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Agronomic characters and genomic markers based assessment of genetic divergence and its relation to heterotic performance in maize

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Abstract

Aim : The present study was undertaken to examine the relation between genetic divergence of some tropical inbred lines of maize and extent of heterosis manifestation in their single cross hybrids.

Methodology : Fifteen inbred lines and three inbred testers along with 45 single cross hybrids were evaluated by adopting randomized complete block design during two seasons. The observations recorded on eight metric traits of the entries in each of three replications were utilized during statistical analysis across the seasons. Relative importance of classification variables was determined by principal component analysis and the phenogram was generated using average taxonomic distance. Molecular profiling of inbred lines was carried out by employing 28 SSR primer pairs covering all the chromosomes. Principal coordinate analysis was performed and similarity indices based dendrogram was constructed. Relationship between parental genetic divergence and heterosis was statistically tested.

Results : Significant differences due to parents, hybrids and parent vs. hybrids were observed for all metric characters. Principal component analysis based diagram and average taxonomic distance based phenogram separated the inbred lines into five clusters. Amplification profiles based principal coordinate analysis and hierarchical cluster analysis differentiated the inbred lines into four major clusters. Statistically non-significant correlation of genomic data based genetic similarity or phenotypic data based genetic dissimilarity of inbreds was revealed with grain yield and heterosis of hybrid combinations. Significantly positive correlation was found between heterosis over mid parent or better parent and grain yield of hybrids.

Interpretation : Parental genetic divergence was not linearly or additively related to heterosis manifestation in hybrid. Moderate genetic divergence together with high *per se* performance of inbreds seemed to be a better criterion than consideration of merely extreme genetic diversity as the basis for the choice of parental inbreds during heterotic single cross hybrid development in maize.

Key words: Genetic diversity, Genomic markers, Heterosis, Maize, Principal Coordinate Analysis

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Introduction

Containing enormous variation in morphological and physiological traits, maize is recognized as the most distinctly diverse crop plant species characterized by enormous polymorphism in its genomic sequences (Adeyemo *et al.*, 2011; Smith *et al.*, 2015; Nyombayire *et al.*, 2016). Maize is widely considered as genetically well characterized plant species that has undoubtedly recorded the highest genetic advance in comparison to that attained in other crop plants during the past several decades (Santos *et al.*, 2017). Technological advancement for commercial scale exploitation of the phenomenon of heterosis has been instrumental in attainment of remarkably greater genetic advance in yield of maize (Hu *et al.*, 2017; Sharma *et al.*, 2018). Any systematic effort for further augmentation and exploitation of heterosis essentially requires a very precise knowledge regarding diversity of germplasm simply because of its significant effect and impact on hybrid maize development (Baranwal *et al.*, 2012). Biological manifestation of the phenomenon of heterosis is known to be directly dependent on the presence of directional dominance, but the occurrence of epistatic interaction between different loci having dominance effect also contributes indirectly to this phenomenon (Kaepler, 2012; Kage *et al.*, 2013). Logical explanation of heterosis phenomenon by geneticists has established that the degree of heterosis manifestation primarily depends on the extent of directional dominance and the difference of gene frequency in the parents at all loci involved in the phenotypic expression of the trait under consideration. Genetically diverse background of the parents contributes to differences in gene frequency and ultimately to the phenomenon of heterosis (Stupar *et al.*, 2008).

Analysis of genetic worth of inbred lines and their appropriate and effective utilization in hybrid development basically require precise information regarding magnitude of heterosis manifestation in their hybrid combination. Conventional approach based hybrid maize development programs suffer from the limitations imposed by expensive and time consuming exercise in the identification of parental inbred lines that produce heterotic hybrid combination. Potential of maize hybrids in relation to grain yield heterosis is not necessarily and accurately reflected by the performance of inbred lines simply because relative performance of parental inbreds determines the degree of heterosis in hybrid (Chen, 2010). Therefore, degree of heterosis manifestation is confined to the concerned hybrid combination (Betran *et al.*, 2003). Efficiency of hybrid maize breeding program can be effectively enhanced by purposive utilization of heterosis predictors of single cross hybrids or evaluation of potential hybrid combinations generated by crossing of inbred lines.

Invariably, in almost all hybrid maize development programs, accurate prediction of performance of hybrid combinations is essentially regarded as an activity of primary interest. Choice of parental lines, which would produce heterotic combination, helps the maize breeders to obviate the necessity of

testing the potential parents by making all possible crosses. Widely supported view point suggests greater probability of obtaining heterotic hybrids, if more genetically diverse parents are utilized in hybridization program. Accordingly, in hybrid maize development programs, the choice of inbred lines with greater genetic divergence as prospective parents is taken as fundamental consideration for producing heterotic hybrids having higher potential for augmentation of grain yield. Underlying basis of this consideration is high yield potential along with desirable agronomic characteristics of such hybrids because of cumulative action of previously selected favorable genetic loci (Amorim and Souza, 2005). However, the magnitude of directional dominance in addition to the parental genetic divergence is instrumental in determining the magnitude of heterosis in single cross hybrids. Consequently, the extent of parental genetic divergence does not exhibit linear or additive relationship with the degree of heterosis manifestation in many cross combinations (Fernandes *et al.*, 2015). Keeping all these in view, the present study was undertaken to investigate the nature and degree of genetic divergence among some tropical inbred lines and testers of maize by analysis of characters and microsatellite markers based data and to elucidate the relation between parental genetic divergence and magnitude of heterosis manifestation in their hybrids.

Materials and Methods

Experimental materials consisted of 18 parental lines comprising 15 inbred lines and three inbred testers and 45 single cross hybrids generated by crossing all inbred lines to all testers. The parental lines (Table 1) and single cross hybrids were evaluated following the recommended agronomic practices during *rabi* (2014-15) and *kharif* (2015) seasons in randomized complete block design with three replications at Dholi Crop Research Farm under Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, India. Each entry was allotted two rows plot and distances between rows and plants within a row were maintained at 75 and 25 cm, respectively. While raising the crop, randomization of parental lines and experimental hybrids was done independently in homogenous and contiguous plots.

Phenotypic observation and data analysis: Statistical analyses of data recorded on eight agronomic characters (Table 2) were carried out to evaluate the nature and extent of divergence among the parental lines and the magnitude of heterosis manifestation in experimental hybrids. While recording the observation, plant height was mensurated in centimeter from the ground level to the base of flag leaf, whereas ear height was determined in centimeter from the ground level to the base of ear. The measurement on the length of ear was registered in centimeter to represent the ear length. Grain yield was quantified in gram. The average value computed on the basis of observations taken for these eight agronomic characters on five competitive plants selected randomly per entry in each replication was calculated. Numerical value obtained for eight agronomic characters under consideration over the seasons was subjected to statistical analysis. Heterosis was worked out as per cent increase or

decrease in the mean value of hybrid over mid parent and better parent for the character under consideration and statistical significance of the numerical value was determined with *t* test.

Metric characters based divergence and cluster analysis:

Using eight metric characters based similarity correlation matrix, principal component analysis was performed (Rohlf, 2000) to determine the relative importance of classification variables. Spatial distribution pattern of inbred lines in two-dimensional scatter plot (Fig. 1) along orthogonal coordinate axes was examined to deduce the inter-relationship among inbred lines. Average taxonomic distance (Sokal, 1961) was computed as dissimilarity coefficient for determining the parental genetic divergence using numerical taxonomic approach (Sneath and Sokal, 1973). Tree building in the analysis was performed by adopting distance matrices based sequential agglomerative hierarchical nested (SAHN) clustering module and the phenogram (Fig. 2) was constructed by un-weighted pair group method using arithmetic average (UPGMA).

Genomic markers based divergence and cluster analysis:

Total genomic DNA was extracted from the four to five leaf stage seedlings of parental lines following a standardized protocol (Punya et al., 2017). Selective amplification of primer specific region in genomic DNA template was conducted using 28 SSR primer pairs. The panel of primer pairs covered all the chromosomes existing in the genome. Microsatellite primers specific polymorphism and allelic diversity revealed by these primer pairs, namely, phi 227562, bnlg 1429, umc 1297, nc 133, phi 083, phi 029, phi 053, umc 1266, umc 1136, phi 072, phi 093, nc 130, umc 1332, umc 1152, bnlg 118, bnlg 1136, umc 1083, phi 034, phi 116, umc 1304, umc 1161, phi 115, phi 014, phi 065, phi 084, umc 1367, umc 1196 and umc 1179, were utilized for elucidation of molecular level differentiation and divergence among the inbred parents. Analysis of genetic similarity of pairwise combinations of inbreds was performed by calculating the similarity coefficient (Jaccard, 1908) based on the presence and absence of microsatellite bands. Sequential agglomerative hierarchical non-overlapping (SAHN) clustering module was adopted for cluster analysis and the dendrogram (Fig. 3) was generated by un-weighted pair-group method using arithmetic mean (UPGMA). Principal coordinate analysis was conducted to obtain a two-dimensional ordination of SSR primers dependent genetic profiles of the entries under evaluation. NTSYS-pc software (Rohlf, 2000) was used for data analysis.

Relation between genetic divergence and heterosis: The interrelationships between genotypic performance and heterosis of single cross experimental hybrids and genetic distance among parental lines were elucidated by testing the significance of correlation coefficients.

Results and Discussion

Statistically significant differences due to parents, hybrids and parent vs. hybrids were revealed by the analysis of variance

for the design of experiment carried out for eight agronomic characters under both the seasons and also across the seasons. Therefore, remarkably greater extent of variability for each of the eight quantitative characters was observed among the inbred lines (Ihsan et al., 2005) more or less consistently across the seasons. The inference derived from analysis of variance is also evident from a perusal of range of mean values presented across the seasons (Table 2). Among the eight quantitative characters under consideration, only plant height and number of kernels per ear exhibited significant positive correlation ($r = 0.607$ and 0.726 , respectively), whereas days to brown husk had significant negative correlation ($r = -0.611$) with grain yield of inbred lines. All phenological characters showed significantly positive association among themselves. Only plant height among agro-morphological characters was found to be positively correlated with ear height, ear length and number of kernels per ear ($r = 0.847$, 0.637 and 0.482), whereas it exhibited negative significant correlation with days to brown husk ($r = -0.695$). Negative significant correlation was also recorded between ear height and days to brown husk ($r = -0.532$). Similarly, number of kernels per ear exhibited negative significant association with days to brown husk ($r = -0.469$). Positive significant association of number of kernels per ear with ear length has also been reported earlier (Hemavathy et al., 2008; Dutta et al., 2017).

Eigen values from first, second and third principal component axes accounted for 50.7, 19.3 and 12.6% with cumulative contribution of 82.7% to total variance present, respectively. Eigen vectors from the first principal component axis indicated that days to brown husk, days to 50% silking and days to 50% tassel emergence were the important characters that contributed positively (Table 3). This shows that phenological characters were the important classification variables with positive contribution, while agro-morphological characters such as, plant height, ear height, ear length, number of kernels per ear and grain yield contributed negatively. From the second principal component axis also, phenological characters appeared to be the important classification variables and positive contributors. In addition to phenological characters, plant height, ear height, ear length and grain yield had substantial positive contribution to variance from the second principal component axis. Similarly, the variance explained by the third principal component axis was mainly contributed by number of kernels per ear and grain yield among agro-morphological characters and days to 50% tassel emergence among phenological characters. Additionally, ear height, plant height, days to 50% silking and ear length seemed to be negative contributors to the variance explained by third principal component axis. The importance of some of these characters as main contributors to genetic divergence among inbred lines of maize has been demonstrated earlier (Dutta et al., 2017; Saiyad and Kumar, 2018). Spatial distribution patterns of inbred lines in principal component analysis based two-dimensional ordinations along orthogonal coordinate axes clearly discriminated the inbred lines into five genotypic clusters (Fig. 1), reflecting appreciably wide range of variability for most of the traits (Zaidi et al., 2013; Sood et al., 2015; Dutta et al., 2017).

Fig. 1: Principal component analysis based distribution pattern of 18 maize inbred lines evaluated for 8 agronomic characters.

Fig. 2: Hierarchical clustering pattern of 18 maize inbred lines evaluated and phenotyped for 8 agronomic characters.

Sequential agglomerative cluster analysis (Sneath and Sokal, 1973) based phenogram using taxonomic distance as the measure of dissimilarity exhibited the inter-relationships amongst the inbred lines, which were basically discriminated into five clusters (Fig. 2). Therefore, principal component analysis and sequential agglomerative cluster analysis yielded similar results in respect of phenological and agro-morphological characters based discrimination and classification of inbred lines. Numerical value of taxonomic distance for pair wise combinations of inbred lines ranged from 0.59 between least divergent pair (CML 373 x CML 163) to 2.06 for the pair of most divergent inbred lines (CML 467 x CML 163). A perusal of phenogram clearly indicated that inbred lines HKI 1105 and CML 115 were relatively more similar with respect to the phenotypic expression of eight quantitative characters recorded in the present study. Similarly, the inbred lines CML 471, HKI 162, CML 196, LM 13, CML 115, HKI 586, HKI 1105 and HKI 323B appeared to be comparatively more closely related since all these inbred lines were accommodated into the same cluster. Reflecting a relatively greater extent of similarity, inbred lines CML 468 and CML 469 occupied the same cluster. It is also clearly evident that inbred lines CML 467, CML 470 and DH 2012 exhibited recognizably different properties in comparison to rest of the inbred lines. Employing a panel of 28 SSR markers, which targeted microsatellite specific genomic regions, genetic polymorphism was observed in the form of presence or absence of bands besides variation in the number and position of bands among inbreds. Ample molecular level genetic variation among the inbreds was inferred on the basis of magnitudes of similarity coefficients, which ranged from 0.000 to 0.360.

The range of similarity coefficients provided greater confidence for discrimination and classification of inbred lines and elucidation of their genetic relation. The magnitude of similarity

coefficient between two distinct testers CML 165 and CML 161 was found to be maximum, reflecting relatively close similarity of these two testers in respect of the sequence of nucleotides at the primer binding sites and the length of genomic regions covered and targeted by the panel of primer pairs used during amplification profiling in the present study.

Hierarchical classification based on analysis of amplification patterns discriminated the inbreds and accommodated them into four major clusters (Fig. 3). The distribution pattern of inbreds into these four major clusters was observed to be non-homogenous. Cluster I consisted of four inbred lines including the inbred lines that had same source. Exhibiting more or less a similar trend, cluster II and Cluster IV consisted of two and nine inbred lines, collected from same source. Principal coordinate analysis based spatial two-dimensional distribution patterns of targeted genomic regions dependent genetic profiles of 18 inbred lines (Fig. 4) almost completely corroborated the results of hierarchical clustering pattern displayed by the dendrogram (Fig. 3). The variances explained by first five principal coordinates were 10.3, 8.4, 7.7, 6.9 and 6.4%, respectively, with a cumulative variance of 39.7%. Principal coordinate analysis also indicated that 18 inbred lines were discriminated and classified into four multi-genotypic groups. In general, the inbred lines were not found to be clustered around the centre but mostly located on the extremes of two-dimensional scatter plot and classifications of inbred lines were observed to be source of collection dependent up to a considerably greater extent. It was interesting to observe that all CML inbreds, with exception of three CML inbred testers, were accommodated into a single major group, though they were well dispersed on scatter plot indicating that they were divergent. This group consisted of 50% of inbred lines subjected to molecular

Fig. 3: Hierarchical clustering pattern of 18 maize inbred lines genotyped by using 28 SSR markers.

Fig. 4: Two-dimensional spatial distribution pattern of 18 maize inbred lines genotyped by using 28 SSR markers.

characterization. The CML inbred testers, however, were not dispersed as CML inbreds. Ample molecular variance exhibited among inbred lines within sources of collection signified that the sources of origin of inbreds had relatively smaller contribution to total molecular level variances and the maximum variation was detected among the inbreds within the sources of collection (Nyaligwa *et al.*, 2015; Richard *et al.*, 2016).

Interestingly, cluster analysis based on both phenotypic data and genotypic data showed closer mutual relation of inbred lines CML 468 and CML 469. Experimental results provided the basis to derive this inference because these two inbred lines belonged to same cluster in both phenotypic and genotypic data based cluster analysis. Similarly, the extent of similarity between inbred lines HKI 323B and HKI 586 was found to be considerably greater as these two inbreds occupied same cluster in both the cases. All the three testers were included in same cluster in both

the cases. However, complete correspondence was not noticed in respect of the clustering pattern of inbreds that resulted from cluster analysis based on quantitative characters and microsatellite markers. The result, in general, indicated that microsatellite markers more efficiently discriminated the maize inbred lines than morphological characters, which is in agreement with earlier reports (Beyen *et al.*, 2005; Pabendon *et al.*, 2008; Shah *et al.*, 2010; Shiri *et al.*, 2014).

Only sixteen heterotic hybrids including six hybrids, namely, HKI 162 × CML 161, HKI 323B × CML 161, CML 471 × CML 161, CML 471 × CML 165, CML 469 × CML 165, CML 468 × CML 161 (Table 4) were found to be statistically at par with respect to grain yield per plant and recorded significantly higher grain yield per plant than general mean of all hybrid combinations. Commercial exploitation of heterosis necessarily requires appropriate consideration of the degree of heterosis

Table 1: Source of inbred lines and testers of maize

Sl. No.	Inbred line	Source	Sl. No.	Inbred line	Source
01.	CML 467	CIMMYT, Mexico	10.	LM 13	SRI, Coimbatore
02.	CML 468	CIMMYT, Mexico	11.	DH 2012	TCA, Dholi
03.	CML 469	CIMMYT, Mexico	12.	HKI 162	CCS HAU, Hisar
04.	CML 470	CIMMYT, Mexico	13.	HKI 323B	CCS HAU, Hisar
05.	CML 471	CIMMYT, Mexico	14.	HKI 586	CCS HAU, Hisar
06.	CML 373	CIMMYT, Mexico	15.	HKI 1105	CCSHAU, Hisar
07.	CML 115	CIMMYT, Mexico	16.	CML 161*	CIMMYT, Mexico
08.	CML 196	CIMMYT, Mexico	17.	CML 165*	CIMMYT, Mexico
09.	CML 465	CIMMYT, Mexico	18.	CML 163*	CIMMYT, Mexico

* Testers

Table 2: Range of variation in the mean performance of inbreds and testers of maize

Character	Mean	Standard deviation	Range
DT	086.2	03.2	082.6 - 095.0
DS	087.3	02.3	084.2 - 091.1
DH	114.9	01.7	112.6 - 118.8
PH	134.9	13.1	103.1 - 155.0
EH	058.9	11.6	036.8 - 081.5
EL	015.9	01.5	012.6 - 018.9
KN	308.5	32.6	232.3 - 365.9
GY	051.8	09.8	038.0 - 075.2

DT: Days to 50% tassel emergence; DS: Days to 50% silking; DH: Days to 75% brown husk; PH: Plant height; EH: Ear height; EL: Ear length; KN: Number of kernels per ear; GY: Grain yield per plant

Table 3: Eigenvectors from the first three principal component axes for eight characters

Character	Principal component axis		
	1	2	3
DT	0.410	0.756	0.366
DS	0.638	0.625	-0.233
DH	0.871	0.308	0.030
PH	-0.865	0.326	-0.302
EH	-0.740	0.227	-0.474
EL	-0.564	0.522	-0.156
KN	-0.717	0.080	0.506
GY	-0.772	0.226	0.472

DT: Days to 50% tassel emergence; DS: Days to 50% silking; DH: Days to 75% brown husk; PH: Plant height; EH: Ear height; EL: Ear length; KN: Number of kernels per ear; GY: Grain yield per plant

manifestation in grain yield in conjunction with mean performance of hybrids. Taking this into account while assessing the potential of hybrid combinations, the results of the present study showed that mean performance of hybrids more or less closely reflected the degree of heterosis manifestation in grain yield consistent with the reports of earlier researchers (Ali *et al.*, 2007; Dubey *et al.*, 2009; Singh *et al.*, 2010).

While analyzing the statistical relation of genotypic data based genetic similarity of parental inbreds with grain yield per plant, heterosis over mid parent and heterosis over better parent of hybrid combinations, numerically non-significant association ($r = 0.052, -0.067$ and 0.049) was revealed. Similarly, the phenotypic

data based genetic dissimilarity of parental inbred lines was not found to be correlated to grain yield per plant, heterosis over mid parent and heterosis over better parent of hybrid combinations ($r = 0.161, 0.116$ and 0.023). Statistically non-significant values were obtained for correlation coefficients. However, positive significant correlation was recorded between grain yield per plant and heterosis over mid parent ($r = 0.776$), grain yield per plant and heterosis over better parent ($r = 0.701$) and between heterosis over mid parent and heterosis over better parent ($r = 0.954$). Earlier, it has been reported that higher grain yield is not invariably recorded in hybrids having higher parental genetic distance (Guimaraes *et al.*, 2007). Accordingly, it was emphasized that inbreds having high mean performance

Table 4: Heterotic potential along with parental genetic divergence of high yielding hybrid combinations

Hybrid	Heterosis (%)		Grain yield per plant	Genetic distance	Genetic similarity
	Mid parent	Better parent			
CML 469 x CML 163	79.65**	73.30**	87.07	1.64	0.015
HKI 162 x CML 161	76.15**	72.58**	89.95	1.18	0.059
CML 115 x CML 163	84.53**	72.20**	80.88	1.41	0.000
CML 468 x CML 161	89.03**	66.49**	83.30	0.98	0.015
CML 471 x CML 161	66.98**	59.65**	87.55	1.41	0.042
HKI 1105 x CML 163	65.05**	51.30**	73.90	1.44	0.082
CML 115 x CML 161	52.04**	47.44**	73.77	1.01	0.045
HKI 323B x CML 161	59.47**	46.41**	87.58	1.47	0.043
CML 469 x CML 165	62.60**	37.56**	87.07	1.71	0.015
CML 471 x CML 165	44.60**	34.93**	85.40	1.13	0.029
HKI 586 x CML 161	38.40**	25.30**	77.30	1.27	0.045
CML 115 x CML 165	39.60**	21.61**	76.98	1.14	0.049
HKI 1105 x CML 165	33.47**	18.24**	74.83	1.04	0.069
HKI 162 x CML 165	28.87**	17.50**	74.40	0.91	0.032
CML 465 x CML 163	21.83**	15.94**	75.47	1.08	0.014
CML 196 x CML 165	32.85**	16.66**	73.82	1.01	0.048

for grain yield and intermediate genetic distance should be preferably crossed for exploitation of heterosis than inbred lines with intermediate yield and wide genetic distance (Dutta *et al.*, 2004; Ghosh *et al.*, 2018).

Hybrid combination HKI 162 x CML 161, which had moderate parental genetic distance based on either genomic markers or metric characters, recorded the highest grain yield in the present study (Table 4). Similarly, the hybrid combinations CML 468 x CML 161 and CML 469 x CML 163, with relatively lesser parental genetic distance, registered high heterosis in grain yield. Apparently, the higher parental genetic distance did not necessarily contribute to high grain yield of hybrid combination in accordance with the reports of some of the earlier researchers. Experimental evidence indicating the presence of non-linear correlations between genetic distance and hybrid performance seems to be inconsistent (Sant *et al.*, 1999) however, existence of a strong positive correlation between yield and heterosis over mid parent or better parent completely corroborates previous findings (Drinic *et al.*, 2002; Betran *et al.*, 2003; Devi and Singh, 2011).

Purposeful utilization of parental lines with different heterotic patterns is a heterosis breeding strategy that allows exploration of and capitalization on heterosis in maize (Vieira *et al.*, 2009). It is well established that the extent of heterosis manifestation is dependent on genetic divergence and the involvement of genes with non-additive effects in the phenotypic expression of desirable characteristics. Thus, divergent and adaptively superior individuals are preferentially selected while choosing parents. In this context, precise analysis of genetic divergence effectively assists in the choice of parental inbreds to be used in breeding programs for producing superior hybrid combinations. It is generally taken into consideration that heterotic expression directly depends on parental genetic divergence and crossing of more divergent parents increase the chances of generating more promising and heterotic hybrid combinations (Paterniani *et al.*, 2008).

Experimental results of the present study provides a base to infer that extreme parental genetic divergence does not ensure very high extent of heterosis. Thus, it appears to be a better approach to consider moderate level genetic divergence along with high *per se* performance of inbreds as criterion and to avoid the inbreds with extremely low or excessively high genetic divergence during the selection of parental inbreds for heterotic hybrid development in maize.

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