Genotypic and morphological diversity analysis in high altitude maize (*Zea mays L.*) inbreds under Himalayan temperate ecologies

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Abstract

The present investigation was carried out to generate information on genetic divergence of maize (*Zea mays* L.) inbreds and to identify elite lines to develop potential hybrids for high altitude ecologies. Data were recorded for thirteen (13) quantitative traits viz. germination (%), root depth, shoot length, days to 50 % pollen shed, days to 50% silking, plant height, ear height, ear length, ear girth, number of kernels per row, 100 seed weight, grain yield per plant. The tested genotypes expressed significant variability with high estimates of heritability (broad sense) for all the traits revealing that these traits are amenable to genetic improvement. Grain yield per plant exhibited positive and significant correlation with plant height, ear height, ear width, number of kernels per row, number of kernel rows and 100 seed weight at both the levels which exhibit a strong possibility for improvement of grain yield per plant by selection for these traits. Genetic divergence based on thirteen quantitative traits grouped thirty maize inbred lines into four clusters as per Mahalanobis D2 analysis employing Tocher's method which revealed wide diversity in elite genotypes owing to the involvement of diverse parental lines in the hybridization programme. Grain yield per hectare was the main factor contributing to total divergence (41.16%) which needs to be considered for highlighting clusters to be chosen for the purpose of further selection.

KeyWords *D2 analysis, genetic diversity, maize (Zea mays L.), morphological characterization, correlation*

Introduction

Maize (*Zea mays* L.) is the third most widely grown cereal after wheat and rice at global level (Gami et al., 2017), commercially valued economic crop of universal importance widely used in poultry and cereal food industries. It is the only cereal crop of American origin that is cultivated in tropical and subtropical regions throughout the world (Abate 2017). Total global maize production for the year 2013-14 was 959 million tons which is more than that of wheat (709 million tons) and rice (473 million tons) (GMR, 2014). The increasing use of maize as a staple food reflects higher yields per hectare, compared with wheat, rice and barley. In India maize is grown on an area of 8.7 million ha with a production and productivity of 22.3 million tons and 2.6 tons per hectare respectively with the contribution of 8.65 % to the country's total food grain production (Anonymous 2013). About 28% of maize produced is used for food purpose, 11% as livestock feed, 48% as poultry feed and 12% in wet milling industry (Anonymous, 2012). Maize is the second most important crop after rice in the state of Jammu and Kashmir, India. It is cultivated on an area of 302 thousand hectares with annual production and productivity of 527 thousand tons and 1.5 tons per hectare respectively. The average productivity of maize under high altitude conditions of Kashmir valley is very low (1.05 tons per hectare) when compared to national productivity of 2.2 tons per hectare (Najeeb et al., 2012). Major constraint to increase maize production includes the predominance of cultivated landraces and lack of potential hybrids suitable for high altitude conditions. Characterization of maize inbred lines for agro-morphological traits has been widely used to provide knowledge of genetic diversity in maize germplasm. In addition, it also facilitates in broadening the genetic base of vital maize genetic resources for sustaining genetic improvement and also guides in making the choice of parents for the development of new

hybrids with high seed production potential (Ihsan et al., 2005). Genetic and phenotypic diversity of maize germplasm adapted to a wide range of environmental conditions has been significantly reduced during the decades of maize cultivation. Due to intensive artificial selection and breeding the genetic diversity has decreased as the selection favors alleles responsible for few significant agronomic traits (Buckler et al., 2006). As a consequence, the majority of elite inbred lines exploited for development of maize hybrids in current breeding programs in temperate climate are developed from few ancestral inbred lines. Information regarding the genetic diversity and the relationship among maize inbred lines played a significant role in improvement of new cultivars as it facilitates planning crosses for hybrid and inbred line development, assigning lines to heterotic groups, and protecting the plant variety (Hallauer et al., 1988; Pejic et al., 1998). The present study was aimed to provide information about genetic variability and diversity among maize inbred lines developed under high altitude temperate ecologies (1800 - 2250 m amsl) based on agro-morphological traits which will be helpful to maize breeders for further exploitation in subsequent breeding programs.

Materials and Methods

The experiment was conducted during the year 2015 at Research Farm of Mountain Crop Research Station Larnoo, SKUAST-Kashmir. The research farm is situated at an altitude of 2290 m amsl with latitude and longitude of 33º 37' and 75º 22' respectively with clay loam type of soil. The maximum and minimum temperatures ranged from -10°C to 35°C. A set of 30 fixed maize inbred lines suitable for high altitude areas (1800- 2250 m amsl) were selected for the present study. The seed of inbred lines was sown in 4 rows of 3m length with 60 x 20 cm of inter and intra row spacing respectively. The experiment was performed in a randomized block design with three replications. Sowing was done on 15th April, 2016. The land was prepared with 2-3 ploughings with the addition of farmyard manure (FYM) @ 15 t/ha and 120 kg N, 60 kg P2O5, 30 K2O and 15-20 kg ZnSo4/ha. However, full doses of P, K and Zn and half of N fertilizer was applied at sowing time and the remaining quantity of nitrogen were applied in two equal splits, each at the time of first weeding and hoeing 30 days after sowing (DAS), and second dose at the time of second hoeing (50 DAS).

Observations on agro-morphological and seed traits (quantitative traits) viz., germination (%), root depth (cm), shoot length (cm), plant height (cm), ear height (cm), ear length (cm), ear width (cm), days to 50% tasseling, days to 50% silking, number of kernel rows, number of kernels per row, grain yield (g) and 100 seed weight (g) were recorded as per the directorate of maize research descriptor (Sujay and Singh, 2001).

The mean values of ten randomly selected and tagged plants from each entry were used for recording all the observations except days to 50% cent tasseling and 50% silking which were recorded on whole plot basis. Analysis of variance for pooled data was carried out following Singh and Chaudhary, (1985). The genetic divergence was computed using the procedure as described by Rao (1952) and Singh and Chaudhary (1985).

Results and Discussion

Analysis of variance

All the genotypes tested expressed significant variability for all the thirteen yield and yield attributing

Table 1 Estimates of phenotypic and genotypic coefficient of variation for yield and yield component traits in maize (*Zea mays* L.)

Traits	Phenotypic variance	Genotypic variance	Phenotypic coefficient of variation (PCV)	Genotypic coefficient of variation (GCV)
Germination (%)	23.18	21.80	5.28	5.12
Root depth (cm)	3.24	3.16	28.65	28.3
Shoot length (cm)	0.57	0.52	38.48	36.72
Plant height (cm)	460.37	457.36	16.95	16.89
Days to 50% tasseling	39.62	37.13	6.01	5.82
Days to 50% silking	39.24	36.39	5.82	5.61
Ear height (cm)	168.96	166.21	25.44	25.24
Ear length (cm	0.8	0.69	7.12	6.63
Ear width (cm)	0.28	0.24	12.46	11.59
No. of kernel rows	5.74	3.29	16.72	12.66
No. of kernels per row	39.53	26.42	25.97	24.56
Grain yield /plant (g)	702.48	699.75	40.23	40.15
100 seed weight (g)	19.62	19.08	20.39	21.1

quantitative traits (Table 1). Days to 50% tasseling ranged from 86.6 to 114.3 with a mean of 104.52 days. The mean ear length ranged from 11.1 to 14.6 cm with mean ear length 12.48 cm. The mean ear width of genotypes was 4.25 cm with a range of 3.3 to 5.7 cm. Mean number of kernels per row were 21.17 with a range of 11.6 - 33.3. Grain yield per plant ranged from 28.9 to 114.3 g with a mean of 63.78 g and mean 100 seed weight was 21.08 g with a range of 14.5 - 28.9 g.

The estimates of phenotypic variation and phenotypic coefficient of variation were observed to be higher in magnitude than the corresponding estimates of genotypic variation and coefficient of variation. The magnitude of phenotypic and genotypic coefficient of variation (Table 1) was low (<10.0%) for germination (%), days to 50% tasseling, days to 50% silking and ear length (cm) and high (>20.0%) for root depth (cm), shoot length (cm), ear height (cm), number of kernels per row, grain yield plant /plant.

Table 2 Estimates of heritability, genetic advance, and expected genetic gain for yield and yield component traits in maize (*Zea mays* L.)

Traits	Heritability (broad sense)	Genetic advance	Expected genetic gain (% of mean)
Germination (%)	0.94	9.32	10.23
Root depth (cm)	0.97	3.62	57.61
Shoot length (cm)	0.91	1.42	72.17
Plant height (cm)	0.99	43.91	34.69
Days to 50% tasseling	0.93	12.15	11.61
Days to 50% silking	0.92	11.96	11.12
Ear height (cm)	0.98	26.34	51.57
Ear length (cm)	0.86	1.6	12.71
Ear width (cm)	0.86	0.94	22.22
No. of kernel rows	0.57	2.83	19.76
No. of kernels per row	0.89	10.01	47.87
Grain yield /plant (g)	0.99	54.38	82.55
100 seed weight (g)	0.97	8.87	40.84

Estimates of heritability (broad sense) were high (>50.0%) for all the traits in environment taken (Table 2). High value of heritability of 99.0% was exhibited by plant height (cm) and grain yield per plant (g) followed by 98.0% in case of ear height (cm), 97.0% in case of root depth (cm) and 100 seed weight (g). Genetic advance was estimated at 5% of selection intensity (Table 2) and converted into expected genetic gain (% of mean). The estimates revealed that the expected genetic gain was high (>30.0%) for root depth (cm), shoot length (cm), plant height (cm), ear height (cm), number of kernels per row, grain yield plant-1 and 100 seed weight whereas, it was moderate (20.0-30.0%) for ear width (cm) and low (<20.0%) for germination (%), days to 50% tasseling, days to 50% silking, ear length (cm) and number of kernel rows in the same environment.

In the present study the presence of significant genetic variation for all the characters in the high altitude maize inbreds coupled with moderate to high phenotypic and genotypic coefficient of variation for most of the yield attributing traits has revealed that these traits are amenable to genetic enhancements (Shahrokhi and Khorasani 2013; Ahmed 2013). Estimates of heritability are of considerable practical importance to breeders as they help in information of an efficient and pragmatic breeding program. Heritability (broad sense) estimates are informative as they indicate relative importance of genotypic and environmental contribution to variability exhibited and reliance that can be placed on phenotypic value during selection. Estimates of heritability (broad sense) accompanied by high genetic advance for most of the traits in the material indicated the preponderance of additive gene action for the expression of these traits which is fixable in subsequent generations and are under the control of additive genetic effects. The results are in conformity with findings of Noor et al. (2010); Aminu and Izge (2012); Bello et al., 2012; Anshuman et al. 2013.

Variability studies provide information on the extent of improvement possible in different characters, but they do not highlight the extent and nature of relationship existing between various contributing and economically important traits. Hence, knowledge regarding association of various characters among themselves and with economic traits is necessary for making indirect selection for improvement of these economical traits. Correlation studies pave way to know the association between highly heritable traits with most economic characters and gives better understanding of contribution of each trait in development of ideal genotype.

Estimates of phenotypic and genotypic correlation coefficients

Correlation coefficients were estimated at the phenotypic and genotypic levels among quality, yield and yield component traits in the same environment (Table 3). Genotypic correlation coefficients were higher in magnitude, though similar in direction than their corresponding correlation coefficients at phenotypic level. Days to 50% tasseling exhibited positive and significant correlation with days to 50% silking at both levels and had negative and significant correlation with grain yield plant-1 at both the levels but had negative and significant correlation with number of kernels at genotypic level only. Days to 50% silking exhibited negative and significant correlation with grain yield per plant at both the levels but exhibited negative and significant correlation with number of kernel rows

Table 3 Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficient for yield and yield component traits in maize (*Zea mays* L.).

Parameters	Germina-tion (%)	Root depth (cm)	Shoot length (cm)	Plant height (cm)	Ear height (cm)	Ear length (cm)	Ear width (cm)	Days to 50% tasseling	Days to 50% silking	Grain yield/plant (g)	No. of kernel rows	No.of kernels/row	100 seed weight (g)
Germination (%)	--	-0.35*	-0.17	0.12	0.09	0.07	-0.02	-0.27	-0.29	0.02	0.06	0.16	-0.23
Root depth (cm)	-0.34**	--	0.62**	0.05	-0.04	-0.12	0.33*	0.10	0.13	0.19	0.16	0.21	0.05
Shoot length (cm)	-0.15	0.58**	--	-0.23	-0.30*	-0.04	0.03	-0.03	-0.03	-0.18	-0.03	0.01	-0.40*
Plant height (cm)	0.12	0.05	-0.22*	--	0.39*	0.24	-0.01	0.06	0.07	0.39*	-0.05	0.44**	0.28
Ear height (cm)	0.08	-0.04	-0.28**	0.38**	--	0.03	0.11	-0.28	-0.25	0.62**	0.17	0.46**	0.55**
Ear length (cm)	0.06	-0.11	-0.04	0.22*	0.04	--	-0.20	-0.16	-0.16	0.26	-0.03	0.17	0.33*
Ear width (cm)	-0.01	0.31**	0.01	0.05	0.09	-0.20	--	-0.18	-0.17	0.41*	0.32*	0.27	0.18
Days to 50% tasseling	-0.24*	0.10	-0.02	0.06	-0.26*	-0.15	-0.15	--	0.99**	-0.36*	-0.37*	-0.24	-0.11
Days to 50% silking	-0.25*	0.12	-0.01	0.06	-0.23*	-0.14	-0.13	0.99**	--	-0.33*	-0.34*	-0.21	-0.10
Grain yield/plant (g)	0.02	0.19	-0.17	0.39**	0.62**	0.24*	0.38**	-0.34**	-0.31**	--	0.63**	0.77**	0.61**
No. of kernel Rows	0.01	0.12	-0.01	-0.04	0.12	-0.08	0.24*	-0.28**	-0.26*	0.49**	--	0.47**	0.08
No. of kernels/row	0.16	0.19	0.02	0.42**	0.43**	0.15	0.23*	-0.22*	-0.19	0.73**	0.15	--	0.09
100 Seed weight (g)	-0.21*	0.09	-0.39**	0.28**	0.54**	0.30**	0.17	-0.09	-0.08	0.60**	0.01	0.09	--

at genotypic level only. Ear height exhibited positive and significant correlation with plant height, grain yield plant-1, number of kernels per row and 100 seed weight at both the levels. Grain yield per plant exhibited positive and significant correlation with plant height, ear height, ear width, number of kernels per row, number of kernel rows and 100 seed weight at both the levels but exhibited negative and significant association with days to 50% tasseling and days to 50 percent silking at both the levels. Number of kernel rows exhibited a significant and positive association with grain yield at both the levels but showed significant and positive association with ear width and number of kernels per row at genotypic level. Number of kernel rows also recorded a significant and negative association with days to 50% tasseling and days to 50% silking at genotypic level. 100 seed weight recorded a significant and positive association with ear height, ear length and grain yield per plant at both the levels. Due to the significant and positive association of economically important trait i.e. grain yield plant-1 with plant height, ear height, ear width, number of kernels per row, number of kernel rows and 100 seed weight, there is strong possibility of improvement of grain yield plant-1 by selection for these traits. However, there was a negative and significant correlation of grain yield plant-1 with days to 50% tasseling and days to 50% silking which are in conformity with earlier findings

(Beyene et al., 2005.; Rafiq et al. 2010). Similarly positive and non-significant association of plant height with ear length and 100 seed weight are in accordance with findings of Munawar et al. (2013).

Estimation of genetic divergence

Genetic divergence was estimated based on yield and yield attributing quantitative traits. The results revealed that all the 30 genotypes were grouped into 4 clusters (Table 4) as per Mahalanobis D2 analysis employing Tocher's method (Rao, 1952) with maximum number

Table 4 Distribution of different maize (*Zea mays* L.) genotypes into clusters based on D2 statistics (Clustering by Tocher's Method)

Traits	Heritability (broad sense)	Genetic advance
I	24	SMI460, SMI222, SMI105, SMI39-2-4, SMI39-3, SMI216, SMI 67, CML141, SMI462, CML128-1, SMI61, SMI42-5, SMI168, SMI14-3, SMI135-7, SMI20, SMI11, SMI126, W3, CML 190,SMI31, SMI39-5-1-1, SMI74, SMI130
II	4	SMI125, SMI82, SMI 52, SMI114-1
III	1	SMI510
IV	1	W5

Table 5 Average inter-cluster and intra-cluster D2 values among maize genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	**278.62**	773.02	442.34	1265.71
Cluster II		**263.16**	1546.43	984.23
Cluster III			0.00	2309.56
Cluster IV				0.0

of genotypes in cluster I (24) followed by cluster II (4) whereas, cluster III and IV were mono-genotypic.

The mean intra and inter-cluster distance (D2) values for the same environment (Table 5) revealed that cluster I had highest intra-cluster distance (D2) value of (278.62) followed by cluster II (263.16). The inter-cluster distance (D2) value was highest (2309.56) between cluster III and IV followed by cluster II and cluster III (1546.43), cluster I and cluster IV (1265.71), cluster II and cluster IV (984.23) cluster I and cluster II (773.02) and between cluster I and III (442.34).

Table 6 Cluster means for yield and yield component traits in maize (*Zea mays* L.)

Traits	Clusters			
	I	II	III	IV
Germination (%)	90.83	91.33	97.00	93.00
Root depth (cm)	6.28	7.20	4.13	4.87
Shoot length (cm)	1.95	1.85	3.43	1.67
Plant height (cm)	122.25	138.92	101.00	206.67
Ear height (cm)	48.38	68.33	35.67	62.33
Ear length (cm)	12.46	12.82	13.30	13.73
Ear width (cm)	4.16	4.94	3.90	3.80
Days to 50% tasseling	105.85	97.92	99.00	107.00
Days to 50% silking	108.78	101.08	101.33	110.00
Grain yield/plant (g)	58.24	114.11	29.30	92.78
No. of kernel rows	14.09	16.80	12.00	12.87
No. of kernels/ row	19.33	25.33	14.67	33.33
100 seed wt. (g)	21.01	27.14	17.18	21.70

Cluster means for different traits in the same environment (Table 6) revealed that the magnitude of differences among the mean of traits for clusters was significant. The range of variation in cluster means for germination percentage was 90.83 in cluster I to 97.00 in cluster III. Maximum mean ear height (cm) was recorded in cluster II (68.33) and minimum in cluster III (35.67). The lowest mean ear width (cm) was expressed by cluster IV (3.80)

and highest by cluster II (4.94). Mean days to 50% silking ranged from 101.08 in cluster II to 110 in cluster IV. The mean number of kernel rows ranged from 12 in cluster III to 16.80 in cluster II. Cluster IV recorded maximum mean number of kernels per row (33.33) and cluster III has minimum number (14.67). Maximum mean grain yield plant-1 (g) was recorded in cluster II (114.11) and in cluster IV (92.78) and minimum in cluster III (29.30). Cluster II had maximum 100 seed weight of 27.14 g and cluster III with minimum of 17.18 g.

The percent contribution of a trait towards the total divergence revealed that grain yield/plant was the main factor contributing to total divergence accounting (47.59%) in the same environment followed by plant height (18.39%), root depth (10.34%), ear height (8.97%) , germination percentage (5.29%), days to 50% tasseling (3.45%). The minimum contribution towards divergence was from ear width (0.64%) followed by ear length (0.69%), shoot length (0.69%), number of kernels per row (1.84%) and 100 seed weight (2.3%).

In order to classify large number of potential genotypes into few number of homogeneous clusters, the D2 statistic of Mahalnobis (1936) has been widely used (Reddy et al., 2013) because it permits precise comparison among all possible pair of populations in any group before effecting actual crosses. The grouping of all the genotypes into four clusters (Table 4) with maximum number of genotypes in cluster I (24) followed by cluster II (4), cluster III (1) and IV (1) revealed wide diversity in elite genotypes due to the involvement of diverse parental lines in the hybridization program at different research centers and selection under different environmental situations. The pattern of group constellations in the present study, suggested that geographical diversity was not an essential factor to group the genotypes from a particular source or origin into one particular cluster. This means that, geographical diversity, though important, was not the only factor in determining the genetic divergence. Genetic diversity is the outcome of several factors, including geographical diversification. Therefore, selection of parents should be based on genetic diversity rather than geographical diversity and statistical distance (D2) presented the index of genetic diversity among these clusters.

Parental lines selected from these individual groups showing high inter-cluster distance are likely to produce superior progenies and hybrids (Singh et al., 2009). The minimum inter cluster distance between cluster I and cluster II and between cluster I and III indicates narrow genetic diversity among the genotypes. The similarity in the base material from which they have been evolved might be the cause of genetic uniformity

and genotypes in these clusters were somewhat similar in genetic constitution and hybridization between these groups may not generate sufficient variability. Inter-crossing of genotypes from divergent groups would lead to greater opportunity for crossing over, which release hidden variability by breaking linkage. Emphasis should be laid on characters contributing maximum D values for choosing the cluster with the purpose of further selection and choice of parents for hybridization (Marker and Krupaker, 2009). The highest cluster means for different traits was observed in different clusters (Table 6). For earliness as reflected through days to 50% tasseling (97.92) and days to 50% silking (101.08) cluster II was found to be good. Cluster IV showed high mean values for plant height (206.67), ear length (13.73), and number of kernels/row (33.33). High mean values for ear height (68.33), ear width (4.94), number of kernel rows (16.80), 100 seed weight (27.14) and grain yield per plant (114.11) were found in cluster II. These observations suggested that none of the clusters contained genotypes with all the desirable traits, which could be directly selected and utilized. The hybridization between genotypes of different clusters would be rewarding for the development of desirable genotypes. Recombination breeding between genotypes of different clusters has also been suggested by Alom et al. (2003), Datta et al. (2004), Beyene et al. (2005), Datta and Mukherjee (2004) and Sharma et al. (2012). Cluster means of different clusters identify the characters to be chosen for hybridization. If a breeder's intention is to improve seed yield and oil content, parents which are highly divergent with respect to these characters should be selected. Among the different traits recorded, grain yield/plant was the main factor contributing to total divergence (47.59%) which was in conformity with earlier findings (Beyene et al., 2005). Other traits imply that in order to select genetically diverse genotypes for hybridization, the material should be screened for important traits like plant height, root depth and ear height. Also the traits contributing maximum towards D2 value need to be given more emphasis for attributing to the clusters with the purpose of further selection. The choice of parents for hybridization should be based on their per se performance. There exists an urgent need to promote maize breeding to meet the increasing demands for maize grain and its products. In this context, maize hybrid breeding remains the choice of methods considering its success over years. A logical way to start any breeding program is to survey the genetic variation present in the available germplasm resources. Classification of total variability into its heritable and non-heritable components such as phenotypic and genotypic coefficient of variations, heritability estimates and expected genetic advance is of great importance for understanding the genetic make-up of any breeding material under improvement. Genetic diversity is normally assessed by common morphological traits. These plant characters form the basis for the breeder's selection of promising plant material. Our study concluded that the selection of genotypes for hybridization may be done from the clusters which show high inter-cluster-distance besides care should be taken while selecting parents based on extent of divergence with respect to a particular trait of interest.

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