Maydica

Genetic diversity in interspecific hybridization derived advanced maize inbred lines

Anjali Joshi[1], [2]*, Sneha Adhikari[1], [3], Narendra Kumar Singh[1]

1 Department of Genetics and Plant Breeding, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar, US Nagar (Uttarakhand), India

2 Genetics and Tree Improvement Division, Arid Forest Research Institute, Jodhpur (Rajasthan), India

3 Indian Institute of Wheat & Barley Research, Flowerdale, Shimla (Himanchal Pradesh), India

* Corresponding author: E-mail: anjali999aj@gmail.com

Keywords: *Teosinte, maize, PCA, cluster, genetic diversity*

Abstract

Genetic diversity in maize is incessantly being reduced due to modern breeding practices. This necessitates the creation of diverse pre-breeding lines with desirable allelic introgression from wild germplasm. With a view to enhance maize germplasm, 169 teosinte derived maize inbred lines were developed and studied to assess the genetic diversity in 14 morphological traits and to classify the lines into different clusters. Analysis of variance revealed significant variation amongst the genotypes for all the traits. High heritability with high genetic advance were observed for anthesis silking interval, flag leaf length, plant height, ear per plant, ear length, ear diameter, number of kernel row/ear, number of kernel/row and thousand kernel weight; high heritability and moderate genetic advance were observed for days to anthesis and days to silking while for flag leaf width, node bearing cob and grain yield/plant moderate heritability and high genetic advance were observed. Principal component analysis revealed that first five components had greater than one eigenvalue and accounted for 66.50% of the total phenotypic variation. The values of Euclidean dissimilarity matrices ranged from 6.28-366.88 and genotypes were grouped into fourteen clusters at a Euclidean distance of 62.5. The cluster 8 had early maturing genotypes; cluster 8, 10 and 11 had genotypes with shorter anthesis silking interval and cluster 2, 3 and 4 possessed genotypes superior with respect to yield contributing traits. A significant positive correlation of 0.499 was observed between morphological and molecular data indicating that the two data sets reflect the same genetic diversity pattern and can be utilized simultaneously to capture diversity present in maize germplasm

Abbreviations

MDS- Multidimensional scaling;
PCA- Principal component analysis;
DA-days to anthesis;
DS- days to silking;
ASI- Anthesis–Silking Interval;
FLL- flag leaf length;
FLW- flag leaf width;
PH- plant height;
NBC- node bearing cob;
E/P- ear per plant;
EL- ear length;
ED- ear diameter; KR/E- number of kernel row/ear;
K/R- number of kernel/row
TKW- thousand kernel weight;
GY/P- grain yield per plant;
BIL-Backcross inbred line;
CTAB-Cetyltrimethyl ammonium bromide;
SSR-simple sequence repeats;
UPGMA-unweighted pair group method with arithmetic averages;
PC-Principal component;
GA- Genetic advance.

Introduction

Genetic diversity refers to the degree of differentiation that is found between individuals belonging to either same or to different species. Besides being of evolutionary significance, it is extremely important from plant breeding point of view. In crop breeding program, diverse nature of germplasm serves as allele reservoir and provides novel as well as rare alleles for the purpose of breeding. Improving climatic resiliency of the cultivars is also heavily dependent on diverse allelic forms, may it be from primary, secondary or tertiary gene po-

ols. Genetic diversity not only ensures long-term viability of plant populations, but is also the underlying cause of many agriculturally important phenomena like transgressive segregation and heterosis. With the progression of time, natural variability in different crop species have been declining at a very fast pace. The main drivers of this depletion are faulty breeding practices that focused on improvement of few traits especially yield and yield contributing traits, utilization of few elite parents in varietal development and globalization of few outstanding varieties thereby leading to large areas being cultivated by few uniform genotypes.

Tremendous phenotypic and molecular diversity is present in maize genome (Buckler *et al.*, 2006) with exotic and elite maize genotypes being more diverse at DNA sequence level than most wild plants of other genera (Wright and Gaut, 2005). However, on account of being sourced from a restricted set of ancestral population (Yu *et al.*, 2007) and human directed selection (Van Heerwaarden *et al.*, 2012) today's elite maize genotypes are characterized by lower genetic variance when compared to the progenitor populations from which they have evolved (Lu *et al.*, 2009). Reduced genetic variance restricts the potential to breed for new market demands, resistance to new pathogens, and adaptability to changing environments (Cooper *et al.*, 2014). Due to lack of operation of domestication bottlenecks, extensive genetic variation is available in different wild relatives of maize (Vigouroux *et al.*, 2005; Singh *et al.*, 2021; Sahoo *et al.*, 2021). Teosinte for example displays high genetic variation and is proved to be the source of diverse alleles providing resistance against number of biotic and abiotic stresses in maize including leafhopper, fall army worm (Bernal *et al.*, 2015), maize spotted stalk borer (MSSB) (Niazi *et al.*, 2014), red flour beetle (Joshi *et al.*, 2021), gray leaf spot, corn smut (Chavan and Smith, 2014), southern corn leaf blight, northern corn leaf blight and corn leaf spot to name a few.

The breeding goals will be easier to address if the enormous genetic variation present in wild progenitors becomes available to breeders in a form, they can use in their breeding programs. Teosinte (*Zea mays* ssp. *parviglumis*) being wild progenitor of maize (Doebley *et al.*, 1984) is interfertile with maize, produces viable hybrids (Singh *et al.*, 2017) and in certain parts of the world is still believed to be exchanging genes with maize naturally. The variability present in teosinte can be tapped by generation of diverse prebreeding lines which will serve as a donor of novel genetic variation and can be utilized to breed for high value characteristics such as nutritional quality, abiotic and biotic stress resistance. Creation of diverse lines may prove to be a futile exercise if proper characterization of generated lines is not undertaken. An understanding of genetic relationship among inbred lines in general and wild-derived lines in particular will be useful in allocating lines to specific heterotic groups which is important for planning of hybridization (Hallauer and Miranda, 1988).

Multivariate analytical techniques simultaneously analyze multiple measurements on each individual under investigation and are being widely used for genetic diversity analysis based on morphological, chemical, or molecular marker data. Among different multivariate analytical techniques, multidimensional scaling (MDS) techniques particularly Principal component analysis (PCA) and cluster analysis appears to be more useful and are being commonly employed (Mohammadi and Prasanna, 2003). PCA has been widely used in plant sciences for reduction of variables and grouping of genotypes whereas cluster analysis helps in grouping of individuals based on different characteristics such that individuals with similar descriptions are assembled into the same cluster (Hair *et al.*, 1995). On the basis of clustering of genotypes, hybridization programs may be initiated (Azad et al, 2012).

Taking into consideration the narrow genetic base of germplasm in maize breeding programs (Liu *et al.*, 2016) and the immediate need to diversify maize germplasm in order to meet future breeding demands (Kumar *et al.*, 2019; Adhikari *et al.*, 2019; Adhikari *et al.*, 2021) an investigation was undertaken with an objective to genetically enhance maize germplasm through teosinte (*Zea mays* ssp *parviglumis*) genome introgression and creation of teosinte introgressed maize inbred lines. The introgressed lines were further used, to validate the variability among lines due to allelic introgression from teosinte, to carry out PCA to identify the traits contributing maximum to the total phenotypic variation, to construct pair wise distance matrix and classify inbred lines into different clusters on the basis of data on morphological traits in order to facilitate their utilization for crop improvement programs and also to find out the relationship between morphological and molecular distances utilizing different measures of distance/similarity.

Material and methods

Genetic material

Genetic material was developed at N. E. Borlaug Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. Teosinte (Z. mays ssp. *parviglumis*), wild progenitor of maize, was used as pollen parent and a promising maize inbred DI 103 used as seed parent were crossed to generate F_1 seeds. The F_1 plants raised from F_1 seeds were backcrossed to the maize inbred DI 103 and the

resultant BC_1F_1 was subsequently selfed for four consecutive generations leading to the development of BC_1F_5 population constituting of 169 backcross inbred lines (BILs).

Experimental design and observations procedure

The 169 BC_1F_5 lines along with both parents were evaluated in Randomized Complete Block Design with two replications. Each BIL was sown in a single row which was 2 m long and was at a distance of 75 cm from the adjacent row. Data on fourteen morphological characters namely, days to anthesis (DA), days to silking (DS), Anthesis–Silking Interval (ASI), flag leaf length (FLL), flag leaf width (FLW), plant height (PH), node bearing cob (NBC), ear per plant (E/P), ear length (EL), ear diameter (ED), number of kernel row/ear (KR/E), number of kernel/row (K/R), thousand kernel weight (TKW) and grain yield per plant (GY/P) were recorded. DA and DS were computed as number of days after sowing required by 50% plants in a row to attain anthesis and silking, respectively. For characters namely FLL, FLW, PH, NBC and E/P, the values averaged over five randomly selected and tagged plants per genotype in each replication was used for analysis. For traits namely EL, ED, KR/E and K/R five randomly selected ears harvested from five randomly selected and tagged plants of each line were used for analysis.

DNA extraction and genotyping

Genomic DNA of each line of the BIL population was extracted from leaves of 30 days old plants using CTAB (Cetyltrimethyl ammonium bromide) method of Doyle and Doyle (1990) with some minor modifications. DNA quality was ensured by electrophoresis of stock DNA in 0.8% agarose gel while quantification was done with spectrophotometer (Systronics PC Based Double Beam Spectrophotometer 2202). The DNA stocks were then diluted to a working concentration of 200 ng/µl and stored for further PCR amplification. Out of the total 168 microsatellite markers screened on parental lines, 76 were found to be polymorphic between maize line DI 103 and teosinte accession. These 76 simple sequence repeats (SSR) markers were then used for genotyping of 169 $_{BC1F5}$ lines. Amplification of DNA was done in 13.8 µl reaction mixture consisting of 3 µl DNA template (200 ng/µl), 0.35 µl dNTPs mix (2.5 mM each), 0.25 µl Taq DNA polymerase (3U/µl), 1.5 µl reaction buffer with 15mM $MgCl_2$ (10X), 1.5 µl of both forward and reverse primer (40 ng/µl) and 7.2 µl deionized water. The PCR amplification product was resolved in horizontal gel electrophoresis assembly using 3% agarose gel. The amplicon profiles of each BIL were visualized and photographed with ultra violet light in gel documentation unit (Alpha Innotech Corporation, USA) after 75% of gel run.

Data analysis

Analysis of variance for fourteen morphological traits was done with the help of XLSTAT software (Addinsoft, 2019). Heritability in broad sense (h^2_b) was estimated according to the formula suggested by Johnson *et al.* (1955). Genetic advance was calculated following formula given by Johnson *et al.* (1955) and genetic advance in percent of mean was calculated by the formula given by Comstock *et al.* (1952). Computation of heritability and genetic advance % mean was done with Microsoft Excel. The mean values of each trait were used to perform PCA, cluster analyses and dendrogram construction. On the basis of morphological traits BILs were classified into different clusters using unweighted pair group method with arithmetic averages (UPGMA) clustering algorithm with dissimilarity between lines expressed as Euclidean distance. Euclidean distance between two individuals i and j, having observations on morphological characters (p) was denoted by x_1, x_2, …, x_p and y_1, y_2,…, yp for i and j, respectively, and was calculated with the following formula:

$$d(i, j) = [(x_1 - y_1)^2 + (x_2 - y_2)^2 + (x_p - y_p)^2]^{1/2}$$

Marker data were recorded with 76 SSR markers for each genotype. The data were recorded in binary format where '1' referred to the presence of a specific allele at the locus while '0' referred to the absence of the same allele. Molecular data was subsequently used for the construction of Jaccard's similarity coefficient matrices based on the formula:

$$GD_J = 1 - [N_{11}/(N_{11} + N_{10} + N_{01})]$$

Where, N_{11} was the number of bands/alleles present in both individuals; N_{10} was the number of bands/alleles present only in the individual i; N_{01} was the number of bands/alleles present only in the individual j.

The computation of PCA, Euclidean distance matrices, construction of dendrogram, calculation of cophenetic correlation coefficient and application of Mantel's test (Mantel, 1967) were done with the help of PAST (Paleontological STatistics) software (Hammer *et al.*, 2001).

Results and discussion

Assessment of genetic variability amongst BILs for different morphological traits

Analysis of variance showed presence of significant differences amongst the genotypes for all the traits indicating the presence of genotypic differences and suggesting the importance of selection for the im-

Table 1 - Analysis of variance, mean, range, heritability and genetic advance for fourteen morphological traits in maize.

Characters	Maize (DI 103)	Teosinte	BC1F5		Mean sum of square treatment	Mean sum of square Error	Heritability (h^2)	Genetic advance % mean
			Mean±SEM	Range				
DA	55.00	83.00	58.33±1.63	48-72	34.99**	5.32	73.60	11.67
DS	56.50	78.00	57.91±1.63	46-69.5	48.5**	5.31	80.26	14.81
ASI	-1.50#	5.00	2.5±0.35	-7-6#	2.899**	0.24	84.70	87.33
FLL	31.50	24.50	33.19±0.83	8.75-79.67	256.96**	1.36	98.95	69.79
FLW	4.78	3.70	4.15±0.26	1.5-7	2.76**	1.4	32.69	23.41
PH	98.43	241.50	165.44±4.39	61-248.67	1980.58**	38.61	96.18	38.05
E/P	1.42	266.00	2.44±0.25	1.17-5.83	0.843**	0.123	74.53	43.74
NBC	4.35	6.06	6.24±0.53	2.33-8.83	1.983**	0.566	55.59	20.71
EL	14.45	4.10	10.62±0.80	5.25-18.17	9.79**	1.27	77.03	35.14
ED	3.43	0.73	2.65±0.19	1.17-3.92	0.4**	0.073	69.13	26.18
KR/E	12.83	2.00	10.13±0.66	2.67-17.33	6.78**	0.86	77.49	30.81
K/R	14.00	3.67	17.91±1.23	3.5-36.83	87**	3.03	93.27	71.96
TKW	192.25	64.85	138.34±2.85	69.45-254.50	2813.86**	16.27	98.85	55.37
GY/P	62.5	135	39.94±3.64	4.42-240.83	1429.55**	406.61	44.28	51.64

** Significant at 0.01 probability level.

negative sign indicates anthesis occurred before silking.

SEM- Standard error mean, DA- days to anthesis, DS- days to silking (DS), ASI- Anthesis–Silking Interval, FLL- flag leaf length, FLW- flag leaf width, PH- plant height, E/P- ear per plant, NBC- node bearing cob, EL- ear length, ED- ear diameter, KR/E- number of kernel row/ear, K/R- number of kernel/row, TKW- thousand kernel weight, GY/P- grain yield per plant.

provement of these traits. Ghimire *et al.* (2015) also observed significant variations among the genotypes for grain yield, ear weight, number of kernel/row, number of kernel/ear, ear length, ear girth, plant height and ear height. Significant genotypic differences for average number of cobs per plant, average cob length, average number of grains per cob, average number of grains per plant, average number of rows in a cob and hun¬dred grain weight were also observed by Dutta *et al.* (2017) while evaluating 84 maize genotypes. In the present investigation DA ranged from 48-72 days while DS ranged from 46-69.5 days. MT 148 was the best genotype with respect to DA as it required minimum number of days to anthesis (48 days) followed by MT 39 (48.5 days) and MT 52 (48.5 days). MT 39 was also earliest for silking (46 days) followed by MT 52 (46.5 days) and MT 34 (47 days). MT 39 and MT 52 were earliest for both anthesis and silking wherein silk emerged prior to anthesis. While MT 146 took highest number of days for both anthesis and silking. Lines that require lesser number of days for both anthesis and silking can find their significance in breeding for early maturing varieties. ASI noted in materials ranged from -7 to 6 days. Ngugi *et al.* (2013) also reported range of ASI from -2 to 10 days while studying ASI usefulness in developing drought tolerant maize. MT 1, MT 9, MT 14, MT 15, MT 17, MT 29, MT 35, MT 47, MT 50, MT 56, MT 60, MT 64, MT 73, MT 77, MT 85, MT 93, MT 94, MT 118, MT 120, MT 129, MT 139, MT 155 showed an ASI of 1 day and MT 29 had ASI of 0 day. MT 195 showed highest difference between anthesis and silking of 7 days wherein anthesis was followed by silking. FLL ranged from 8.75-79.67 cm while FLW ranged from 1.5-7.0 cm. The lowest FLL and FLW were displayed by MT 5 and MT 124 while genotypes MT 94 and MT 103 showed highest FLL and FLW, respectively. Larger leaf area is the key to increasing or sustaining maize yield, however smaller leaves in the middle and upper canopy of the plants can result in larger canopy openness thereby improving light penetration at high densities (Huang *et*

Table 2 - Factor loadings, Eigen values and cumulated total variation of the first five principal components

Variables	PC 1	PC 2	PC 3	PC 4	PC 5
DA	-0.294	-0.064	0.477	0.185	0.358
DS	-0.382	-0.042	0.534	0.082	0.024
ASI	0.238	-0.023	-0.230	0.136	0.501
FLL	0.108	0.582	0.103	0.328	-0.009
FLW	0.147	0.604	0.035	0.247	0.004
PH	0.310	0.253	0.208	-0.361	-0.061
E/P	0.042	0.182	0.199	-0.155	0.384
NBC	0.063	0.056	0.395	-0.492	-0.158
EL	0.388	-0.075	0.259	0.094	-0.206
ED	0.272	-0.218	0.249	0.330	0.123
KR/E	0.320	-0.314	0.110	0.279	-0.061
K/R	0.405	-0.185	0.205	0.088	-0.118
TW	0.133	-0.066	-0.029	-0.178	0.582
GY/P	0.258	0.010	0.010	-0.374	0.172
Eigen value	3.19	1.89	1.66	1.37	1.21
Cumulated variation (%)	22.77	36.24	48.08	57.88	66.50

DA- days to anthesis, DS- days to silking (DS), ASI- Anthesis–Silking Interval, FLL- flag leaf length, FLW- flag leaf width, PH- plant height, E/P- ear per plant, NBC- node bearing cob,EL- ear length, ED- ear diameter, KR/E- number of kernel row/ear, K/R- number of kernel/row, TKW- thousand kernel weight, GY/P- grain yield per plant, PC 1- Principal component 1, PC 2- Principal component 2, PC 3- Principal component 3, PC 4- Principal component 4, PC 5- Principal component 5.

al., 2017). Hence smaller leafed genotypes viz., MT 5 and MT 124 can be useful in developing varieties suitable for high density plantings. Plant height ranged from 61-248.67 cm. MT 11 and MT 183 were the shortest and tallest genotypes, respectively. Plant height in maize is an important agronomic trait as it is highly heritable, easy to measure, and influences stalk lodging (Li *et al.*, 2007). Previous research had shown that plant height correlates highly with biomass and grain yield and so can be effectively used for estimating biomass (Salas Fernandez *et al.*, 2009; Han *et al.*, 2019) and grain yield (Yin *et al.*, 2011; BarreroFarfan *et al.*, 2013; Geipel *et al.*, 2014). NBC ranged from 2.33-8.83. NBC along with internode length determines the height of ear in maize plant. Genotypes MT 131 (2.33) had the lowest NBC. Genotypes characterized by lower NBC and lesser internode length are desirable as it is associated with lower ear height which along with high top height/plant height ratio lowers the center of gravity of plant leading to enhanced lodging resistance (Li *et al.*, 2007). Ear/plant ranged from 1.17-5.83 and MT 69 with 5.83 E/P showed highest prolificacy. The EL ranged from 5.25-18.17 cm with largest length recorded for MT 60. The ED varied from 1.17-3.92 cm and MT 106 showed the largest diameter. The KR/E for the population ranged from 2.67-17.33 with MT 77 showing the highest number of kernel rows per ear. The K/R ranged from 3.5-36.83 while TKW and GY/P ranged from 69.45-254.50 g and 4.42-240.83 g, respectively. Genotypes MT 92, MT 183 and MT 176 showed highest K/R, TKW and GY/P, respectively. Higher estimates of E/P, EL, ED, KR/E, K/R and TKW are important as these are the major yield contributing traits in maize that can be selected for indirect improvement of maize yield.

Heritability is classified as low (below 30%), medium (30-60%) and high (above 60%) as suggested by Johnson *et al.* (1955). Heritability was high for DA, DS, ASI, FLL, PH, E/P, EL, ED, KR/E, K/R and TKW while for FLW, NBC and GY/P moderate heritability estimates were observed. Heritability ranged from 44.28%-98.95% and it was highest for FLL (98.95%) followed by TKW (98.85 %), PH (96.18%), K/R (93.27%), ASI (84.70%), DS (80.26%), KR/E (77.49%), EL (77.03%), E/P (74.53%), DA (73.60%), ED (69.13%), NBC (55.59%), GY/P (44.28%) and FLW (32.69%). Genetic advance as percent mean is categorized as low (0-10%), moderate 10-20% and high (≥20%) as suggested by Falconer and Mackay (1996). GA was high for ASI, FLL, FLW, PH, E/P, NBC, EL, ED, KR/E, K/R, TKW and GY/P while it was moderate for DA and DS. Values of genetic advance percent mean ranged from 87.33% to 11.67%. Highest GA was observed for ASI (87.33%) followed by K/R (71.96%) and FLL (69.79%) while it was lowest for

Table 3 - Distribution of genotypes in different clusters

Cluster	No. of genotypes	Genotypes
1	1	Teosinte
2	1	MT 61
3	1	MT 183
4	1	MT 176
5	18	MT 3, MT 14, MT 27, MT 28, MT 34, MT 37, MT 45, MT 50, MT 57, MT 62, MT 64, MT 69, MT 85, MT 119, MT 128, MT 143, MT 144, MT 190
6	6	MT 25, MT 152, MT 160, MT 164, MT 174, MT 175
7	15	MT 1, MT 2, MT 46, MT 47, MT 58, MT 86, MT 106, MT 112, MT 131, MT 147, MT 149, MT 172, MT 185, MT 187, Maize
8	7	MT 19, MT 24, MT 60, MT 94, MT 108, MT 120, MT 127
9	2	MT 70, MT 99
10	61	MT 5, MT 10, MT 16, MT 17, MT 20, MT 22, MT 26, MT 30, MT 35, MT 39, MT 40, MT 44, MT 52, MT 55, MT 59, MT 68, MT 72, MT 77, MT 78, MT 80, MT 82, MT 83, MT 87, MT 90, MT 91, MT 92, MT 96, MT 100, MT 102, MT 103, MT 105, MT 114, MT 117, MT 121, MT 122, MT 123, MT 124, MT 129, MT 130, MT 133, MT 134, MT 136, MT 137, MT 139, MT 140, MT 141, MT 150, MT 153, MT 156, MT 159, MT 167, MT 168, MT 169, MT 170, MT 178, MT 179, MT 182, MT 184, MT 188, MT 191, MT 195
11	52	MT 6, MT 8, MT 12, MT 13, MT 15, MT 18, MT 29, MT 33, MT 41, MT 42, MT 51, MT 56, MT 63, MT 65, MT 66, MT 67, MT 71, MT 73, MT 74, MT 79, MT 81, MT 84, MT 88, MT 89, MT 93, MT 95, MT 97, MT 98, MT 101, MT 107, MT 109, MT 111, MT 115, MT 116, MT 118, MT 125, MT 126, MT 132, MT 135, MT 138, MT 145, MT 146, MT 148, MT 151, MT 155, MT 158, MT 162, MT 165, MT 166, MT 177, MT 181, MT 161
12	3	MT 9, MT 110, MT 173
13	2	MT 32, MT 53
14	1	MT 11

DA (11.67%). High heritability and high genetic advance were observed for ASI, FLL, PH, E/P, EL, ED, KR/E, K/R and TKW; high heritability and moderate genetic advance were observed for DA and DS while moderate heritability and high genetic advance were observed for FLW, NBC and GY/P. Greater magnitude of broad sense heritability coupled with higher genetic advance for all the traits studied provided evidence for existence of high additive gene effects in controlling these traits. Traits with high heritability and high genetic advance could be improved by plant selection based on phenotypic performance (Akbar *et al.*, 2003; Sadat *et al.*, 2010), traits with high heritability but low genetic advance indicated the presence of non-additive (dominant/epistatic) gene action in controlling these trait (Akbar *et al.*, 2003). Such traits could be improved by development of hybrids. High heritability and GA were also observed by Mahmood *et al.* (2004) for grain yield/ plant, plant height and days to anthesis; by Kinfe and Tsehaye (2015) for plant height and grain yield. High heritability for plant height was also observed by Peiffer *et al.* (2014). Bekele and Rao (2014) recorded high genetic advance with higher heritability for 100 seed weight which is in accordance with the present findings. Rahman *et al.* (2015) also found the same result for 1000-kernel weight. These traits can therefore, be improved through simple or progeny selection methods. High heritability and moderate GA were observed for DA and DS. Similar results for DS were also reported earlier by Mahmood *et al.* (2004). Hence improvement for both DA and DS can be achieved by careful selection based on plant phenotype.

Principal component analysis

Multivariate analysis is used to analyze a group of data with more than one variable. One of the multivariate analyses technique is PCA, which is used to decide the contribution of different characters on total variability

Table 4 - Mean values of clusters for different traits.

Cluster	DA	DS	ASI	FLL	FLW	PH	E/P	NBC	EL	ED	KR/E	K/R	TW	GY/P
1	83.00	78.00	5.00	24.50	3.70	241.50	266.00	6.06	4.10	0.73	2.00	3.67	64.85	135.00
2	56.00	59.00	-3.00#	38.67	4.93	223.75	2.83	6.17	10.67	2.83	12.67	22.67	167.50	132.67
3	58.00	55.00	3.00	36.92	4.62	248.67	2.00	7.50	13.67	2.75	11.33	29.67	254.50	111.50
4	56.00	50.50	5.50	31.50	4.42	229.92	2.33	7.67	12.92	2.83	11.67	24.25	238.50	240.83
5	55.91	54.64	1.39	33.59	4.29	199.47	2.80	6.69	11.62	2.72	10.54	21.69	155.96	56.64
6	57.50	55.67	1.83	36.60	4.90	206.40	2.58	6.28	13.08	3.04	10.94	23.36	199.89	35.39
7	57.50	56.27	1.23	27.14	3.62	149.18	2.24	5.93	10.10	2.85	10.06	17.19	201.44	52.71
8	55.93	54.21	1.71	42.96	4.88	201.06	2.48	6.69	13.38	2.82	10.90	24.69	95.15	70.74
9	59.75	59.25	0.50	35.64	4.31	241.75	1.75	7.08	14.92	2.63	10.50	14.83	93.40	30.00
10	58.35	58.73	-0.38#	32.25	4.06	161.84	2.39	6.25	10.50	2.56	10.19	17.08	105.82	34.37
11	59.54	59.35	0.19	33.87	4.16	152.02	2.46	6.04	9.72	2.60	9.65	16.39	149.26	25.56
12	59.67	58.67	1.00	46.69	4.62	125.85	2.22	6.06	10.83	2.58	11.22	12.83	115.33	70.61
13	57.75	56.00	1.75	26.15	3.80	91.25	1.83	4.83	10.17	2.63	10.00	10.71	136.83	49.50
14	66.50	63.00	3.50	9.70	2.98	61.00	2.17	7.00	10.75	2.58	8.00	20.33	193.75	13.67

negative sign indicates anthesis occurred before silking.
DA- days to anthesis, DS- days to silking (DS), ASI- Anthesis–Silking Interval, FLL- flag leaf length, FLW- flag leaf width, PH- plant height, E/P- ear per plant, NBC- node bearing cob, EL- ear length, ED- ear diameter, KR/E- number of kernel row/ear, K/R- number of kernel/row, TKW- thousand kernel weight, GY/P- grain yield per plant.

to easily determine the character that can represent a genotype (Afuape *et al.*, 2011). PCA was performed on fourteen characters of different scales therefore a correlation matrix standardizing the original data set was preferred for analysis. Results of the PCA analysis showed five principal components having Eigen values more than 1.00 (Table 2) *i.e.*, PC 1 (3.19), PC 2 (1.89), PC 3 (1.66), PC 4 (1.37) and PC 5 (1.21) which together explained 66.50% of the total phenotypic variation present in the data. The Eigen value for all five principal components ranged from 1.21-3.19. The principal component 1 (PC 1) accounted for 22.77% of the total phenotypic variability with major contribution from K/R (factor loading- 0.405) followed by EL (factor loading- 0.388), DS (factor loading- 0.382) and KR/E (factor loading- 0.320). The principal component 2 (PC 2) covered 13.468% of the total variation and major contributors were FLW (factor loading- 0.604), FLL (factor loading- 0.582), KR/E (factor loading- 0.314) and PH (factor loading- 0.253). The principal component 3 (PC 3) and principal component 4 (PC 4) contributing 11.84% and 9.8% of the total phenotypic variation were mainly influenced by DS (factor loading- 0.534), DA (factor loading- 0.477), NBC (factor loading- 0.395), EL (factor loading- 0.259) and NBC (factor loading- 0.492), PH (factor loading- 0.361), ED (factor loading- 0.330), FLL (factor loading- 0.328), respectively. The principal component 5 (PC 5) contributed only 8.61% of the total phenotypic variation and major contributors were TW (factor loading- 0.582), ASI (factor loading- 0.501), E/P (factor loading- 0.384) and DA (factor loading- 0.358). These results revealed that yield contributing traits (K/R, EL, KR/E, ED), maturity (DA, DS), flag leaf size (FLL, FLW) and plant size (PH and NBC) were the key traits having largest contribution to the total phenotypic variation amongst teosinte derived maize population. In a similar experiment pertaining to multivariate analysis of genetic diversity of 75 maize genotypes, Syafii *et al.* (2015) identified four major principal components explaining 67.27% and 71.85% of the total phenotypic variation in maize sole cropping system and maize-Albizia cropping system, respectively. In accordance with the study conducted by us, maximum variation was contributed by leaf area, leaf area index, days to tasseling and days to harvesting under both the cropping systems. Another experiment conducted by Dutta *et al.* (2017) revealed that three principal components having greater than one eigen values contributed 76.6% of the total variation amongst eighty-four genotypes of maize with yield per ha, aver¬age number of grains per plant, cobs per plant contributing maximum to the total variation. The distribution pattern of 169 maize inbred based on

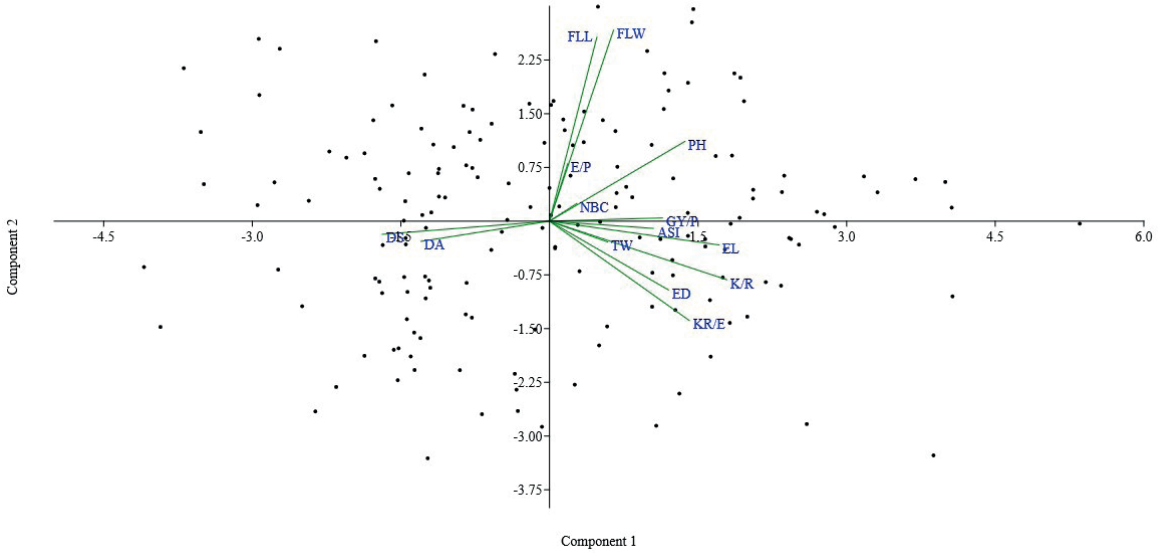


Fig. 1 - Biplot representing distribution pattern of 169 maize inbreds.

first two principal components is presented in fig 1. The biplot (Fig 1) also provides an insight into the direction of correlation that exist between different variables. GY/P had positive correlation with ASI, EL, NBC, PH, TW, K/R, ED, KR/E, E/P, FLW and FLL while negative correlation with DA and DS. A negative correlation was evident from an obtuse angle between GY/P vector and DA, DS vector. Dutta *et al.* (2017) also showed that yield was positively associated with average number of cobs per plant, average cob length, average number of grains per cob, average number of grains per plant and hundred grain weight. A high correlation between yield and hundred grain weight was also observed by Hemavathy *et al.* (2008). Corke and Kannenberg (1998) and Mohammadi *et al.* (2003) also observed high correlation between grain yield and num¬ber of rows per ear. Grain yield being controlled by polygenes is a quantitative trait (Bello and Olaoye, 2009) and have low heritability. Selection for yield alone is not much effective and improvement in yield requires indirect selection for other traits that are positively and highly correlated with yield (Muhammad et al, 2003). In the present investigation, the traits found to be positively correlated with yield and having high heritability are ASI, EL, PH, TW, K/R, ED, KR/E, E/P, and FLL. These traits are assumed to have great potential and can be given due

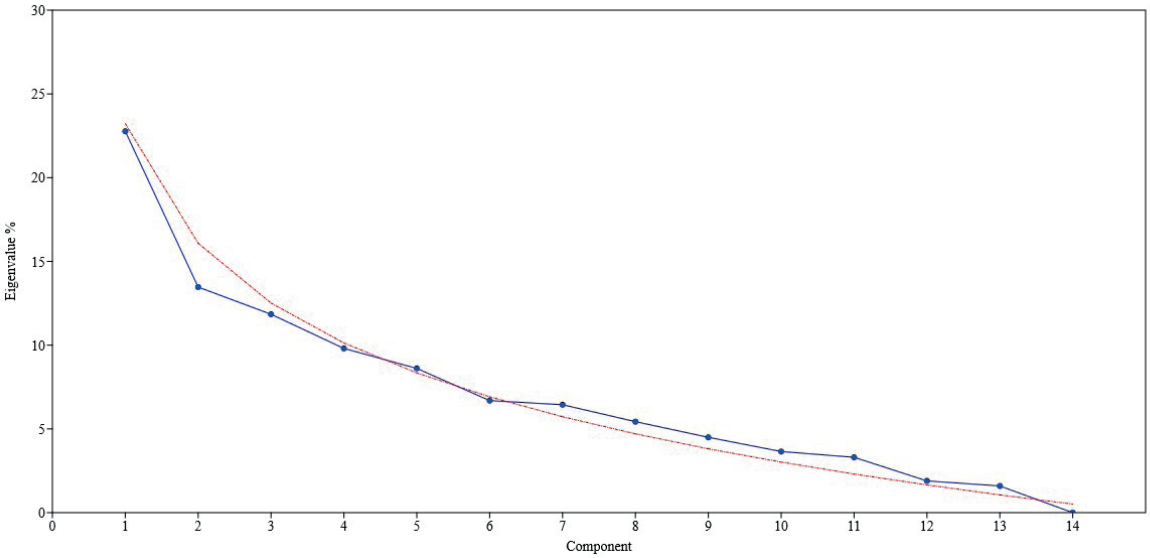


Fig. 2 - Scree plot representing fourteen principal components.

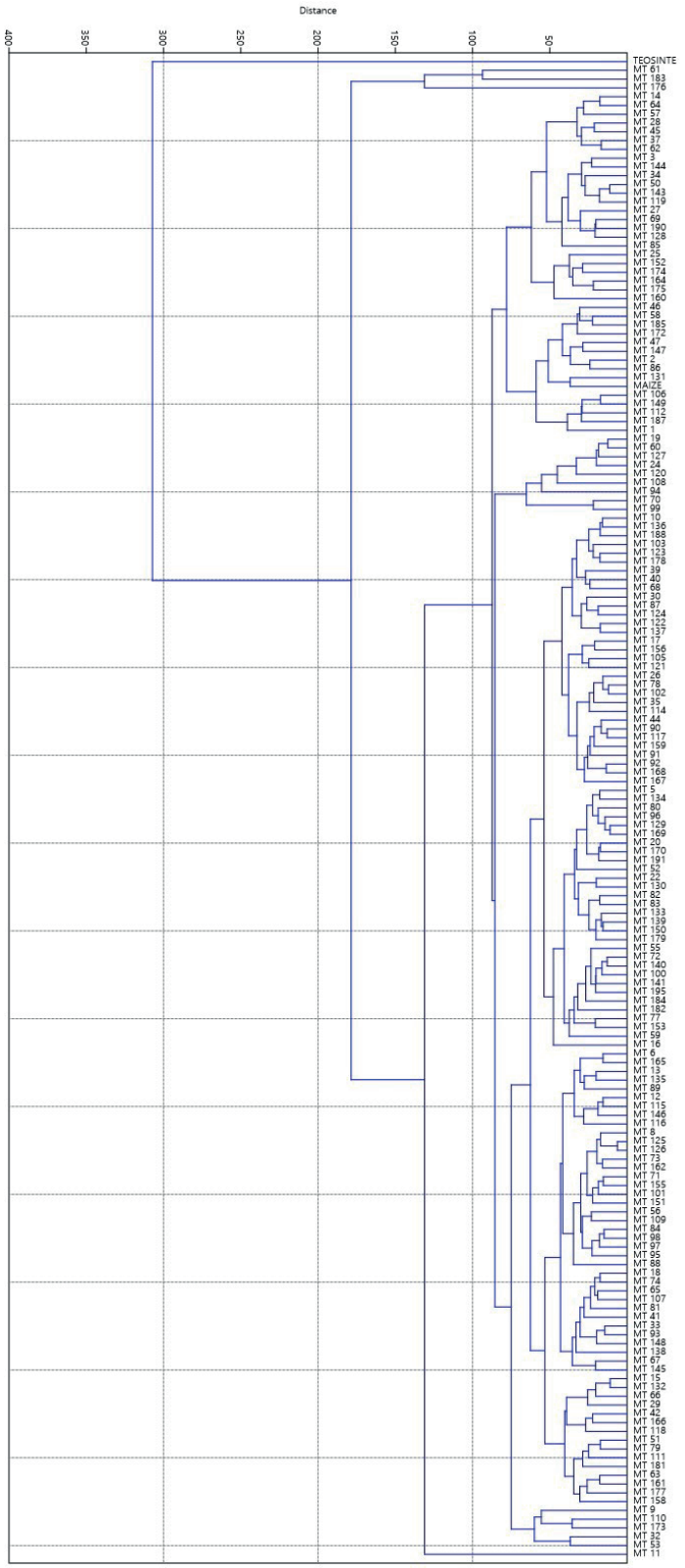
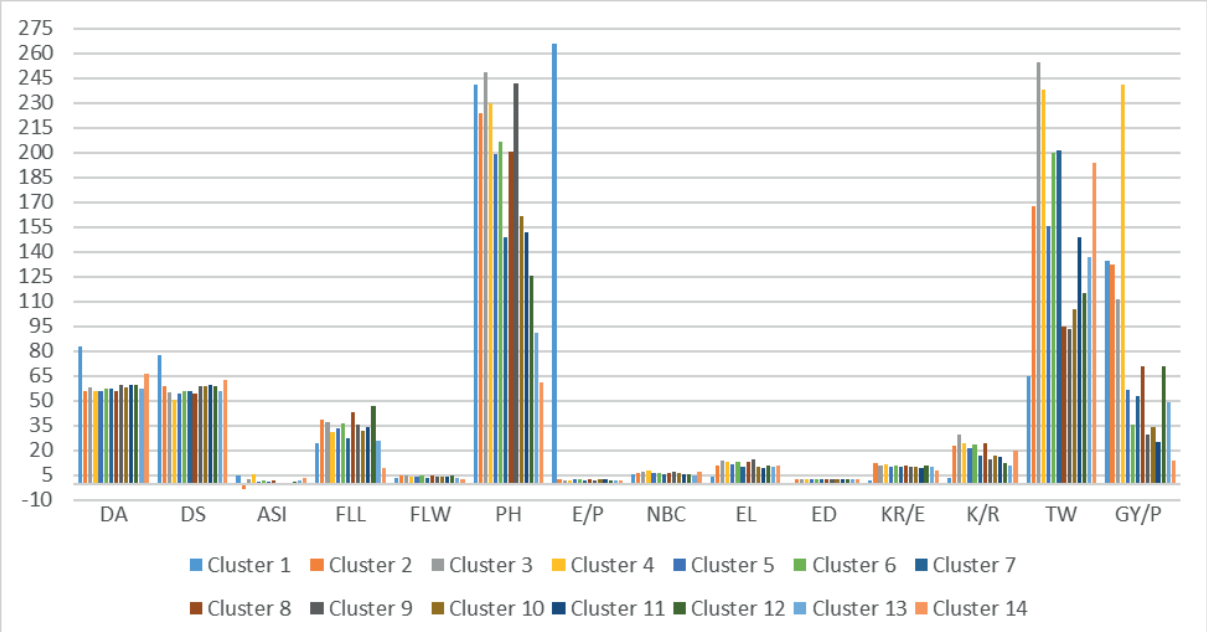


Fig. 3 - Dendrogram from UPGMA clustering of 169 inbred lines and two parents based on Euclidean distance.



DA- days to anthesis, DS- days to silking (DS), ASI- Anthesis–Silking Interval, FLL- flag leaf length, FLW- flag leaf width, PH- plant height, E/P- ear per plant, NBC- node bearing cob, EL- ear length, ED- ear diameter, KR/E- number of kernel row/ear, K/R- number of kernel/row, TKW- thousand kernel weight, GY/P- grain yield per plant.

Fig. 4 - Mean values of fourteen morphological traits for different clusters.

attention while planning for grain yield improvement in maize. The scree plot visualizing the amount of variation each principal component captures from the data is depicted in fig 2. The scree plot is not an ideal one as it requires eight principal components for explaining 85% of the total variation present in the data set. PCA therefore might not be the best multivariate analysis technique to visualize this data and when only first five principal components (Eigen value>1) were taken into account for data representation a substantial amount of information was anticipated to be lost.

Classification of genotypes into different clusters on the basis of morphological characteristics

Cluster analysis using unweighted pair group method with arithmetic average (UPGMA) along with construction of Euclidean distance matrices was done. The dissimilarity matrices expressing divergence between genotypes in terms of Euclidean distance ranged from 6.28-366.88. The maximum distance of 366.88 was observed between teosinte and inbred line MT 11 with teosinte being part of cluster 1 and MT 11 of cluster 14. This was followed by a distance of 345.49 between teosinte and MT 1 (cluster 7) and a distance of 344.13 between teosinte and MT 112 (cluster 7). Inbred lines MT 125 and MT 126 showed minimum divergence with the lowest Euclidean distance of 6.28 and both of them were grouped together in cluster 11 followed by MT 132 and MT 15 with a distance of 10.9 and grouped together in cluster 11, and MT 129 and MT 169 grouped together in cluster 10 with distance of 11.0. Clustering of inbred lines based on morphological data is shown in Fig 3.

Dendrogram was checked for agreement using the cophenetic correlation coefficient and a value of 0.769 was obtained. Though cophenetic correlation coefficient value equal to or greater than 0.85 is considered good (Stuessy, 1990), values greater than 0.75 also lies within an acceptable range thereby ensuring the consistency of the dendrogram with the distance matrices (Bohn *et al.* 1999). The lower cophenetic correlation coefficient values obtained in our study may be due to large number of genotypes involved in the analysis. In accordance with our study Rincon *et al.* (1996) also obtained a cophenetic correlation coefficient of 0.91 when 23 individuals and 11 traits were used for dendrogram construction and a coefficient of 0.60 when 68 individuals and 11 traits were considered. Thus, the study indicates that as the number of individuals increases the agreement between phenograms and dissimilarity matrices decreases. The same was also reported by Rohlf and Fisher (1968) who proposed that cophenetic correlation coefficient decreased if the number of individuals increased above 50 and further increase in individuals had no more effect on cophenetic correlation coefficient.

Based on morphological diversity, at a Euclidean di-

stance of 62.5, 171 genotypes including two parents were grouped into fourteen clusters (Table 3). Teosinte, being morphologically very distinct from the maize line DI 103 and derived inbreds, separated into a different cluster at a Euclidean distance of 312.5. The distribution pattern revealed maximum number of genotypes *i.e.*, 61 were present in cluster 10 followed by cluster 11 having 52 genotypes and cluster 5 having 18 genotypes. Cluster 8 was composed of 7 genotypes, cluster 6 of 6 genotypes, cluster 12 of 3 genotypes while two genotypes were present each in cluster 9 and cluster 13. Minimum number of one genotype was placed each in cluster 1, 2, 3, 4 and 14. A study conducted by Chen *et al.* (2008) reported 10 clusters constituting of a total of 186 maize genotypes. Mean performance of different clusters for different traits is shown in table 4 and presented in fig 4. All short duration genotypes with reduced days to anthesis and silking were grouped together in cluster 8. The mean DA for cluster 8 was 55.93 days while 54.21 days were required for silking with a short ASI of 1.71 wherein silking preceded anthesis. Cluster 8 also constituted of genotypes with high flag leaf length (42.96 cm) and high K/R (24.69). K/R was one of the important traits determining plant yield. However, cluster 8 was also characterized with smaller grains and lower test weight (95.15 g). Shortest interval between anthesis and silking of 0.19 days was a characteristic of cluster 11 which constituted of 52 genotypes. Positive ASI in cluster 11 signified that it constituted of genotypes were silking occurred prior to anthesis. Sixty-one members of Cluster 10 had an ASI of -0.38 days and was next to cluster 11 in having short ASI. It however consisted of genotypes in which anthesis occurred prior to silking. Drought stress in maize in particular is associated with delayed emergence of silk resulting in an increased ASI. The increased ASI leads to gap between availability of pollen grains and receptivity of stigma (silk emergence) leading to non-synchrony and decreased fertilization, reduced kernel set and increased barrenness (Hall *et al.*, 1984). Therefore, maize genotypes which maintains a short ASI during moisture stress conditions are capable of retaining high grain yield (Bruce *et al.*, 2002). This approach is being routinely used by CIMMYT to improve and breed for drought tolerant maize genotypes (Banziger *et al.*, 2000). Association of short ASI with grain yield under stressed conditions was also evident from a study conducted earlier by Duvick (1997) in which he showed that yield of maize hybrids released from 1930 to 1991 for stress environment increased by 53 kg/ha/yr with an accompanied reduction in ASI by 0.04 days/yr, an increase in harvest index by 0.1%/yr and rise in ear/plants by 0.002 number/yr. Therefore, genotypes of cluster 8, 10 and 11 can be evaluated in drought stress conditions to validate stability of lower ASI following which they can be used further in stress resistance breeding programs.

Cluster 14 was characterized by reduced flag leaf length (9.70 cm) and flag leaf width (2.98 cm). Both these traits are associated with reduced leaf area. Besides lower leaf area cluster 14 was also characterized with reduced height (61 cm). In a study conducted by Lambert *et al.* (2014) it was found that maize leaf area is an important factor in manipulating plant densities as genotypes with lower leaf area are more tolerant to higher plant densities compared to those having higher leaf area. In fact, reduced leaf area provides better light penetration to lower leaves specially to leaves around ear which lead to better photosynthate accumulation and hence higher yields compared to plant with higher leaf area. Going by the same logic of reduced mutual shading of plants and increased light penetration, another plant architecture trait *i.e.*, reduced leaf angle, governed by teosinte-borne *UPA2* allele, upon its transfer to modern maize, substantially increased the yield due to increase in plant density (Tian *et al.*, 2019). Both reduced height and lower leaf area were characteristics of genotypes belonging to cluster 14 and hence their potential can be explored in breeding varieties suitable for high density plantings. Cluster 14 was however, poor in other yield contributing traits as ED (2.58 cm), KR/E (8) and GY/P (13.67 g). Cluster 13 had genotypes with shorter height (91.25 cm) and lower values for EL (10.17 cm) and K/R (10.71). With respect to yield contributing traits cluster 2 (E/P- 2.83, KR/E -12.67 and GY/P 132.67g), cluster 3 (EL-13.67 cm, K/R - 29.67 and TW -254.50 g) and cluster 4 (KR/E -11.67, TW -238.50 g and GY/P -240.83 g) were considered to be superior. Hence, for the improvement of yield through manipulation of yield contributing traits, genotypes should be selected from cluster 2, 3 and 4. Therefore, after proper combining ability assessment, hybridization between individuals belonging to cluster 2, 3 and 4 with individuals of cluster 8 are assumed to be of great significance in breeding genotypes for short duration and higher yield.

Relationship between morphological and molecular distances

Mantel test have long been used in ascertaining correspondence of matrices derived by means of different marker systems over the same set of genotypes. In present study it was used to ascertain the correspondence between the distance matrices calculated with the help of both morphological and molecular data. Mantel test revealed significant positive correlation of 0.499 between morphological and molecular marker based analysis. In accordance with our finding, Beyene

et al. (2005) also reported a correlation of 0.43 between morphological and SSR marker data and a correlation of 0.39 between morphological and AFLP marker data while assessing genetic diversity in maize germplasm. The correlation obtained was much lower in several other crop species as r=0.08 in faba bean (Ouji *et al.*, 2016), r=0.217 in sesame (Reed and Frankham, 2001) and r=0.31 in castor germplasm (Rukhsar Patel *et al.*, 2017). Moderate positive correlation between molecular and phenotypic distances observed in our investigation indicated that phenotypic variability can to a limited extent be attributed to genetic factors however the effect of environment cannot be ignored. This justification might be acceptable owing to the fact that morphological traits used to classify genotypes were polygenic in nature and variation in such traits were significantly affected by environment (Smith and Smith, 1992). Oligogenic or polygenic control of anthesis silking interval, culm diameter, days to anthesis, days to silking, ear diameter, ear length, kernel row/ear, kernel weight, leaf length, leaf width, plant height and tassel branch number was also evident from their approximately normal distribution (Chen *et al.*, 2019) while studying teosinte Nested Association Mapping population. Therefore, the presence of genotype X environment interaction also might be one of the reasons responsible for lower correlation between morphological and molecular distances. The presence of moderate positive correlation indicates that both morphological and molecular marker data likely reflects the same pattern of genetic diversity and both of them may be utilized simultaneously and in conjugation to capture actual genetic diversity present in maize germplasm.

Conclusions

The results of the study indicate diversification in teosinte-derived maize inbred lines leading to significant differences for all the fourteen traits. The promising lines displaying desirable magnitude of a specific trait can therefore be used as donor in maize breeding programs. The magnitude of heritability and genetic advance would also be useful in assessing the most suitable breeding method to be adopted for improvement of the trait concerned. All the characters studied in present investigation showed greater magnitude of both heritability as well as genetic advance; therefore, selection based on phenotypic performance would be sufficient to bring about an improvement for the traits concerned. The investigated genotypes were successfully classified into 14 discrete clusters with different mean values. The genotypes belonging to different clusters were genetically more diverse compared to genotypes belonging to the same cluster. Therefore, by making crosses between genotypes from different clusters, plant breeders have greater chances of obtaining superior high-yielding hybrids from fewer crosses. However, the clustering has to be augmented with specific combining ability data in order to ascertain that genotype pairs which are to be used as parental lines for hybrid development also possesses good specific combining ability. The PCA helped in identification of traits which were most important in determining variation amongst different genotypes. The correlation between morphological and molecular data depicts correspondence between the two data sets. Therefore, traits identified by PCA and contributing maximum to the phenotypic variation can be used in conjugation with molecular markers to better classify genotypes. The present investigation therefore indicates that teosinte can be used successfully in diversification of maize germplasm through allelic introgression. Such germplasm can play significant role in maize improvement program.

Acknowledgments

The Innovation in Science Pursuit for Inspired Research (INSPIRE) scholarship of the Department of Science and Technology, Ministry of Science and Technology, Government of India received by the first author for her PhD degree programme as well as the technical and financial support from AICRP maize during the entire investigation is duly acknowledged.

References

Addinsoft, 2019. XLSTAT statistical and data analysis solution. New York, USA.

Adhikari S, Joshi A, Singh NK, 2019. Phenotypic characterization and microsatellite marker analysis of elite maize inbred and teosinte (*Zea mays* ssp. *parviglumis*) accession. Pantnagar Journal of Research 17(2):123-128.

Adhikari S, Joshi A, Kumar A, Singh NK, 2021. Diversification of maize (*Zea mays* L.) through teosinte (*Zea mays* subsp. *parviglumis* Iltis & Doebley) allelic introgression. Genetic Resources and Crop Evolution. https://doi.org/10.1007/s10722-021-01170-z.

Afuape SO, Okocha PI, Njoku D, 2011. Multivariate assessment of the agromorphological variability and yield components among sweet potato (*Ipomoea batatas* (L.) Lam) landraces. African Journal of Plant Science 5(2):123-132.

Akbar M, Mahmood T, Yaqub M, Anwar M, Ali M, Iqbal N, 2003. Variability, correlation and path coefficient studies in summer mustard (*B. juncea* L.). Asian Journal of Plant Sciences 2 (9):

696-698.
Azad MAK, Biswas BK., Alam N, Alam SKS, 2012. Genetic Diversity in Maize (*Zea mays* L.) Inbred Lines. The Agriculturists 10 (1): 64-70.
Banziger M, Edmeades GO, Beck D, Bellon M, 2000. Breeding for drought and nitrogen stress tolerance in maize: From theory to practice. Mexico, D.F.: International Maize and Wheat Improvement Centre(CIMMYT).
Barrero Farfan ID, Murray SC, Labar S, Pietsch D, 2013. A multi-environment trial analysis shows slight grain yield improvement in Texas commercial maize. Field Crops Research 149: 167–176.
Bekele A, Rao TN, 2014. Estimates of Heritability, Genetic Advance and Correlation Study for Yield and It's Attributes in Maize (*Zea Mays* L.). Journal of Plant Sciences 2: 1-4.
Bello OB, Olaoye G, 2009. Combining ability for maize grain yield and other agronomic characters in a typical southern guinea savanna ecology of Nigeria. African Journal of Biotechnology 8 (11): 2518-2522
Bernal JS, Melancon JE, Zhu-Salzman K, 2015. Clear advantages for fall armyworm larvae from feeding on maize relative to its ancestor Balsas teosinte may not be reflected in their mother's host choice. Entomologia Experimentalis et Applicata 155: 206–217.
Beyene Y, Botha AM, Myburg AA, 2005. A comparative study of molecular and morphological methods of describing genetic relationships in traditional Ethiopian highland maize. African Journal of Biotechnology 4 (7): 586-595.
Bohn M, Utz HF, Melchinger AE, 1999. Genetic similarities among winter wheat cultivars determined on the bases of RFLPs, AFLPs SSRs and their use in predicting progeny variance. Crop Science 39: 228– 237.
Bruce WB, Edmeades GO, Barker TC, 2002. Molecular and physiological approaches to maize improvement for drought tolerance. Journal of Experimental Botany 53 (366): 13–25.
Buckler ES, Gaut BS, McMullen MD, 2006. Molecular and functional diversity of maize. Current Opinion in Plant Biology 9:172–176.
Chavan S, Smith SM, 2014. A rapid and efficient method for assessing pathogenicity of *Ustilago maydis* on maize and teosinte lines. Journal of Visualized Experiments 83:e50712.
Chen F, Yang K, Rong T, Pan G, 2008. Analysis of genetic diversity of maize hybrids in the regional tests in Sichuan and Southwest China. Frontiers of Agriculture in China 2(2): 162–171.
Chen Q, Yang CJ, York AM, Xue W, Daskalska LL, DeValk CA, Krueger KW, Lawton SB, Spiegelberg BG, Schnell JM, Neumeyer MA, Perry JS, Peterson AC, Kim B, Bergstrom L, Yang L, Barber IC, Tian F, Doebley JF, 2019. TeoNAM: A Nested Association Mapping Population for Domestication and Agronomic Trait Analysis in Maize. Genetics 213 (3):1065-1078.
Comstock R, Robinson H, Gowen J, 1952. Estimation of average dominance of genes, Heterosis 494-516.
Cooper M, Gho C, Leafgren R, Tang T, Messina C, 2014. Breeding drought-tolerant maize hybrids for the US corn-belt: discovery to product. Journal of Experimental Botany 65(21):6191–204.
Corke H and Kannenberg LW, 1998. Selection for veg¬etative phase and actual filling period duration in short season maize. Crop Science 29: 607-612.
Doebley JF, Goodman MM, Stuber CW, 1984. Isoenzymatic variation in Zea (Gramineae). Systematic Botany 9:203-218.
Doyle JJ, Doyle JL, 1990. Isolation of plant DNA from fresh tissue. Focus 12:3-15
Dutta R, Kumar A, Chandra S, Ngachan SV, 2017. Genetic divergence, path coefficient, principal component and cluster analyses of maize genotypes in the mid-altitudes of Meghalaya. Maydica 62 (1): 1-5.
Duvick DN, 1997. What is yield? In: Edmeades GO, Ba¨ nziger M, Mickelson HR, Pen˜ a-Valdivia CB, eds. Developing drought and low-N tolerant maize. El Batan, Mexico: CIMMYT, 332–335.
Geipel J, Link J, Claupein W, 2014. Combined spectral and spatial modeling of corn yield based on aerial images and crop surface models acquired with an unmanned aircraft system. Remote Sensing 6: 10335–10355.
Ghimire B, Timsina D, Nepal J, 2015. Analysis of chlorophyll content and its correlation with yield attributing traits on early varieties of maize (*Zea mays* L.). Journal of Maize Research and Development 1: 134.
Hair JR, Anderson RE, Tatham RL, Black WC, 1995. Multivariate data analysis with readings. 4th edition, Prentice-Hall, Englewood Cliffs, NJ.
Hall AJ, Chimenti N, Trapani F, Cohen de Hunau R, 1984. Yield in water-stressed maize genotypes: association with traits measured in seedling and in flowering plants. Field Crops Research 9:41-46.
Hallauer AR, Miranda JB, 1988. Quantitative

genetics in maize. breeding. 2nd edition, Iowa State University Press, Ames, IA.
Hammer O, Harper DAT, Ryan PD, 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9.
Han L, Yang G, Dai H, Xu B, Yang H, Feng H, Li Z, Yang X, 2019. Modeling maize above-ground biomass based on machine learning approaches using UAV remote-sensing data. Plant Methods 15:10.
Hanson WD, 1961. Heritability, Statistical Genetics and Plant Breeding, National Academy of Science, National Research Council, Washington 125-140.
Hemavathy AT, Balaji K, Ibrahim SM, Anand G, Sankar D, 2008. Genetic variability and correlation studies in maize (*Zea mays* L.). Agricultural Science Digest 28 (2): 112-114.
Huang S, Gao Y, Li Y, Xu L, Tao H, Wang P, 2017. Influence of plant architecture on maize physiology and yield in the Heilonggang River valley. The Crop Journal 5 (1): 52-62.
Johnson HW, Robinson HF, Comstock RE, 1955. Estimation of genetic and environmental variability in soybean. Journal of Agronomy 47:314-318.
Joshi A, Adhikari S, Singh NK, 2021. Mapping genomic regions for red flour beetle (*Triboliumcastaneum* (Herbst)) resistance in teosinte (*Zea mays* L. subsp. *parviglumis* H. H. Iltis&Doebley) derived maize backcross inbred line population. Genetic Resources and Crop Evolution 68(4):1529-1544. https://doi.org/10.1007/s10722-020-01083-3.
Kinfe H, Tsehaye Y, 2015. Studies of Heritability, Genetic Parameters, Correlation and Path Coefficient in Elite Maize Hybrids. Academic Research Journal of Agricultural Science and Research 3(10): 296-303.
Kumar A, Singh NK, Adhikari S and Joshi A, 2019. Morphological and molecular characterization of teosinte derived maize population. *Indian J. Genet.* 79(4):670-677
Lambert RJ, Mansfield BD, Mumm RH, 2014. Effect of leaf area on maize productivity. Maydica 59: 58-64.
Li YL, Dong YB, Niu SZ, Cui DQ, 2007. The genetic relationship among plant-height traits found using multiple-trait QTL mapping of a dent corn and popcorn cross. Genome 50: 357–364.
Liu Z, Garcia A, McMullen MD, Flint-Garcia SA, 2016. Genetic Analysis of Kernel Traits in Maize-Teosinte Introgression Populations. G3 6(8): 2523–2530.
Lu Y, Yan J, Guimarães CT, Taba S, Hao Z, Gao S, Chen S, Li J, Zhang S, Vivek BS, Magorokosho C, Mugo S, Makumbi D, Parentoni SN, Shah T, Rong T, Crouch JH, Xu Y, 2009. Molecular characterization of global maize breeding germplasm based on genome-wide single nucleotide polymorphisms. Theoretical and Applied Genetics 120: 93–115.
Mahmood Z, Malik SR, Akhtar R, Rafique T, 2004. Heritability and genetic advance estimates from maize genotypes in shishilusht a valley of krakurm. International Journal of Agriculture & Biology, 6 (5): 790-791.
Mantel N, 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.
Mohammadi SA, Prasanna BM, 2003. Analysis of genetic diversity in crop plants-salient statistical tools and considerations. Crop Science 43: 1235-1248.
Mohammadi SA, Prasanna BM, Singh NN, 2003. Se¬quentional path model for determining interrela¬tionships among grain yield and related charac¬ters in maize. Crop Science 43:1690-1697.
Muhammad BA, Muhammad R, Muhammad ST, Amer H, Tariq M, Muhammad SA, 2003. Character association and path coefficient analysis of grain yield and yield Components in maize. Pakistan Journal of Biological Sciences 6 (2): 136-138.
Ngugi K, Cheserek J, Muchira C, Chemining'wa G, 2013. Anthesis to Silking Interval Usefulness in Developing Drought Tolerant Maize. Journal of Renewable Agriculture 1 (5): 85-90.
Niazi IAK, Rafique A, Rauf S, Da Silva JAT, Afzal M, 2014. Simultaneous selection for stem borer resistance and forage related traits in maize (*Zea mays* ssp. maysL.) × teosinte (*Zea mays* ssp. *mexicana* L.) derived populations. Crop Protection 57: 27–34.
Ouji A, El Bok S, Syed N, Flavell A, Rouaissi M, Younes M, Gazzah M, Kharrat M, 2016. A comparative study between molecular and agro-morphological methods for describing genetic relationships in Tunisian faba bean populations. Journal of new sciences, Agriculture and Biotechnology 27(8): 1513-1518.
Peiffer JA, Romay MC, Gore MA, Flint-Garcia SA, Zhang Z, Millard MJ, Gardner CAC, McMullen MD, Holland JB, Bradbury PJ, Buckler ES, 2014. The Genetic Architecture Of Maize Height. Genetics 196 (4): 1337-1356.

Rahman S, Mia KMMM, Tamanna Q, Hassan L, Haque Md, 2015. Assessing genetic diversity of Maize (*Zea mays* L.) genotypes for agronomic traits. Research in Agriculture Livestock and Fisheries 2: 53.

Reed DH, Frankham R, 2001. How closely correlated are molecular and quantitative measures of genetic variation? A meta-analysis. Evolution 55 (6):1095–1103.

Rincon F, Johnson B, Crossa J, Taba S, 1996. Cluster Analysis, An Approach To Sampling Variability In Maize Accessions. Maydica 41: 307-316.

Rohlf F, Fisher DR, 1968. Tests for hierarchical structure in random data sets. Systematic Zoology 17: 407-412.

Rukhsar Patel MP, Parmar DJ, Kalola AD, Kumar S, 2017. Morphological and molecular diversity patterns in castor germplasm accessions. Industrial Crops and Products 97: 316-323.

Sadat HA, Nematzadeh GA, Jelodar NB, Chapi OG, 2010. Genetic evaluation of yield and yield components at advanced generations in rapeseed (*B. napus* L.). African Journal of Agricultural Research 5 (15): 1958-1964.

Sahoo S, Adhikari S, Joshi A, Singh NK, 2021. Use of Wild Progenitor Teosinte in Maize (*Zea mays* subsp. mays) Improvement: Present Status and Future Prospects. Tropical Plant Biology. https://doi.org/10.1007/s12042-021-09288-1.

Salas Fernandez MG, Becraft PW, Yin Y, Lübberstedt T, 2009. From dwarves to giants? Plant height manipulation for biomass yield. *Trends Plant Sci.* 14, 454–461.

Singh NK, Joshi A, Sahoo S, Prasad B, 2021. Resistance Breeding and Exploitation of Wild Relatives for New Resistance Sources. In: Singh K.P., Jahagirdar S., Sarma B.K. (eds) Emerging Trends in Plant Pathology. Springer, Singapore. https://doi.org/10.1007/978-981-15-6275-4_10.

Singh NK, Kumar A, Chandra H, Pal K, Verma SS, 2017. Enhancement of Maize Allelic Diversity using Wild Relative Teosinte (*Zea mays* ssp. *Parviglumis*). Indian Journal of Plant Genetic Resources 30(3): 253–257.

Smith JSC, Smith OS, 1992. Measurement of genetic diversity among maize hybrids; a comparison of isozymic, RFLP, pedigree, and heterosis data. Maydica 37:53–60.

Stuessy TF, 1990. Plant taxonomy. The systematic evaluation of comparative data. Columbia University Press, New York.

Syafii M, Cartika I, Ruswandi D, 2015. Multivariate Analysis of Genetic Diversity among some Maize Genotypes under Maize-Albizia Cropping System in Indonesia. Asian Journal of Crop Science 7: 244-255.

Tian J, Wang C, Xia J, Wu L, Xu G, Wu W, Li D, Qin W, Han X, Chen Q, Jin W, Tian F, 2019. Teosinte ligule allele narrows plant architecture and enhances high-density maize yields. Science365(6454): 658-664.

Van Heerwaarden J, Hufford MB, Ross-Ibarra J, 2012. Historical genomics of North American maize. Proceedings of the National Academy of Sciences of the United States of America 109:12420–12425.

Vigouroux Y, Mitchell S, Matsuoka Y, Hamblin M, Kresovich S, Stephen J, Smith C, Jaqueth J, Smith OS, Doebley J, 2005. An Analysis of Genetic Diversity Across the Maize Genome Using Microsatellites. GENETICS 169 (3): 1617-1630.

Wright SI, Gaut BS, 2005. Molecular population genetics and the search for adaptive evolution in plants. Molecular Biology and Evolution 22:506-519.

Yin XH, Mcclure MA, Jaja N, Tyler DD, Hayes RM, 2011. In-season prediction of corn yield using plant height under major production systems. Agronomy journal103: 923–929.

Yu Y, Wang R, Shi Y, Song Y, Wang T, Li Y, 2007. Genetic diversity and structure of the core collection for maize inbred lines in China. Maydica 52: 181–94