Plant Archives Vol. 24, Special Issue (GABELS), 2024 pp.387-392 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.SP-GABELS.056

GENETIC DIVERSITY STUDIES BASED ON ISOZYME PROFILE IN MAIZE INBREDS (*ZEA MAYS* L.)

Ranju Kumari[1], Jyoti Kumari*, A. K. Singh[2] and Divya Tiwari[3]

[1*]Department of Plant Breeding and Genetics, Nalanda College of Horticulture, Noorsarai, Nalanda, Bihar, India
[2]K.V.K., Saraiya, D.R.P.C.A.U., Bihar, Pusa, Samastipur, Bihar, India
[3]Nalanda College of Horticulture, Noorsarai, Nalanda, Bihar, India
*Corresponding author email: jyotikashu23@gmail.com

ABSTRACT

The present study employed horizontal starch gel electrophoresis to analyze isozyme patterns and assess genetic diversity among eight maize inbred lines: CML 142, CML 144, CML 150, CML 176, CML 186, CM 300, CM 400, and CM 600. Six isozyme systems (peroxidase, esterase, acid phosphatase, catalase, amylase, and alcohol dehydrogenase) were utilized for characterization. The genetic distances among the inbred lines were calculated based on the presence or absence of bands, using Nie and Li coefficients. The resulting dendrogram, generated via the unweighted pair group method with arithmetic mean (UPGMA), classified the inbreds into five groups (A, B, C, D, and E). Notably, CM 600 exhibited the least similarity to the other inbreds, with a similarity coefficient of 0.4615 with CML 186, while the highest coefficient (0.8511) was observed between CML 142 and CML 144. The similarity coefficients ranged from 0.46 to 0.85, indicating moderate levels of diversity among the evaluated inbreds. The study concludes that isozyme pattern-based diversity groups could be effectively utilized in hybridization programs to obtain heterotic hybrids. Moreover, the electrophoresis technique demonstrated speed, precision, and minimal environmental effects, further supporting its suitability for genetic diversity analysis in maize breeding programs.

***Keywords*:** Maize, Inbreds, Genetic diversity, Isozyme

Introduction

Maize is one of the most important crops after rice and wheat. It is used as food and for making industrial products in the form of starch, pharmaceuticals, alcoholic beverages, oil, cosmetics, textiles and fuel like ethyl alcohol. With a high yield potential, it has become a model crop among cereals and is therefore called the queen of cereal crops (Stanley *et al.,* 2020). Now a day local cultivar of maize has been replaced by improved and uniform cultivars with higher yield which created narrow the genetic base of maize. Due to this many challenges have been faced by breeders in identifying superior germplasm sources that will provide inbreds with superior heterosis in single cross combinations. Assessment of germplasm diversity based on traditional methods relying on morphological markers have limitations due to environmental influences and genetic complexities and the unknown genetic control of the traits (Mannetji, 1984; Smith and Smith, 1989). Thus, there is need to study the diversity in maize to successfully use its characteristics. Isozymes have been extensively used to examine the genetic variability in breeding materials since the 1978s (Tanksley and Orton, 1983). Several studies revealed the associations of alleles at isozyme loci with agronomically useful traits and used s for more efficiently improving certain agronomic traits such as grain yield.

To achieve this, isozyme analysis, a biochemical method that assesses genetic diversity more accurately. Isozymes, or multiple forms of enzymes that catalyse the same reaction but have different structures, serve as

genetic markers reflecting the underlying genetic diversity. By studying isozyme patterns of important enzymes like amylase, catalase, esterase, peroxidase, acid phosphatase, and alcohol dehydrogenase, genetic variability among maize cultivars and parental lines can be evaluated more effectively (Mauria *et al*., 2000; Sanchez *et al*., 2000). Each isozyme reflects specific biochemical functions within the plant. For instance, catalase and peroxidase play crucial roles in scavenging reactive oxygen species, protecting the plant from oxidative stress (Anderson *et al*., 1995). Esterase, on the other hand, is involved in hydrolyzing reactions and is valued for its high allele polymorphism, making it a useful marker in genetic studies (Resende *et al*., 2004). Studies utilizing isozyme analysis have provided insights into the genetic diversity of maize populations, evolutionary relationships, and even the genetic purity of seed lots (Doebley, 1990; Lu *et al*., 2002; Salgado *et al*., 2006; Hamill and Brewbaker, 2008). In the context of heterosis breeding, where superior hybrid performance is sought, isozyme analysis helps assess the genetic diversity among inbred lines, aiding in the selection of parental combinations likely to exhibit strong hybrid vigour. Genetic diversity using molecular marker have been studied by many researchers (Kamara *et al*., 2020; Kumar *et al*., 2022 and Stagnati *et al*., 2022). Keeping in view of heterosis breeding in the present study, isozyme analysis offers a rapid, inexpensive, and reliable method for assessing genetic diversity in maize breeding programs, contributing to the development of high-performing hybrids essential for sustaining agricultural productivity.

Materials and Methods

The present experiment conducted to study genetic divergence using isozyme patters among eight maize inbred lines, including five Quality Protein Maize (QPM) and three non-QPM maize lines: CML 142, CML 144, CML 150, CML 176, CML 186 and CM 300, CM 400, CM 600 respectively. The experimental materials were obtained from AICRP, Dholi Centre under Rajendra Agricultural University, Bihar, Pusa. Six isozymes viz., peroxidase (PRX, E.C.1.11.1.7), esterase (EST, E.C.3.1.1.2), catalase (CAT, 1.11.1.6), amylase (AMY, E.C. 3.2.1.1.) acid phosphatase (ACP, E.C 3.1.3.2) and alcohol dehydrogenase (ADH, E.C. 1.1.1.1.) patterns were studied in germinating coleoptile tissue of eight inbred lines. The horizontal starch gel electrophoresis technique of smithies (1955) was used to study the isozyme patterns using discontinuous buffer system as described by Poulik (1957). This system has better resolving power (Brewer and Singh, 1970) which results from gradation of molecular sieving effect of starch gel matrix besides electrophoretic separation. Besides this, isozyme of more than one enzyme can be studied at a time because two slices can be stained separately for different enzymes. Protocols outlined by Shield et al (1983) with some minor modifications were used for extraction and electrophoretic separation of isozyme. Various steps involved in the starch gel electrophoresis were hydrolysis of starch, preparation of buffer system comprising gel buffer, electrode buffer, sodium acetate buffer, phosphate buffer, sodium thiosulphate buffer, preparation of gel, extraction and application of the sample, running of gel, slicing the gel and enzymatic staining. The gel was stained following the procedure prescribed for peroxidase (Veech, 1969), catalase (Thorup, 1961), amylase (Vellejos, 1983), esterase, Acid phosphatise and alcohol dehydrogenase (Tanksley, 1979). The anodal bands were designated with prefix 'A' and cathode bands with prefix 'C'. Symbol 'A' and 'C' are entirely operational as net change of a molecule that varies with the pH. 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. A band having same mobility, in different tissues at a particular stage of development in different genotypes, carries the same number and has been treated as the same isozyme. Relative mobility (Rm) was calculated as the ratio of the distance of the band from the origin to the distance of the dye front. Depending on staining intensity, isozyme band (electromorphs) were characterized into light intensity (+), moderate intensity (++) and dark intensity (+++).

Analysis of isozyme 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 (Nei and Li, 1979). The method used for tree building in the analysis involved sequential agglomerative hierarchical non-overlapping clustering based on similarity coefficients. The dendrograms based on similarity indices were obtained by unweighted pair group method (UPGMA) using arithmetic 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.

$$\text{Nei and Li's coefficient} = 2a/(2a + b + c)$$

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

Results and Discussions

Six isozymes viz., peroxidase, esterase, catalase, amylase, acid phosphatise and alcohol dehydrogenase patterns were studied and used for evaluating the genetic divergence among the inbreds. Total number of bands obtained and band number of each inbred for different isozymes patterns were presented in table 1. Presence or absence of isozyme bands was also used as marker for characterizing the inbreds by earlier workers (Mauria *et al*., 2000; Baishya *et al*., 2003). A total of five anodal bands, PRX-1 (Rm- 0.20), PRX-2 (Rm-0.27), PRX-3 (Rm-0.46), PRX-4 (Rm-0.53) and PRX-5 (Rm- 0.67) and five cathodal bands, PRX -1C (Rm-0.18), PRX-2C (Rm-0.28), PRX-3C (Rm-0.35), PRX-4C (Rm-0.55) and PRX-5C (Rm- 0.65) of peroxidase isozyme were observed. The mobility values (Rm) indicated a wide range of variability in molecular weights for peroxidase bands (Table 1). Thus, it appeared that peroxidase can be considered as useful biochemical marker for the characterization of maize inbreds. The results of the present study was in agreement with the earlier reports (Yang *et al*., 1995; Hamilla and Brewbaker, 2008). Only two esterase bands EST-1 (Rm- 0.67) and EST-2 (Rm-0.73) were present in all the eight inbreds. Observable variation was recorded in respect of mobility indicating a wide range of variation in molecular weight of esterase bands. Thus, it is evident that isozyme analysis provided information regarding the existence of genetic variability among the inbreds under consideration in this study. Similar information was also provided earlier by several researchers (Yang *et al*., 1995; Lu *et al*., 2002; Salgado *et al*., 2006 and Pereira *et al*., 2008).

Altogether six acid phosphatase bands viz., ACP-1, ACP-2, ACP-3, ACP-4, ACP-5 and ACP-6 (Rm-0.37, 0.40, 0.45, 0.53, 0.60 and 0.63) were visualized on gel. In case of alcohol dehydrogenase seven anodal, ADH-1, ADH-2, ADH-3, ADH-4, ADH-5, ADH-6 and ADH-7 (Rm- 0.09, 0.13, 0.16, 0.23, 0.53, 0.67 and 0.73) and five cathodal bands viz., ADH-1C, ADH-2C, ADH-3C, ADH-4C and ADH-5C (Rm- 0.17, 0.20, 0.25, 0.38 and 0.47) were observed. . In addition to differences in the number of bands, observable variations were also recorded in respect of mobility of bands in both enzymes (Table 1). Differences in mobility values indicated a wide range of variability in molecular weight of acid phosphatase and alcohol dehydrogenase isozyme and can be considered as the basis of characterization of maize inbreds (Table 1). Similar observation was also reported earlier (Haider *et al*., 2000; Mauria *et al*., 2000; Zlokolica and Milosevic, 2001; Lu *et al*., 2002; Salgado *et al*. 2006; Cortes *et al*., 2007)

Five amylase bands Amy-1, Amy-2, Amy-3, Amy-4 and Amy-5 (Rm – 0.01, 0.04, 0.1, 0.16 and 0.26) were observed in eight inbreds. In addition to differences in the number of bands, observable variations were also recorded in respect of mobility. Six catalase isozyme bands, Cat-1, Cat-2, Cat-3, Cat-4, Cat-5 and Cat-6 with Rm value 0.22, 0.24, 0.28, 0.30, 0.32 and 0.34 were observed (Table 1). The differential mobility exhibited by amylase and catalase bands gave an indication of their different molecular weight. Variation in mobility and number of bands may be considered as the biochemical marker for characterization of maize inbred lines. This observation is in agreement with the earlier report documented from the studies conducted by several researchers (Yang *et al*., 1995; Lu *et al*., 2002; Salgado *et al*., 2006). Presence of a particular isozyme in one inbred and its absence in other may be due to the fact that structural gene responsible for production of particular isozyme, has mutated resulting in complete loss of its activity in some inbreds. The altered electrophoretic mobility, as revealed by the position of bands, reflects a change in net charge of the protein molecule. This is possible when the substituted amino acid carries a charge that is different from one it replaces. This change may be caused by mutation in structural gene. Another possibility of its presence and absence of bands be due to change in regulatory gene which did not allow the structural gene for the isozyme to be expressed in some inbreds. These findings align with previous research documenting similar observations in maize and emphasize the utility of isozyme analysis as a tool for characterizing genetic diversity among maize inbred lines.

Analysis of genetic divergence

Combined analysis of six isozyme systems was done for assessment of divergence in present studies. Based on the presence or absence of bands, the genetic distance was computed by using the similarity coefficients. Inbreds were classified into five groups, A, B, C, D and E by drawing the Phenon level at forty similarity units and allowing only inbreds with increasing similar pattern of isozymes to be clustered together. Cluster A accommodated three inbreds, namely, CML 142, CML 144 and CML 176 while the

cluster B accommodated two inbreds, namely, CML 150 and CM 300. Cluster C and D was mono genotypic contained only one inbred in each. Inbred CM 400 was accommodated in cluster D while cluster E accommodated inbred CM 600. Isozyme patterns of inbreds CML 142, CML 144 and CML 176 of cluster A were most similar followed by isozyme patterns of inbreds CML 150 and CM 300 of cluster B (Table 4.29). Analysis (Fig. 1 and Table 2) revealed that isozyme patterns in the inbred CM 600 were least similar to the isozyme patterns obtained in other inbreds. Similarity coefficient between CM 600 and CML 186 was the least (0.4615) whereas; the highest similarity coefficient (0.8511) existed between CML 142 and CML 144 (Table 3). Similarity coefficients ranged from 0.46 to 0.85, indicating moderate levels of diversity present among the inbreds under evaluation in the present study (Table 3). Isozyme patterns based genetic diversity studied among landraces/populations and evolution of the maize have also been reported earlier (Mauria *et al*., 2000; Lu *et al*., 2002; Revilla *et al*., 2003).

Table 1: Isozyme banding patterns in maize inbreds

Inbreds	Band number present in inbreds					
	PRX (10)	**EST (2)**	**ACP(6)**	**ADH (12)**	**CAT (6)**	**AMY (5)**
CML 142	1, 2, 3, 4, 5, 1c, 3c, 4c	1, 2	1, 4	3, 4, 5, 6, 1C, 4C	6	2, 3, 4, 5
CML 144	1, 2, 3, 4, 5, 1c, 3c, 4c	1, 2	2, 4	5, 6, 1C, 4C	5	2, 3, 4, 5
CML 150	1, 2, 3, 4, 5, 1c, 3c, 4c	1, 2	3, 5	5, 6, 1C, 4C	4	2, 3, 4
CML 176	1, 2, 3, 4, 5, 1c, 3c, 4c	1, 2	2, 5	4, 5, 7, 2C,	3	2, 3, 4, 5
CML 186	1, 2, 3, 4, 5 .3c	1, 2	2, 5	2, 5, 6, 7, 1C, 5C	2, 3	4, 5
CM300	1, 2, 3, 4, 1C	1, 2	3, 5	1, 5, 6, 7, 1C, 4C	2	1, 3, 4, 5
CM400	1, 2, 3, 4, 1C, 4C, 5C	1, 2	3, 5	2, 5, 7, 3C	1	2, 3, 4, 5
CM600	1, 2, 3, 4, 2C, 3C, 4C	1, 2	3, 6	3, 5, 7, 1C, 4C	1	1, 3

Parenthesis (-) indicate total number of bands present in maize inbreds ; C – Cathodal bands

Table 2: Composition of clusters based on six isozyme patterns in maize

Cluster	No. of entries	Composition of cluster
A	3	CML 142, CML 144, CML 176
B	2	CML 150, CM 300
C	1	CML 186
D	1	CM 400
E	1	CM 600

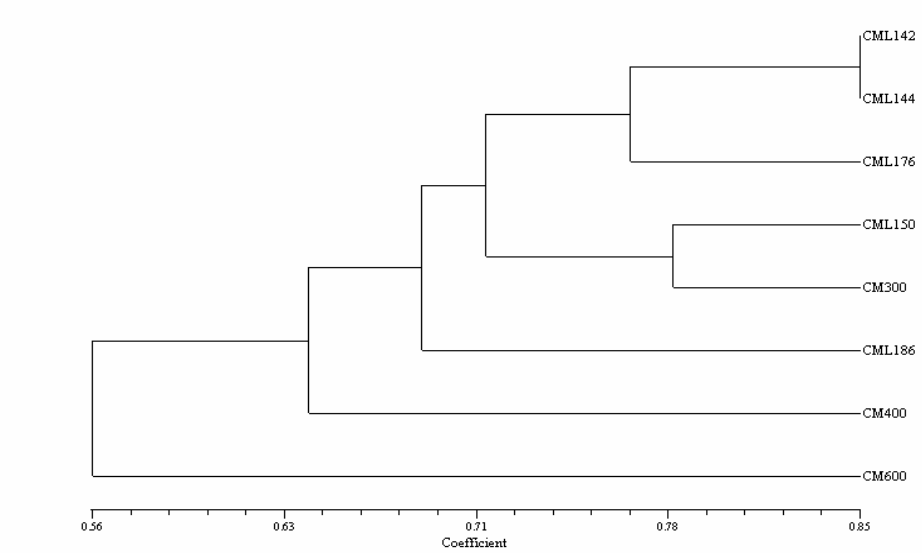

Fig. 1: Dendrogram based on Nei and Li (1979) similarity coefficient for six isozyme system of eight inbreds of maize

Table 3: Nei and Li (1979) similarity coefficient in eight inbreds of maize for six isozyme systems

	CML142	CML 144	CML 150	CML 176	CML 186	CM 300	CM 400	CM 600
CML142	1.0000							
CML 144	0.8511	1.0000						
CML 150	0.7556	0.7727	1.0000					
CML 176	0.7556	0.7727	0.7619	1.0000				
CML 86	0.6512	0.7619	0.7000	0.7000	1.0000			
CM 300	0.6818	0.6512	0.7805	0.6341	0.6154	1.0000		
CM 400	0.6818	0.7442	0.6341	0.6341	0.5128	0.6500	1.0000	
CM 600	0.6364	0.5581	0.6341	0.4878	0.4615	0.6000	0.5500	1.0000

Acknowledgement

The present investigation is part of a Ph.D. research project! Acknowledging the support from the All India Coordinated Project on Maize, Dholi Centre, RAU, Pusa, Bihar, demonstrates gratitude for the resources and assistance provided, which undoubtedly contributed to the success of the research. Collaborative efforts like this are crucial for advancing scientific knowledge and addressing complex challenges in agriculture.

References

Anderson, D., Prasad, K. and Stewart, R. (1995). Changes in isozyme Profiles of catalase, peroxidase and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. *Plant Physiology*, 109: 1247 – 1257

Baishya S., Sachdev A., Johari R.P., Mehta S.L. (2003). Studies on proteins and isozymes in aromatic and non-aromatic rice. *Indian J. of Plant Physiol.,* 8: 323-328.

Cortes, J.O.M., Torres T.C., Victoria D.E., Orozco A.M., Penaloza J.R. and Varela A.S. (2007). Differentiation among maize (*Zea mays* L.) landraces from the Tarasca Mountain Chain, Michoacan, Mexico and the *Chalqueno* complex. *Genetic Resource and Crop Evolution*, 54: 309-325.

Doebley, J.F. (1990). Molecular evidence and the evolution of maize. *Econ. Bot.,* 44: 6-27.

Hamill, D.E. and Brewbaker, J.L. (2008). Isoenzyme polymorphism in flowering plants. IV. The peroxidase isoenyzmes of maize (*Zea mays* L.). *Physiologia Plantarum,* 22(5): 945-958.

Haider, A.S., El-Shanshoury, A.R. and Haider, A. (2000). Variation of storage proteins and isozymes with maize inbred lines. *Biologia Plantaum,* 43(2): 199-203.

Kamara, M.M., Rehan, M., Ibrahim, K.M., Alsohim, A.S., Elsharkawy, M.M., Kheir, A. (2020). Genetic diversity and combining ability of white maize inbred lines under different plant densities. *Plants*, 9: 1140

Kumar, A., Longmei, N., Kumar, P. and Kaushik, P. (2022). Molecular Marker Analysis of Genetic Diversity in Maize: A Review. *OBM Genetics*, 6(1): 150;

Lu, H., Li, J.S., Liu, J.L. and Bernardo, R. (2002). Allozyme polymorphisms of maize populations from South Western China. *Theor. Appl. Genet.,* 104: 119-126.

Mannetji, L. (1984). Consideration on the taxonomy of the genus stylosanthes. (Eds Stac, V.M. and Edye L.A.) Academy Press, Sydney.

Mauria, S., Singh, N.N., Mukherjee, A.K and Bhat, K.V. (2000). Isozyme characterization of Indian maize inbreds. *Euphytica,* 112 (3): 253-259.

Nie, M. and Li, W.H. (1979). Mathematical model for studying genetic variations in terms of restriction endomocleses. *Proc. Natl. Acad. Science,* USA, 76: 5269-5273.

Pereira, L.K., Scapim, C.A., Mangolin, C.A., Fatima, M. de, Machado P. de S., Pacheco, C.A.P. and Mora, F. (2008). Heterozygosity following half sib recurrent

Poulik, M.D. (1957). Starch gel electrophoresis in a discontinuous system of buffer. *Nature*, 180: 1477-1479.

Resende, A.G., Vidigal-Filho, P.S. and Machado, Maria.de Fatima, P.S. (2004). Esterase polymorphism marking cultivars (*Manihot esculenta* Crantz.). *Brazilian Archives of Biology and Technology*, 47(3): 347-353: 347-353

Salgado, K.C.P. de C., Vieira, M.D.G.G.C., Pinho, E.V. de R.V., Guimaraes, C.T., Pinho R.G.V. and Souza, L.V. de. (2006). Genetic purity certificate in seeds of hybrid maize using molecular markers. *Revista Brasileira de Sementes*, 28(1): 169-175

Sanchez, G.J.J., Goodman, M.M., Bird, R.M. and Stuber, C.W. (2006). Isozyme and morphological variation in maize of five Andean countries. *Maydica*, 51 (1): 25-42.

Shields, C.R., Orton, T.J. and Stuber, C.W. (1983). An outline of general resource needs and procedures for electrophoresis separation of active enzymes from plant tissues. *In*: Isozymes in Plant Genetic

Smith, J.S.C. and Smith, O.S. (1989). The description and assessment of distance between inbred lines of maize: II. The utility of morphological, biochemical and genetic descriptors and a scheme for the testing of distinctness between inbred lines. *Maydica*, 34: 151-161.

Smithies, O. (1955). Zone electrophoresis in starch gels: group variation in the serum proteins of normal human adults. *Biochem. J.,* 61: 629-641.

Stagnati, L., Soffritti, G., Martino, M., Lanubile, A., Desiderio, F. and Ravasio, A. (2022). Morphological and genetic characterization of local maize accessions from Emilia Romagna region, Italy. Sustainability. 14: 91.

Stanley, A., Menkir, A., Paterne, A., Ifie, B., Tongoona, P. and Unachukwu, N. (2020). Genetic diversity and population structure of maize inbred lines with varying levels of resistance to striga hermonthica using agronomic trait-based and SNP markers. Plants, 9: 1223.

Tanksley, S.D. (1979). Linkage, chromosomal association and expression of Adh-1 and Pgm-2 in tomato. *Biochem. Genet.,* 17: 1159-1167.

Tasksley, S.D. and Orton, T.J. (1983). Isozymes in plant genetics and breeding. Part IA and IB. Elsevier, Amsterdam

Thorup, O.A., Strole, W.B. and Leavell, B.S. (1961). A method for the localization of catalase on starch gels. *J. Lab. Clin. Med.,* 58: 122-128.

Vellejos, C.E. (1983). Enzyme activity staining. In: Tanksly S.D. and orton T.J. (eds). *Isozymes in Plant Genetics and Plant Breeding*, Part A. Elsevier Science Publishers. BV Amsterdam. pp 469-515.

Veech, J.A. (1969). A localization of peroxidase in infected tobacco susceptible and resistant to blank shark. *Phytopathology*. 59: 566-71.

Yang, T.X., Duan, Z.X., Guo, L.Q., Liu, X.Z. and Si Yang. (1995). Isozyme studies in predicting the potential yield of heterosis in maize. *Acta Botanica Sinica,* 37 (6): 432-436.

Zlokolica, M. and Milosevic, M. (2001). Isozymes as genetic markers in maize breeding. *Biologia Plantarum,* 44 (2): 207-211.