

## Cluster Analysis of Maize Inbred Lines

Jiban Shrestha

National Maize Research Program, Rampur, Chitwan, Nepal.

Correspondence: [jibshrestha@gmail.com](mailto:jibshrestha@gmail.com)

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### ABSTRACT

The determination of diversity among inbred lines is important for heterosis breeding. Sixty maize inbred lines were evaluated for their eight agro morphological traits during winter season of 2011 to analyze their genetic diversity. Clustering was done by average linkage method. The inbred lines were grouped into six clusters. Inbred lines grouped into Clusters II had taller plants with maximum number of leaves. The cluster III was characterized with shorter plants with minimum number of leaves. The inbred lines categorized into cluster V had early flowering whereas the group into cluster VI had late flowering time. The inbred lines grouped into the cluster III were characterized by higher value of anthesis silking interval (ASI) and those of cluster VI had lower value of ASI. These results showed that the inbred lines having widely divergent clusters can be utilized in hybrid breeding programme.

**Keywords:** Cluster, diversity, Maize inbred lines

### सारांश

हार्डिब्रिड ब्रिडिङका लागि मकैका ईनब्रेड लाइनहरूको विविधताको अध्ययन महत्वपूर्ण हुन्छ। सन् २०११ हिउँदमा मकैका ६० वटा ईनब्रेड लाइनहरूलाई तिनको क्लस्टर एनलाईसिस द्वारा विविधता छुट्टाउने कामका लागि राष्ट्रिय मकैवाली अनुसन्धान कार्यक्रम, रामपुर, चितवनमा परिक्षण संचालन गरिएको थियो। ईनब्रेड लाइनहरूलाई ६ वटा क्लस्टरमा विभाजन गरियो। क्लस्टर २ मा पर्ने ईनब्रेड लाइनहरू अग्लो र धेरै पातको संख्या भएको पाईयो। क्लस्टर ३ मा पर्ने ईनब्रेड लाइनहरू होचो बोटहरू र थोरै पातको संख्या भएको भएको पाईयो। त्यसैगरी क्लस्टर ५ मा पर्ने ईनब्रेड लाइनहरू चाडै फूल फूलने र क्लस्टर ६ मा पर्ने ईनब्रेड लाइनहरू ढिलो फूल फूलने रहेको पाईयो। क्लस्टर ३ मा पर्ने ईनब्रेड लाइनहरूमा भाले र पोथी फूल फूलने दिनको अन्तर धेरै र क्लस्टर ६ मा पर्ने ईनब्रेड लाइनहरूमा सो कम रहेको पाईयो। यी नतिजाहरूले के देखाउछ भने क्लस्टरमा विविधता भएको मकैका ईनब्रेड लाइनहरूलाई हार्डिब्रिड जातहरूको विकासको लागि प्रयोग गर्न सकिन्छ।

### INTRODUCTION

The morphological data has an important role in the management of genetic resources that are conserved in *ex situ* gene-banks (Sanchez et al 2000 and Bioversity International 2007). To study relationships among cultivars, there are many tools including various types of molecular markers are now available; however, in the description and classification of germplasm the morphological characterization is the first step (Smith and Smith 1989). The characterization on morphological variability is important tool to identify accessions with desirable traits like earliness, disease resistance, or improved ear trait etc. The characterization and grouping of germplasm helps the breeders to avoid duplication in sampling populations. The variation occurred in anthesis-silking interval (ASI) provides an opportunity to identify suitable inbred lines for hybrid maize development and production. Inbred lines having similar plant height, ear height can be used for development of synthetic varieties. Cluster analysis is frequently used to classify maize (*Zea mays* L.) accessions and can be used by breeders and geneticists to identify subsets of accessions which have potential utility for specific breeding or genetic purposes (Rincon et al 1996). The main aim of using a cluster technique in plant breeding trials is to group the varieties into several homogeneous groups such that those varieties within a group have a similar response pattern across the locations. Multivariate analysis based on principal component analysis (PCA) is mostly used to evaluate the magnitude of genetic diversity among the germplasm (Brown-Guedira 2000). The principal component (PC) analysis divides the total variance into different factors. Classifying genotypes based on their agronomic traits with multivariate techniques could reduce the time period and expenditure for crop improvement. Information on cluster analysis on maize inbred lines is not sufficient in Nepal. Therefore this study was carried out with the objectives to characterize and choose the plant traits that best explain genetic variation; and group sixty maize inbred lines according to eight morphological traits.

### MATERIALS AND METHODS

Sixty maize inbred lines were evaluated in 2011 at Rampur, Chitwan, Nepal to assess their morphological diversity. The location of the experimental site was situated at 27° 40' N latitude, 84° 19' E longitude with an altitude of 228 m. The soil was generally acidic, light textured and sandy loam. The total rainfall during the crop season was 607 mm, while air temperature average maximum 28°C and minimum 16°C temperature with average relative humidity of 97% (NMRP 2011). The experiment was conducted in an alpha lattice design with two replications. The inbred lines were planted in individual plot area of 6 m<sup>2</sup> (2 rows of 5 m in length) with spacing of 25 cm × 60 cm (plant to plant × row to row). All intercultural operations were carried out as per standards adopted by National Maize Research Program, Rampur, Chitwan, Nepal. The data on days to 50% tasseling, days to 50% silking, Anthesis silking interval (ASI), number of leaves, plant height, ear height, tassel length, no. of tassel branches number were recorded. The collected data were subjected

to descriptive statistics and principle component analysis was done using statistical software packages of Minitab ver.17. Cluster analysis was performed using average linkage method.

## RESULTS

In the present study, genetic diversity was analyzed among 60 maize inbred lines (Table 2) on the basis of 8 agro morphological traits. The results of descriptive analysis (Table 1) showed that anthesis silking interval (ASI) had the highest variation (58.93%) followed by tassel branches number (34.95%), ear height (29.84%) and plant height (20.06%). Among the traits, the leaf number showed the lowest variation (10%). The mean values of days to 50% tasseling, days to 50% silking, ASI, number of leaves, plant height, ear height (ear position), tassel branches and tassel length were 60.33, 63.83, 3.5, 11.00, 143.94, 66.07, 10.34 and 37.09, respectively. Among the tested inbred lines the 50% tasseling was highest 74 days in RML-17 and lowest 47 days in RML-8. Similarly 50% silking was highest 77 days in RML-17 and lowest 50 days in RL-105 and RML-8. Anthesis-silking interval (ASI) varied from 9 days (L-17) to -6 days (RL211, RL105 and RL-170). Number of leaves varied from 13.33 (RL-189) to 8.33 (L-17). Plant height ranged from 211 cm (RL-180) to 95 cm (L-17), similarly ear height ranged from 111 cm (RML-62) to 25 cm (RML-6).

**Table 1.** Descriptive statistics of agro-morphological traits of 60 maize inbred lines at Rampur, Chitwan, Nepal in 2011 winter

Statistics	50% Tasseling days	50% Silking days	ASI (days)	No. of leaf	Plant height (cm)	Ear Height (cm)	Tassel branch (n)	Tassel length (cm)
Mean	60.33	63.83	3.5	11.00	143.94	66.07	10.34	37.09
SE Mean	0.89	0.92	0.266	0.14	3.73	2.55	0.46	0.56
SD	6.93	7.136	2.063	1.10	28.87	19.72	3.61	4.40
CV, %	11.49	11.18	58.93	10	20.06	29.84	34.95	11.87

SE Mean=Standard error of mean, SD=standard deviation and CV=Coefficient of variation

The observed variation in plant and ear height may be attributed to differences at the genotypic level. Number of tassel branches was highest 19.66 (RL-195) to lowest 4.33 (RL-86). Tassel length was highest 46 cm (RL-85) and lowest 27.66 cm (L-17). Clustering pattern of inbred lines under this study reveals that the inbred lines showed considerable genetic diversity among themselves by occupying six different clusters (Table 2). These inbred lines were grouped based on mainly tasseling and silking days, plant height and ear height, tassel length, tassel branch numbers and no. of leaf. The values fall within clusters are presented in following Table 2.

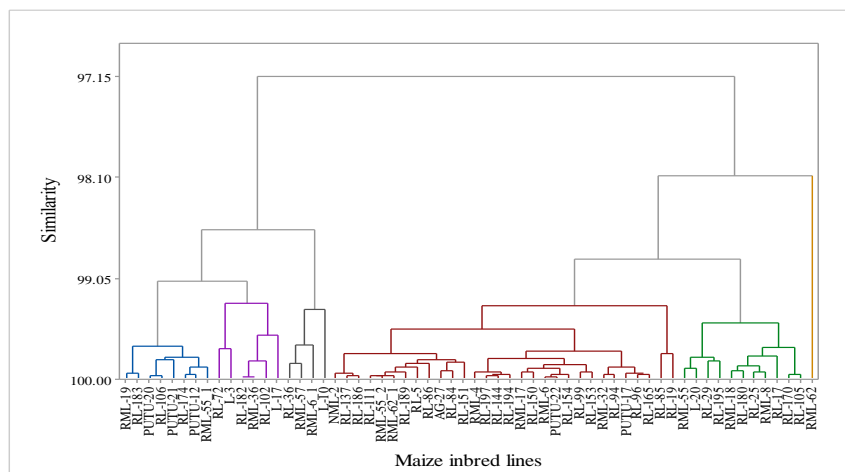
Sixty maize inbred lines were grouped into 6 clusters based on various agro morphological traits (Table 3). Cluster analysis showed that cluster I was comprised of 8 genotypes, cluster II of 6, cluster III of 4, cluster IV of 30, cluster V of 11 and cluster VI of 1 inbred lines (Figure 1). Inbred lines grouped into Clusters II had taller plants with maximum no. of leaves. The cluster III was characterized with shorter plants with minimum no. of leaves. The Inbred lines categorized into cluster V had early flowering where as they grouped into cluster VI had late flowering time. The inbred lines grouped into the cluster III were characterized by highest value of ASI and those of cluster VI had lowest value of ASI.

**Table 2.** Agro-morphological traits of sixty inbred lines within and among six clusters

Variable	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Days to 50 % tasseling	61.63	65	62.11	56	52.6	72
Days to 50% silking	65.18	69	66.22	58.81	55.2	74
Anthesis Silking Interval (days)	3.54	4	4.11	2.81	2.6	2
Number of leaves	11.02	12.66	10.55	11.46	10.93	12.33
Plant height (cm)	145.95	202	108.63	183.6	163.27	150
Ear height (cm)	63.36	90.5	45	94.3	82.36	111
Tassel branch number	11.98	13.33	7.77	11.33	9.90	11.66
Tassel length (cm)	37.37	42.22	35.32	38.26	37.63	35.33

**Table 3.** Grouping of sixty inbred lines into six cluster based on agro-morphological traits

Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
RML-19, PUTU-20, RL-174, RML-55, RL-183, PUTU-12, PUTU-21, RL-106	RL-72, RL-182, RML-36, RL-102, L-3, L-17	RL-36, RML-57, RML-6, L-10	NML-2, RL-137, RML-4, RML-32, RML-17, RL-99, RML-6, RL-111, RL-189, PUTU-17, AG-27, RL-197, RL-5, RL-154, RL-96, RL-144, RL-153, RL-186, RL-150, RL-151, RL-86, PUTU-22, RL-85, RL-165, RL-94, RL-19, RL-84, RML-55, RL-194, RML-62	RML-55, RL-29, RML-18, RL-195, RL-180, RL-25, RL-170, L-20, RL-105, RML-8, RL-17	RML-62



**Figure 1.** Cluster analysis of 60 maize inbred lines evaluated for agro-morphological traits in 2011 winter season at Rampur, Chitwan, Nepal.

### Principal Component Analysis

In this experiment, the principle component (PC) analysis partitioned the total variance into 4 PCs contributing maximum to the total diversity among the genotypes due to the study of various traits. Principal component analysis showed first 4 PCs having Eigen value >1 explaining 79.1% of the total variation (Table 4). The first principal component (PC1) which explained 33.5% variation was associated mainly with number of leaf, stem diameter, plant height, ear height, number of tassel branch and tassel length. The second principal component (PC2) was responsible for about 21.3% of the variation and was mainly related to, tasseling and silking days, number of leaf, stem diameter, plant height, ear height, number of tassel branch and tassel length.

### DISCUSSION

The estimation of genetic diversity and relationships among germplasm accessions facilitates the selection of parents with diverse genetic background which is very essential for breeding program (Murphy et al 1986, Souza and Sorrels 1991). Sokolov and Guzhva (1997) reported significant amount of variability for in maize inbred line populations for their different morphological traits. In this study considerable morphological variation was found mainly due to genetic factors and also subjected to environmental factors (Table 2, Figure 1). Ihsan et al (2005) have also reported that there was substantial variability for days to anthesis among different maize genotypes. Shah et al (2000) have also noticed the different maturity traits among maize populations and variability for different morphological traits in maize. Dijak et al (1999) observed significant amount of variability among long and short stature maize populations for ear and plant height. In the present study, six clusters of maize were formed based on morphological traits (Table 3); these findings were similar to the results founded by Singh et al (2005).

**Table 4.** Eigen analysis and principal component coefficients based on correlation matrix of eight variables measured in 60 maize inbred lines

Variable	PC1	PC2	PC3	PC4
Eigen value	3.0124	1.9198	1.188	1.0005
% of total variance	33.5	21.3	13.2	11.1
Cumulative variance %	33.5	54.8	68	79.1
Tasseling days	-0.294	0.578	-0.218	0.114
Silking days	-0.34	0.558	-0.049	0.197
ASI (days)	-0.191	-0.013	0.563	0.3
No. of leaves	0.289	0.496	0.285	-0.116
Stem diameter (cm)	0.268	0.233	0.444	-0.561
Plant height (cm)	0.472	0.14	-0.261	0.262
Ear height (cm)	0.473	0.065	-0.218	0.334
No. of tassel branch	0.335	0.056	0.363	0.437
Tassel length (cm)	0.215	0.164	-0.326	-0.399

This clustering in natural groups is represented by a dendrogram progressively dividing the accessions into smaller and smaller groups (KC 2001). Khorasani et al (2011), Crossa (1990) and Mostafavi et al (2011) reported that to minimize the plant pool making groups or clusters of under-study maize genotypes is an efficient tool during selection process. In this study there were four principal components (Table 4). Principal component analysis is usually used in plant sciences for the reduction of variables and grouping of genotypes. Among these biometrical procedures, the main edge of principal component analysis (PCA) is that each genotype can be assigned to only one group and it also reflects the significance of the largest contributor to the total variability at each axis of differentiation (Sharma 1998). The principal component analysis divides the total variance into different factors. Principal component reduces the dimensionality of multivariate data by removing interrelations among variables and Eigen value represents the variance of principal component (Matus 1999). The Principal Component Analysis is a powerful tool to obtain parental lines for a successful breeding programme (Akter et al

2009). Kamara et al (2003) used PCA to categorize traits of maize (*Zea mays* L.) that accounted for most of the variance in the data. Chozin (2007) and Mujaju and Chakuya (2008) reported important contribution of the first PCs in total variability while studying different traits. Greenacre (2010) reported that Eigen values (in PCA) have primary importance for numerical diagnostics to assess variation attributed by number of large variables on the dependent structure and their data matrix in a graphical display.

## CONCLUSION

The genetic diversity was observed in agromorphological traits namely days to 50% tasseling, days to 50% silking, anthesis silking interval (ASI), number of leaves, plant height, ear height, tassel length and number of tassel branches number in the tested maize inbred lines. The inbred lines grouped into cluster IV and cluster V were good because of their desirable agromorphological traits. The presence of high level of diversity among the inbred lines grouped into divergent clusters indicated their suitability for hybridization and various crosses can be made among them in breeding program.

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