	149.67	102.35	68.18
NSPP	76.60	147.64	133.10	245.50	399.80	189.29
YPP	102.25	104.10	104.17	101.00	105.10	103.90
TSW	1.71	2.00	3.00	1.70	2.40	2.28
OC	33.95	36.53	36.90	30.30	37.70	36.27

DF=days to flowering, DM=days to maturity, GFP= Grain filling period, PH= plant height, NH=number of heads, NPB=Number of primary branch, NSB=Number of secondary branch, NSH =number of seed per head, NSPP= Number of seed per plant, YPP=yield per plot, TSW=Thousand seed weight and OC= oil content.

3.3. Distance Among Clusters (D2 Analysis)

The standardized Mahalanobis's D^2 statistics showed existence of high genetic distance among the five clusters. Out of 10 possible pairs of clusters, differences between eight pairs were highly significant (P<0.01) and one pairs were significant (P<0.05) while one pair was non-significant (Table 3). The maximum distance was found between cluster four and five (D^2=94.75). Cluster four constitutes three accessions each of them comprise from Illubabor, Shewa and Wellega regions; While, cluster 5 constitute accession from Gonder. The geographic divergence may favor the accumulation of contrasting alleles between the two groups which increase genetic dissimilarity between the clusters. These suggesting that the genotypic materials with maximum inter cluster distance may be used as parents for hybridization program to develop desirable varieties; because crosses between genetically divergent lines will generate heterotic segregates' or broad spectrum of variability in subsequent generations (Khodadadi *et al.*, 2011 and Afroz *et al.*, 2013).

Table3. *Pair-wise generalized squared distances (D^2) values between clusters constituting 100 niger seed genotypes*

Clusters	1	2	3	4	5
1					
2	15.92ns				
3	44.86***	36.69***			
4	27.69**	19.80*	69.15***		
5	67.79***	59.35***	64.45***	94.75***	

ns = non-significant P > 0.05, *= significant at P ≤ 0.05, **= significant at P ≤ 0.01, *** = highly significant at P < 0.001

The second most divergent clusters was cluster three and four (D^2 = 69.15). Cluster three comprises only one accession from Wollo. The lowest divergence was observed between cluster one and two (D^2 = 15.92), which shows the presence of less genetic variability or diversity between this clusters. The present study recorded lowest D^2 values when we compared with the previous study (Parameshwarappa *et al.,* 2009); the highest value they reported was D^2 = 252.150 which is twofold

higher than the current report. But almost equal lowest D^2 value (15.92) was observed. For crossing parents selection should be from clusters with highest genetic distance to obtain highest genetic recombination and transgressive segregates' in the subsequent generation. Crossing of parents from clusters described by significant distances will give maximum genetic segregation and genetic recombination (Pulate *et al.,* 2013). Increasing parental distance mean great number of contrasting alleles at the desired loci therefore, the extent of these loci recombine in the F2 and F3 generation following a cross of distantly related parents has a high potential. According to the present investigation crossing of accessions from cluster four and five followed by cluster three and four would give rise to maximum recombination and segregation of the progenies. However crossing of low distance clusters (cluster 1 and 2) might not give higher heterotic value in F1 and narrow range of variability in the segregating F2 population.

It is valuable to note that in calculating cluster mean, the superiority of a particular accession with respect to a given character could get diluted by other accessions that are grouped in the same cluster but are inferior or intermediate for the character in question. Hence apart from selecting genotypes from the clusters which have higher inter-cluster distance for hybridization one can also think of selecting parents based on the extent of divergence in respect to a character of interest.

3.4. Principal Component Analysis

Principle component analysis is really a reliable tool for successfully selection of parents in breeding program of any crop (Venujayakanth *et al.*, 2017). In order to reduce a large set of variables to a small set and to assess the pattern of variations; a well-known method of dimension reduction, principal component analysis was done by considering all the 12 variables simultaneously. Five of the 12 principal components accounted for more than 81 % the total variation (Table 4). The phenotypic variation reflect in the principal component analysis cannot be explained in terms of few principal components. However previous studies on niger seed genotypes reported different percentage with similar number of PCs. According to Kumar (2016) report on 71 niger seed accessions; 71.82 % of the total variation was explained by five components which are below the present finding. Conversely, Pulate *et al.* (2013) reported that 96.23 % of variation was contributed by the first five principle components.

Table4. *Eigen value, percentage, cumulative variances and Eigen vectors on the first five principal components for 12 traits in 100 niger seed genotypes*

Traits	PC1	PC2	PC3	PC4	PC5
DF	**-0.438**	-0.142	0.184	0.006	0.051
DM	-0.306	0.001	0.033	0.179	**0.651**
GFP	**0.356**	0.186	-0.216	0.119	**0.394**
PH	-0.265	-0.021	**0.433**	-0.322	-0.206
NPB	0.015	**-0.474**	0.258	**-0.462**	**0.300**
NH	**0.377**	-0.022	-0.113	-0.350	0.135
NSB	**0.328**	**-0.352**	-0.017	-0.276	0.149
NSH	0.223	0.123	**0.623**	**0.352**	0.088
NSPP	**0.348**	0.059	**0.486**	0.162	0.125
YPP	0.005	**-0.542**	-0.031	**0.358**	-0.136
TSW	0.302	-0.019	0.094	-0.031	**-0.449**
OC	0.079	**-0.532**	-0.129	**0.398**	-0.049
Eigen value	4.275	2.021	1.472	1.142	0.909
Proportion	0.356	0.168	0.123	0.095	0.076
Cumulative	0.356	0.525	0.647	0.742	0.818

PC1, PC2, PC3, PC4 and PC5 = Principal component 1, 2, 3, 4 and 5 respectively; DF=days to flowering, DM=days to maturity, GFP= Grain filling period, PH= plant height, NH=number of heads, NPB=Number of primary branch, NSB=Number of secondary branch, NSPP= Number of seed per plant, YPP=yield per plot, TSW=Thousand seed weight, OC= oil content.

The contribution of morphological traits in the loading plot showed that number of head, grain filling period, number of seed per plant, days to flowering and days to maturity have strongly influenced the

loading plot of the first component. Whereas yield per plot, number of primary branches and oil content have weak influence. Principal component two strongly influenced by yield per plot, number of primary branches and oil content. However, weak influence for loading plot of the second component were observed by number of heads, thousand seed weight, number of seeds per plant, days to maturity and plant height (Figure 2). The Positive and negative loading shows the presence of positive and negative correlation trends between the components and the variables.

In our experiment the data revealed that traits with high contribution in the first component indicate that they contributing maximum towards divergence. Therefore the divergence in the present materials due to these traits will offer good scope for improvement of niger seed through selection of parents for crossing. It was concluded that the germplasms exhibited a wide range of variability for most of the traits. Some genotypes possessed desirable genes for more than one character and hence could be utilized directly or included in hybridization programs for variety development. Genotypes will be selected on the basis of special objectives and no common criteria can be considered for selection of genotypes.
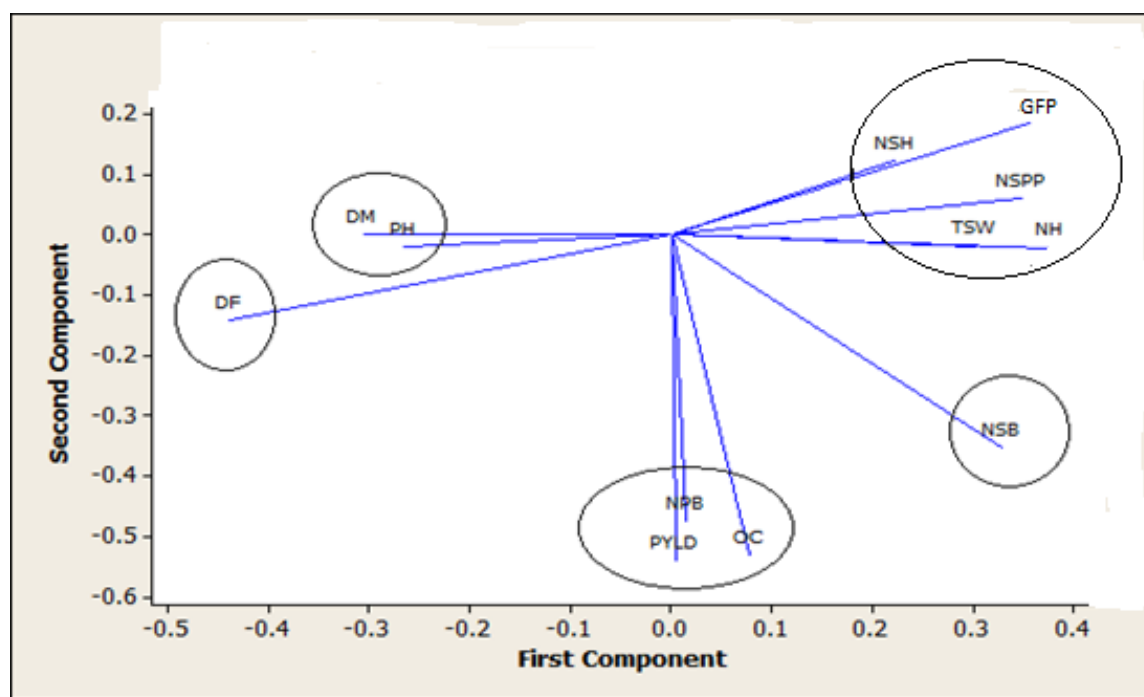
DF=days to flowering, DM=days to maturity, GFP= Grain filling period, PH= plant height, NH=number of heads, NPB=Number of primary branch, NSB=Number of secondary branch, NSPP= Number of seed per plant, YPP=yield per plot, TSW=Thousand seed weight, OC= oil content.

Figure 2 Loading plots of the first two principal components showing the contribution of different morphological traits to the total variation by the two components

In breeding programs, genetic divergence analysis aims to identify parents for the generation of populations with genetic variability and consequent genetic gain in successive selection cycles. In selecting the most divergent parents, it is advisable to select, within each group, parents with higher averages in relation to those characters to be improved, with the aim of achieving the maximum concentration of favorable alleles, according to the selection objectives.

4. CONCLUSION

Genetic distance is very important for hybridization program to get better yield and best recombinant parents. In the present study the multivariate analyses of genetic divergence among genotypes have resulted in the formation of five clusters and have shown the presence of genetic variability for further selection and breeding. We could also found that geographical diversity could not necessarily be an index of genetic variability to group genotypes in to different clusters. Genetic distances among most clusters were significant from which selection of parents may be made for crossing in order to obtain genetic recombination. Genotypes in clusters IV and V possess highest cluster distance and thus; the

genotypes of these two clusters hold great promise as parents in hybridization program to obtain promising heterotic expression in F1 and may create considerable variability in the segregating populations. Significant differences were observed among the genotypes for various traits indicating the scope for selection from diverse genotypes for desired traits.

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