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USE OF PEROXIDASE ISOZYME PATTERNS FOR CHARACTERIZATION OF MAIZE INBREDS (*ZEA MAYS* L.)

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Abstract

The present investigation was carried out to characterization of maize inbred lines by using isozyme as a marker. The horizontal starch gel electrophoresis was used to study the isozyme patterns in eight inbred lines namely, CML 142, CML 144, CML 150, CML 178, CML 186, CM 300, CM 400 and CM 600.. The inbred lines were obtained from AICRP on Maize, Dholi Centre. These inbred lines were characterized by isozymatic pattern of peroxidase. Remarkable extent of polymorphism was visualized in respect of number, mobility and intensity of the bands among the inbreds. Some specific bands were exhibited by a particular inbreds. Morever, this technique was fast, precise and without environmental effects.

Key words : Maize, isozyme, marker, starch gel electrophoresis.

Maize (*Zea mays* L.) in one of the important crop plants in which extensive studies based on isozyme analysis have been conducted and exhaustive information on the method of analysis, genetic control and molecular nature are available (Stuber and Goodman, 1983). Classical identification of cultivars and more so the assessment of germplasm diversity based on standard morphological markers 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 (Mannetji, 1984; Smith and Smith, 1989). Being the manifestation of nearly direct gene products (Proteins) 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. Electrophoretic separation of isozymes has provided a reasonably precise and quantitative approach for the analysis of genetic diversity in many crops including maize (Mauria *et al*., 2000; Baishya *et al*., 2003; Revilla *et al*., 2003).

Depending on the molecular weight of the zwitterions, their mobility in an electrophoretic medium varies. This differential movement is a direct manifestation of genetic make-up responsible for such multiple form of isozymes. Genetically, the production of isozymes of multiple form or molecular weight is accounted to allelic variation of the organisms. Thus, isoenzyme of a particular molecular weight can be considered as direct manifestation of the blue print of the specific gene. 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

Keeping all above into consideration, an attempt was made in the present investigation to characterize the eight inbreds under study on isozyme.

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 inbred lines were characterized by using isozyme pattern of peroxidise. The horizontal starch gel electrophoresis technique was used to study the isozyme polymorphism in germinating coleoptile tissue and 15-20 days old seedling of eight inbred lines. Isozymes, peroxidase (PRX, E.C.1.11.1.7), patterns were studied using technique of smithies (1955) with discontinuous buffer system as described by Poulik (1957). Protocols outlined by Shield et al (1983) with some minor modifications were used for extraction and electrophoretic separation of isozyme. The gel was stained following the procedure prescribed by Veech,

Table-1 : Peroxidase isozyme banding pattern in seedling of maize inbreds.

Genotypes		Anodal					Cathodal				
	Band No.	PRX-1	PRX-2	PRX-3	PRX-4	PRX-1C	PRX-2C	PRX-3C	PRX-4C	PRX-5C	PRX-6C
	Rm Value	0.20	0.46	0.63	0.71	0.18	0.25	0.33	0.42	0.50	0.72
CML 142		+ + +	+ + +	+	+	+ + +	_	_	_	++	++
CML 144		+ + +	+ + +	+	+	+ + +	_	+	_	++	_
CML 150		+ + +	+ + +	+	+	+ + +	_	++	_	++	_
CML 176		+ ++	+ + +	+	+	+ ++	_	++	_	++	+
CML 186		++ +	+ ++	+	+	+++	_	++	++	-	_
CM 300		+ + +	+ + +	+++	+	+++	_	+	_	+	+
CM 400		+ + +	+ + +	+	+	+ ++	_	_	_	++	++
CM 600		+ + +	+ + +	+	+	+++	+ + +	_	_	++	_

+++ Dark intensity, ++ Medium intensity, + Light intensity.

Table-2 : Peroxidase isozyme banding pattern in coleoptile of maize inbreds.

Genotypes		Anodal					Cathodal				
	Band No.	PRX-1	PRX-2	PRX-3	PRX-4	PRX-5	PRX-1C	PRX-2C	PRX-3C	PRX-4C	PRX-5C
	Rm Value	0.2 0	0.27	0.46	0.53	0.67	0.18	0.28	0.35	0.55	0.65
CML 142		+ + +	+ + +	+ +	+ +	+	+ + +	_	+ + +	+ + +	_
CML 144		+ + +	+ + +	+	+	+	+ + +	_	+ + +	+ +	_
CML 150		+ + +	+ + +	+ +	+ +	+	+ + +	_	+ + +	+	_
CML 176		+ +	+ +	+	+	+	+	_	+	+	_
CML 186		++ +	+ ++	+	+	+	_	_	+ +	_	_
CM 300		+ + +	+ + +	+	+	_	+	_	-	_	_
CM 400		+ + +	+ + +	+ +	+ +	_	+ +	_	_	+ + +	+ +
CM 600		+ + +	+ + +	+ + +	+ ++	_	_	+ + +	+ + +	+	_

+++ Dark intensity, ++ Medium intensity, + Light intensity

1969. 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.

RESULTS AND DISCUSSION

Peroxidase isozyme in seedling : The electrophoretic assay for peroxidase activity in 15-20 days old seedling revealed four anodal bands, namely, PRX-1 (Rm-0.20), PRX-2 (Rm-0.46), PRX-3 (Rm-0.63) and PRX-4 (Rm-0.71) and six cathodal bands, namely, PRX-1C (Rm-0.18), PRX-2C (Rm- 0.25), PRX-3C (Rm-0.33), PRX-4C (Rm-0.42), PRX-5C (Rm-0.50) and PRX-6C (Rm-0.72) (Table-1) with intensity varying from light medium to dark. Anodal bands, PRX-1, PRX-2, PRX-3 and PRX-4 were present in all the inbreds with varying intensity. Anodal bands PRX-1 and PRX-2 were present in all the eight inbreds with dark intensity. Whereas, band PRX-3 was observed with light intensity in all the inbreds except inbred CM 300. All inbreds showed light intensity PRX-4 b and Cathodal band PRX-1C was present in all inbreds with dark intensity whereas, band PRX-2C was present only in CM 600 though, with same intensity. In CML-144, CML-150, CML-176, CML 186 and CM 300, band PRX-3C was present with varying intensity from light to medium but absent in inbreds CML-142, CM 400 and CM 600. The imbred CML 186 distinished itself from the rest in that, it exclusively possessed and lacked, the bonds PRX 4C and PRX 5C respectively. Further cathodal band PRX-6C was present in the inbreds, CML-142, CML-176, CM 300 and CM 400, with varying intensities of light to medium.

From a close perusal (Table-1) of the results of peroxidase isozyme analysis in seedling of maize inbreds, it is evident that all the eight inbreds exhibited similarity in respect of presence of five anodal bands and electrophoretic mobility as well as intensity of these five bands, with the exception of inbred CM 300, which had dark intensity for band PRX-4. Among eight inbreds, variation was observed only for cathodal bands. Only three inbreds, namely, CML 144, CML 150 and CML 176, out of eight inbreds exhibited similarity in respect of number, electrophoretic mobility and intensity of cathodal bands. The rest of the inbreds

exhibited variability in respect of presence or absence, mobility and intensity of the cathodal bands.

Peroxidase isozymes in germinating coleoptiles : A total of five anodal bands, namely, 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, namely, 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 (Table-2) were observed. Anodal bands, PRX-1, PRX-2, PRX-3 and PRX-4 were present in all eight inbred lines with intensity varying from light to dark. Variation was observed among the eight inbreds, only for the anodal band PRX-5. This band was present in all QPM lines, with light intensity but absent in the non-QPM lines used in the present study. CM 300, CM-400 and CM 600. Cathodal band PRX-1C was present in all inbred lines with varying intensity from light to dark except in CML 186 and CM 600. Band PRX-2C was present only in inbred CM 600 only with dark intensity. Cathodal band PRX-3C was observed in all except inbreds CM 300 and CM-400. Similarly, band PRX-4C was present in all inbreds except inbreds CML 186 and CM 300. Cathodal band PRX-5C was observed only in the inbred CM 400, with medium intensity.

From a perusal of the data (Table-2) it is evident that as many as five anodal bands and five cathodal bands of peroxidase were visualized among coleoptiles of eight inbreds of maize. In addition to the differences in the number of bands, observable variations were recorded in respect of mobility and intensity. The bands exhibited differential mobility indicating their different molecular weights. The mobility values indicated a wide range of variability in molecular weights for peroxidase bands. Presence or absence of isozyme bands was used as marker for characterizing the inbreds in the studies conducted by earlier workers (Mauria *et al*., 2000; Baishya *et al*., 2003; Revilla *et al*., 2003). Thus, it is evident that isozyme analysis is useful for the characterization of the inbreds under evaluation in this study.

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