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Isozyme Pattern and Morpho-agronomical Traits Based Genetic Divergence Studies in Maize (*Zea mays* L.) Inbreds

Ranju Kumari[1*], A. K. Singh[2] and V. K. Sharma[2]

[1]Department of Plant Breeding and Genetics, Nalanda College of Horticulture, Noorsarai, Nalanda, 803113, (B. A. U., Sabour, Bhagalpur), India.
[2]Dr. Rajendra Prasrad Central Agricultural University, Bihar, Pusa, Samastipur, 848125, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author RK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AKS managed the analyses of the study and managed the literature searches. Author VKS corrected the draft manuscript and provided necessary suggestions in the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was carried out with an objective to study genetic divergence based on morpho-agronomical traits and isozyme pattern in eight maize inbreds. These inbreds were evaluated in randomized block design with three replication for ten morph-agronomical traits. Horizontal starch gel electrophoresis technique used to study the isozyme polymorphism in different tissues of eight inbreds. Analysis of variance revealed significant differences among the inbreds for all the ten morpho-agronomical traits. Nature and extent of genetic divergence for morpho-agronomical traits was measured using average taxonomic distances as a measure of dissimilarity coefficient. Eight inbreds were clustered into four groups (A, B, C, D) based on dissimilarity coefficient. Cluster B and cluster D showed the highest inter cluster distance (2.2422) and the lowest was observed between clusters B and C (1.0401). Cluster A exhibited the highest intra cluster distance (0.8519). Based on inter cluster distances inbreds present in cluster B and D were

**Corresponding author: E-mail: ranjupusa@gmail.com;*

found more diverse consisted of inbred CML 186 and CM 600 respectively. Six isozyme systems were used for characterization and divergence studies based on similarity coefficients. Inbreds were classified into six clusters (A, B, C, D, E and F). The lowest (0.5957) similarity coefficient exist between inbreds CM 600 and CML 176 and the highest (0.8132) existed between inbreds CML 186 and CML 144. Cluster analysis in both cases reflected the moderate level of genetic divergence among the inbred lines but result may not be completely similar, but somewhat distinct and complementary in nature. Isozyme patterns was found to effective in revealing the nature of relationship among the inbred lines Therefore, divergence study using one estimate can't replace the need to evaluate the relationship on the basis of the other which may be to used as parents in hybridization programme.

Keywords: Maize; Zea mays L.; genetic divergence; morpho-agronomical traits; isozymes.

1. INTRODUCTION

Maize, also known as corn, is believed to have derived its name from the word "mahis" meaning source of life. It is growing globally as multipurpose crops like food for man, feed for animals and use in industrial sectors. It has the highest yield potential than any other cereals and grown during the year due to its photo-thermo insensitive character. Though, the production of maize seems unable to meet the demand of maize in the world. Maize germplasm has remarkable exploitable genetic variability in respect of plant, ear and seed, resistance to various stresses for its improvement. Maize breeder has choosen genetically divergent parents for its use in heterosis programme. The reasons behind that genetically diverse parent are expected to produce high heterotic effects and desirable segregants as reported by many workers [1]. But, superior heterotic expression is sometimes also observed in the hybrid using moderately divergent parents associated with high *per se* performance [2,3]. Maize breeders concentrate on maintenance and exploitation of two or more heterotic breeding groups for the development of inbreds and subsequent hybrids.

Electrophoretic separation of isozymes has provided a reasonably precise and quantitative approach for the analysis of genetic diversity in many crops including maize [4,5,6,7]. Presence or absence of isozyme bands was used as marker for characterizing the inbreds in the studies conducted by earlier workers. Classical identification of cultivars and more so the assessment of germplasm diversity based on standard morphological marker in maize has proved to be inadequate because of the existence of wide spectrum of phenotytpic variation, interaction of morphometric and morphologic characters with environment, epistatic interaction and the unknown genetic control of the traits [8,9]. Isozymes are relatively less affected by environmental influence unlike conventional traits. Owning to this advantage isozymes have been used as molecular markers to provide useful data in a broad range of basic and applied research. Six isozyme systems, namely peroxidase, esterase, acid phosphatase, catalase, amylase and alcohol dehydrogenase were used for characterization of eight maize inbred lines. Thus, the objective of the present investigation was to measure the genetic divergence among eight inbred based on morphological traits and isozyme patterns for the development of wide array of single crosses and also assess the supremacy of the technique one.

2. MATERIALS AND METHODS

Materials for the present investigation were included eight inbred lines (CML 142, CML 144, CML 150, CML 176, CML 186, CM 300, CM 400 and CM 600) obtained from AICRP on maize, Dholi Centre at the research farm of Tirhut College of Agriculture, Dholi, under Rajendra Agricultural University, Bihar, Pusa. These inbreds were planted in randomized block design (RBD) with three replication with row to row and plant to plant spacing of 60 cm and 20 cm respectively in plots having three rows per plot in Rabi season. Recommended package of practices was followed for raise a good crop. Observations were recorded for ten morpho-agronomical traits viz., plant height (cm), ear length (cm), ear girth (cm), number of kernel rows per ear, number of kernels per row, 100 kernel weight (g), yield per plant (g), days to 50 per cent tassel emergence, days to 50 per cent silk emergence and days to 50% maturity on five plants chosen randomly in each plot.

2.1 Assessment of Divergence

Numerical taxonomic approach [10] was used for assessing genetic divergence using data on morpho-agronomical traits. An average

taxonomic distance [11] was computed as a measure of dissimilarity. The method for tree building involved sequential agglomerative hierarchical nested (SAHN) clustering based on distance matrices and similarity coefficients. The dendrograms were constructed on the axis of dissimilarity between genotypes and group of genotypes by unweighted pair group method using arithmetic average (UPGMA). Considering forty, fifty and sixty dissimilarity units as cut off point for minimum dissimilarity, the clusters were identified at this phenon level. The standardized data were utilized for the computation of taxonomic distance (d) as follows:

$$djk = \sqrt{\{\Sigma 1/n(X_{ij} - X_{ik})^2\}}$$

Where,

X_{ij} = Mean value of j^{th} entry for the i^{th} character
X_{ik} = Mean value of k^{th} entry for the i^{th} character
n
Σ = Sum over n characters
d = Distance in a phenetic space divided by

2.2 Isozyme Studies

The horizontal starch gel electrophoresis technique was used to study the isozyme polymorphism in different tissues (coleoptiles, seedling and root) of eight inbred lines. Six isozymes viz., peroxidase, esterase, catalase, amylase, acid phosphatase and alcohol dehydrogenase patterns were studied using technique of [12] with discontinuous buffer system as described by [13]. Protocols outlined by [14] with some minor modifications were used for extraction and electrophoretic separation of isozyme. The gel were stained following the procedure prescribed for peroxidase [15], catalase [16], amylase [17], esterase, acid phosphatise and alcohol dehydrogenase [18]. The anodal bands were designated with prefix 'A' and cathode bands with prefix 'C'. A number was also assigned to each band; the closest to the origin is number 1 with more rapidly moving bands being assigned progressively higher numbers.

2.3 Assessment of Divergence

Presence or absence of each band on the gel was scored as '1' or '0' respectively. Similarly between every pair of entries included in the present study was ascertained on the basis of isozyme pattern. The similarity coefficient was estimated as Nei and Li's coefficient [19]. The method used for tree building in the analysis involved sequential agglomerative hierarchical non-overlapping (SHAN) clustering based on similarity coefficients. The dendrograms based on similarity indices were obtained by unweighted pair group method using arithmetic (UPGMA) mean. The level of diversity for isozyme of peroxidase, esterase, acid phosphatase, catalase, amylase and alcohol dehydrogenase by identifying the clusters at appropriate phenon levels.

Nei and Li's coefficient = 2 a /(2a + b + c)

Where,

a = No. of shared bands between j^{th} & k^{th} genotypes
b = No. of bands present in j^{th} genotypes but absent in k^{th} genotypes
c = No. of bands absent in j^{th} genotypes but present in k^{th} genotypes

3. RESULTS AND DISCUSSION

ANOVA revealed significant differences among genotypes for all the traits showing the presence of genetic variability among the genotypes (Table 1). Genetic variability among genotypes of maize was also reported earlier by some workers [20,21,22,23].

3.1 Divergence Analysis Based on Morphological Characters

Mean values of morphological characters were used for evaluating genetic divergence. As shown in dendrogram (Fig. 1 and Table 2) eight inbreds were classified into four clusters (A, B, C, D) at different per cent dissimilarity units as cut off point. The number of clusters obtained in the present study clearly reflected that there was adequate scope for selecting superior and diverse parents for their further exploitation in the breeding programme. Cluster B consisted of two inbreds (CML 144 and CM 300) had the lowest (0.45) dissimilarity coefficient indicating that they were most similar (Table 3). The highest average taxonomic distance (2.3785) was observed between CM 300 and CM 600 followed by CM 300 and CML 186 (2.2706) and CM 600 and CML 144 (2.1058) showing that they were divergent (Table 3). It was found that the inter cluster distance between clusters B and D was

the highest (2.2422), followed by clusters A and C (1.5726), cluster A and D (1.425), clusters C and D (1.325) and between the clusters A and B (1.2283) (Table 4). The highest inter cluster distance between cluster B and D showed greater divergence between inbreds of these two clusters indicating wider differences in their mean values. Similar results indicating appreciable extent of divergence were also reported by earlier workers [3,24]. The least distance was observed between clusters B and C (1.0401) indicating the genetic closeness between the inbreds of these two clusters. The intra cluster distances were found to be smaller than the inter-cluster distances revealed the existence of wide genetic diversity among the inbreds of different groups. The inbreds included in the diverse clusters can be decisively used as promising parents in hybridization programmes to obtain high heterotic response and better segregants in maize in accordance with the earlier report [25].

3.2 Divergence Analysis Based on Isozyme Patterns

Inbreds were classified into six groups (A, B, C, D, E, F) by drawing the phenon line at forty similarity units (Fig. 2, Table 5). Similarity coefficient ranged from 0.5957 to 0.8132, indicating the presence of considerable level of diversity among eight inbreds of maize (Table 6). Moderate level of genetic diversity in Indian maize inbreds was also reported by [4]. The lowest similarity coefficient (0.5957) existed between inbreds CM 600 and CML 176 whereas, the highest similarity coefficient (0.8132) existed between inbreds CML 186 and CML 144. The results revealed that the isozyme patterns in the inbred CM 600 was least similar to the isozyme patterns obtained in other inbreds suggested that inbred CM 600 was most divergent amongst the eight inbreds. While, the isozyme patterns in the inbreds CML 144, and CML 186 of cluster D were least dissimilar followed by the isozyme patterns in the inbreds CML 150 and CML 176 of cluster E.

Isozyme polymorphism has also been used earlier to study genetic diversity in maize by several workers [4,6]. Some workers reported that morphological diversity was not related to the isozyme diversity [26,27]. However, several others [28,29] reported that isozyme data supported the deductions made from agronomic data. Confirming the reports of the earlier workers, the results of the present study also indicated a considerable extent of correspondence between the divergence patterns revealed on the basis of analysis of morpho-agronomical data and isozyme patterns based biochemical data.

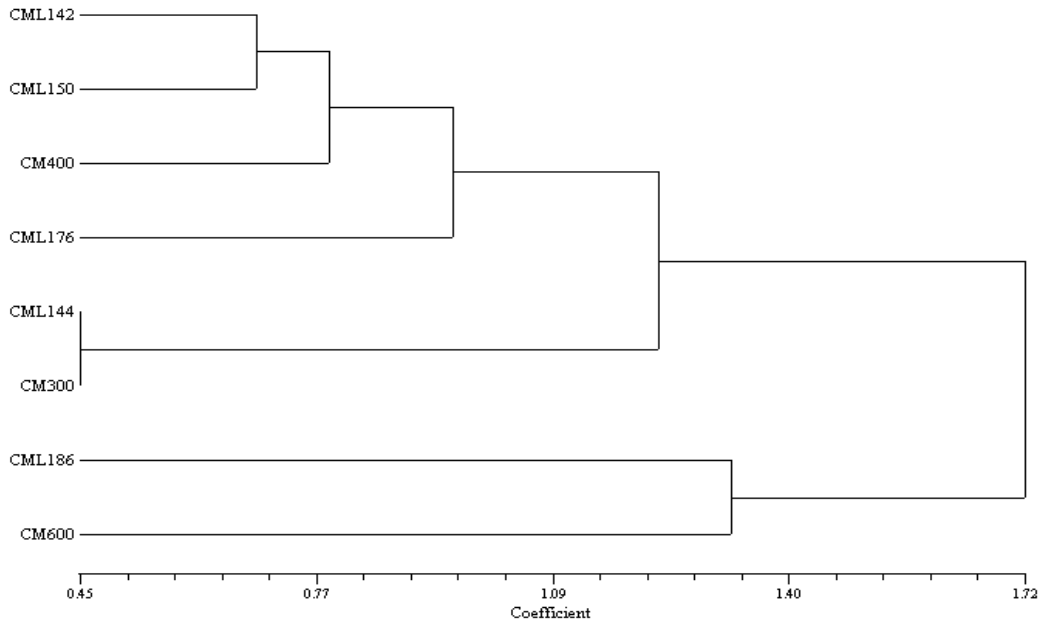

Fig. 1. Dendrogram of maize inbreds based on average taxonomic distance for ten characters

Table 1. Analysis of variance on data obtained for different traits in maize inbreds

Sources of variation	D.F.	Mean sum of Squares									
		Plant height (cm)	Ear length (cm)	Ear girth (cm)	Number of kernel rows per ear	Number of kernels per row	100 Kernels weight (g)	Days to 50% tassel emergence	Days to 50% silk emergence	Days to 50% maturity	Yield per plant (g)
Replications	2	2341.14**	0.01	0.29	0.14	3.72	3.17	0.04	0.16	0.25	0.93
Parents	7	2033.50**	5.04**	3.93**	3.90**	52.94**	10.98*	20.52**	18.48**	47.18**	236.17**

**and ** indicates significant at 5% and 1% respectively*

Table 2. Composition of clusters based on taxonomic distance in average taxonomic approach for cluster analysis using data on ten traits of maize

No. of clusters identified at different phenon level*			Inbreds included in each cluster
60%	50%	40%	
A(4)	A(4)	A(4)	CML 142, CML 150, CM 400, CML 176
B(2)	B(2)	B(2)	CML 144, CM 300
C(1)	C(1)	C(1)	CML 186
D(1)	D(1)	D(1)	CM 600

Figures in parenthesis indicate number of inbreds in the respective clusters.
** Phenon level indicates 60, 50 and 40 units of dissimilarity coefficient*

Table 3. Estimates of average taxonomic distance for ten morphological characters in eight inbreds of maize

	CML142	CML144	CML150	CML176	CML186	CM 300	CM 400	CM 600
CML142	**0.0000**							
CML144	**0.8541**	**0.0000**						
CML150	**0.6892**	0.9832	**0.0000**					
CML176	**0.7983**	1.4799	0.9434	**0.0000**				
CML186	**1.6607**	1.8895	1.4526	1.5950	**0.0000**			
CM 300	**1.1009**	0.4516	1.2481	1.7489	2.2706	**0.0000**		
CM 400	**0.8741**	1.1327	0.6953	1.1115	1.5821	1.2789	**0.0000**	
CM 600	**1.5551**	2.1058	1.5222	1.2321	1.3257	2.3785	1.3906	**0.0000**

Table 4. Average intra-cluster (diagonal) and intra-cluster distance of four clusters of maize

Cluster	A	B	C	D
A	0.8519	1.2283	1.5726	1.425
B		0.4516	1.0401	2.2422
C			0.000	1.3257
D				0.000

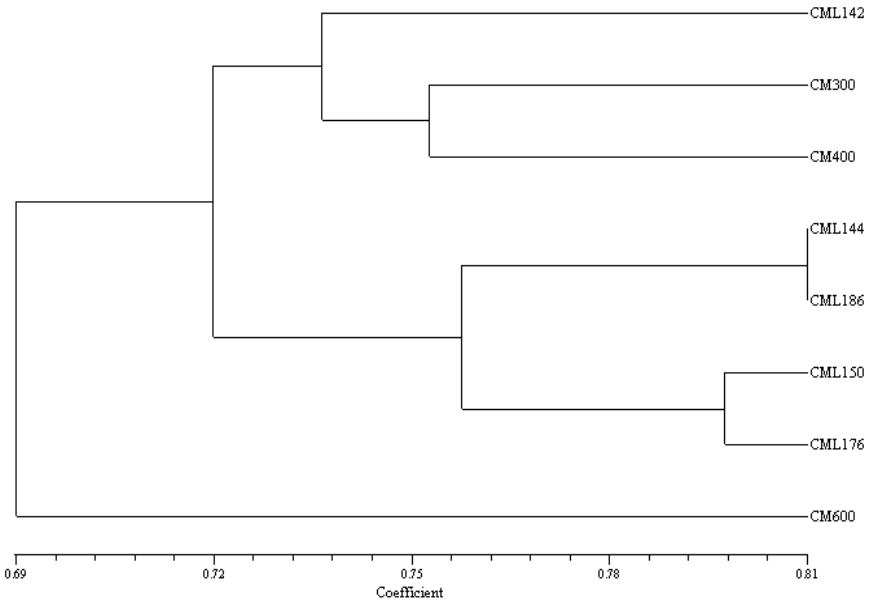

Fig. 2. Dendrogram of maize inbreds based on Nei and Li (1979) similarity coefficient on isozyme systems

Table 5. Composition of clusters based on isozyme patterns in maize

Cluster	No. of entries	Composition of cluster
A	1	CML 142
B	1	CM 300
C	1	CM 400
D	2	CML 144, CML 186
E	2	CML 150, CML 176
F	1	CM 600

Table 6. Nei and Li (1979) similarity coefficient in eight inbreds of maize for six isozyme systems

	CML142	CML144	CML150	CML176	CML186	CM 300	CM 400	CM 600
CML142	1.0000							
CML144	0.7835	1.0000						
CML150	0.7083	0.7957	1.0000					
CML176	0.6869	0.7708	0.8000	1.0000				
CML186	0.6596	0.8132	0.7333	0.7312	1.0000			
CM 300	0.7475	0.7708	0.7789	0.7143	0.7097	1.0000		
CM 400	0.7234	0.8132	0.6889	0.6667	0.6364	0.7527	1.0000	
CM 600	0.6947	0.7391	0.7033	0.5957	0.6517	0.7021	0.7191	1.0000

4. CONCLUSIONS

A relatively less number of isozyme systems were studied in the present study, clustering pattern based on morpho-agronomical data seen to be more effective in objective based discrimination and classification of maize inbred lines. Results of the present study provided the basis to infer that divergence analysis based on morpho-agronomical characters and isozyme pattern may not be completely similar, but somewhat distinct and complementary in nature. Therefore, divergence study using one estimate does not necessarily replace the need to evaluate the relationship on the basis of the other.

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COMPETING INTERESTS

Authors have declared that no competing interest exists.

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