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ORIGINAL ARTICLE

Studies on developmental variation of isoperoxidase and protein profile of *Zea mays* L.

Johnson M[1*], Janakiraman N[2], Chalini K[1], Narayani M[1], Kalaiarasi V[1]

[1] *Department of Plant Biology and Plant Biotechnology, St. Xavier's College (Autonomous), Palayamkottai - 627 002, Tamil Nadu, India.*
[2] *Department of Botany, St. Joseph's College (Autonomous), Tiruchirappalli - 620 002, Tamil Nadu, India.*

Tel: +91 97869 24334; *Fax: + 91 462 2561765*

[*] *Author for Correspondence: E-mail: ptcjohnson@gmail.com*

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The present study was aimed to produce the isoperoxidase and protein marker for the maize cultivars from India and to find the isoperoxidase and protein expression on the various developmental stages. The leaf samples of *Zea mays* L. were harvested on 3rd, 7th, 11th, 15th, 19th, 23rd and 27th d for electrophoretic analysis of isoperoxidase and protein. The total protein and isoperoxidase were isolated using the standard procedure described by Sadasivam and Manickam. Isozyme and protein separation was carried out using 10% non-denaturing polyacrylamide gel electrophoresis. The banding pattern suggested several similarities as well as presence of unique isoperoxidase and protein in each stage. These studies will be useful for the identification of the major proteins present at different developmental stages of *Z. mays*.

Key words: Electrophoresis, isoperoxidase, protein, Zea mays.

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Key words: Electrophoresis, isoperoxidase, protein, Zea mays.

Maize (*Zea mays* L.) an agronomically versatile crop, is also known as corn in other countries. It is the third most important cereal in India after rice and wheat. Maize is utilized in India 48% as poultry feed, 28% human food, 12% industrial products, 11% animal feed and 1% seed (Anonymous, 2007). Maize is serving as a basic raw material for the production of starch, oil and protein, alcoholic beverages, food sweeteners and fuel. India ranks eighth in terms of production and shares about 1.85 per cent of the total maize production of the world (Anonymous, 2004). In India the production of maize is 14.13 million tonnes and the total area under this crop is 7.55 million hectares (Anonymous, 2005). World-wide with its high content of carbohydrate, fats, proteins, some of the important vitamins and minerals, maize has acquired a well deserved reputation as a poor man's

nutricereal (Prasanna, 2001). Besides this, it is also used as industrial starches and in pharmaceuticals as dextrose, maltose, ethanol and corn oil. Maize as a crop has multiple uses and can be used as food at its various stages of development and it is not necessary to wait for the crop to reach maturity. Maize is used as food, as feed for livestock and as raw material for industry.

Electrophoresis is generally employed for characterization and comparison of germplasm as well as evaluation of protein expression at different developmental stages of plants (Turi *et al*., 2010; Smila *et al*., 2007). Identification of protein expression at different developmental stages using simple and inexpensive technique such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been shown advantages in plant physiology especially on growth and developmental stages (Smila *et al*., 2007). Since the 1930s, electrophoresis (Isozymes and SDS-PAGE) in conjunction with the zymogram technique has been used as a tool for the study of heritable and developmental variation because of their relative efficiency and cost effectiveness (Siva and Krishnamurthy, 2005; Johnson, 2007; Smila *et al*., 2007; Johnson *et al*., 2010).

Punita Guria (2006) studied the physico-chemical properties and nutritional quality of *Zea mays*. Isozyme variation studies on Mexican maize (*Zea mays* L. ssp. mays) and the teosintes (*Zea* spp.) the close wild relatives of maize was carried out previously (Wilkes, 1967; Doebley *et al.*, 1984 and 1985). But there is no report on isoperoxidase and protein variation on the various developmental stages of maize from India. Therefore, the present study was aimed to produce the isoperoxidase and protein marker for the maize cultivars from India and to find the isoperoxidase and protein expression on the various developmental stages.

MATERIALS AND METHODS

The young and disease free leaves of *Zea mays* L. were taken for the isoperoxidase and protein separation studies. For electrophoretic analysis of isoperoxidase and protein, the leaf samples of *Z. mays* were harvested on 3rd, 7th, 11th, 15th, 19th, 23rd and 27th d. The isoperoxidase and protein were extracted from leaf tissues at different developmental stages of the maize. 1 g of leaf tissue was taken and homogenized with 3.5 mL of cold homogenizing tris buffer (pH 7.0) in a pre-chilled pestle and mortar for protein. For isoperoxidase, 0.1 M sodium phosphate buffer (pH 7.0) containing 1% polyvinyl pyrrolidone (PVP) were used. The homogenate was centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge at 4°C. The supernatent of protein and isoperoxidase extract was used as the enzyme and protein source.

Isozyme and protein separation were carried out using 10% non-denaturing polyacrylamide gel electrophoresis (Sathasivam and Manickam, 1992). After electrophoresis, for peroxidase, the gel was stained in a solution containing 100 mg benzidine, 4.5 mL acetic acid and 200 µL hydrogen peroxide. The reaction is terminated at the correct time using 7% acetic acid solution. Each band of peroxidase was named by capital letter PRX followed by a number for each isozyme. For protein, the gel was stained with Coomassie brilliant blue. The gels were fixed with 7% acetic acid solution for 30 min; the gels were washed with distilled water and photographed using the gel documentation system, Vilber Lourmat. The Rf value was calculated for the different developmental stages of maize. The zymogram was constructed based on the Rf values of isoperoxidase and proteins.

RESULTS

Isoperoxidase and protein profile for the different developmental stages of 3rd, 7th, 11th, 15th, 19th, 23rd and 27th d of *Zea mays* were observed and the banding patterns were compared. A comparison of isoperoxidase and protein profiles of different developmental stages of maize seedlings suggested several similarities as well as presence of unique isoperoxidase and protein in each stage.

Isoperoxidase

On 3rd d, the isoperoxidase of maize leaves showed seven bands and their Rf values ranged from 0.03 to 0.64 (Fig. 1A; Table 1). On 7th d, the positions of the band were changed and the number of bands also reduced to six and five new bands were observed, viz. MW-Rf 0.06, 0.14, 0.38, 0.46 and 0.54. On 11th d, the number of bands was further reduced to three but the positions of the bands were changed with the Rf values 0.12, 0.43 and 0.51. On 15th d, the number of bands (three) remains same. On 19th d, the number of bands gets increased to four and two new bands were formed with the Rf values 0.08 and 0.19. On 23rd d, the numbers of bands (four) maintained same and no new bands were observed. On 27th d, the number of bands was further increased to five and two new bands were formed (0.04 and 0.50).

In the isoperoxidase system, the MW-Rf 0.03 and 0.32 were present only in 3 d old leaves of *Z. mays*. The MW-Rf. 0.06, 0.14, 0.46 and 0.54 were showed their presence only in 7 d old leaves. The MW-Rf. 0.08 showed its uniqueness only in the 19 d leaves of *Z. mays.* The MW-Rf. 0.04 and 0.50 were present only in 27 d old leaves of *Z. mays*. The 11 d, 15 d and 23 d old leaves were failed to express their identity in the isoperoxidase system. The isoperoxidase MW-Rf. 0.09 was commonly present in 3, 23 and 27 d old leaves of *Z. mays.* The isoperoxidase MW-Rf. 0.12 and 0.43 were shared by 11, 19 and 23 d old leaves of *Z. mays*. Similar to that, the MW-Rf. 0.17 were showed its presence in 3 and 23 d; the MW-Rf. 0.19 in 19 and 27 d; the MW-Rf. 0.38 in 7 and 15 d; the MW-Rf. 0.41 in 3 and 27; 0.51 in 11 and 15 d and 0.58 in 3 and 15 d old leaves of *Z. mays*.

Protein

The protein of maize leaves showed eight bands on 3rd d and their Rf values ranged from 0.03 to 0.74 (Fig. 1B; Table 2). On 7th d, the number of bands increased to nine and eight new bands were observed with the Rf value 0.20, 0.36, 0.50, 0.61, 0.69, 0.72, 0.87 and 0.94. The number of bands (nine) remained same on the 11th d and four new bands were showed viz. MW-Rf 0.38, 0.45, 0.96 and 0.98. On 15th d, the number of bands was reduced to seven and two new bands (MW-Rf 0.23 and 0.52) were occurred. On 19th d, the number of bands gets increased to ten and five new bands were obtained with the Rf value 0.01, 0.34, 0.40, 0.56 and 0.67. The number of bands gets increased to thirteen on 23rd d and seven new bands were observed viz. MW-Rf 0.12, 0.21, 0.30, 0.49, 0.70, 0.83 and 0.92. On 27th d, the number of bands (thirteen) remained same two new bands were obtained with the Rf value 0.54 and 0.80.

In the protein system, the MW-Rf. 0.18 was present only in the 3 d old leaves of *Z. mays.* The MW-Rf. 0.69 and 0.94 were showed their expression only in 7 d old leaves of *Z. mays*. The MW-Rf. 0.98 showed their unique presence only in 11 d old leaves of *Z. mays*. The MW-Rf. 0.01 and 0.40 was present only in 19 d old leaves of *Z. mays* respectively. The MW-Rf. 0.12, 0.21, 0.49, 0.70, 0.83 and 0.92 were present only 23 d old leaves of *Z. mays*. The MW-Rf. 0.54 and 0.80 were showed their presence only in 27 d old leaves of *Z. mays*.

The 15 d old leaves were failed to express their uniqueness in the protein system. The protein MW-Rf. 0.03 was commonly present in 3, 7 and 23 d old leaves of *Z. mays*. The MW-Rf. 0.09, 0.41 and 0.58 were shared by 3 and 27 d old leaves of *Z. mays*. Similar to that, the MW-Rf. 0.20 were showed its presence in 7, 11, 19, 27 d; the MW-Rf. 0.23 in 15 and 19 d; the MW-Rf. 0.30 in 23 and 27 d; the MW-Rf. 0.32 in 3 and 11 d; the MW-Rf. 0.34 and 0.67 were shared by 19 and 27d; the MW-Rf. 0.36 in 7 and 23 d; the MW-Rf. 0.38 and 0.96 were shared by 11 and 15 d; the MW-Rf. 0.45 in 11 and 27 d; the MW-Rf. 0.50 in 7 and 19 d; the MW-Rf. 0.52 in 15 and 23 d; the MW-Rf. 0.56 in 19 and 23; the MW-Rf. 0.61 in 7, 11, 15, 19, 23 and 27 d; the MW-Rf. 0.65 in 3 and 23 d; the MW-Rf. 0.72 in 7, 11 and 19 d; the MW-Rf. 0.74 in 3 and 15 d; the MW-Rf. 0.87 in 7, 11, 15, 23 and 27 d respectively.

Table 1: MW- Rf values and Peroxidase banding profile of maize

MW-RF	Band Positions	Age of the seedlings in Days						
		3	7	11	15	19	23	27
0.03	PRX 1^1	+	-	-	-	-	-	-
0.04	PRX 1^2	-	-	-	-	-	-	+
0.06	PRX 1^3	-	+	-	-	-	-	-
0.08	PRX 1^4	-	-	-	-	+	-	-
0.09	PRX 1^5	+	-	-	-	-	+	+
0.12	PRX 2^1	-	-	+	-	+	+	-
0.14	PRX 2^2	-	+	-	-	-	-	-
0.17	PRX 2^3	+	-	-	-	-	+	-
0.19	PRX 2^4	-	-	-	-	+	-	+
0.29	PRX 3^1	-	+	-	-	-	-	-
0.32	PRX 4^1	+	-	-	-	-	-	-
0.38	PRX 4^2	-	+	-	+	-	-	-
0.41	PRX 5^1	+	-	-	-	-	-	+
0.43	PRX 5^2	-	-	+	-	+	+	-
0.46	PRX 5^3	-	+	-	-	-	-	-
0.50	PRX 5^4	-	-	-	-	-	-	+
0.51	PRX 6^1	-	-	+	+	-	-	-
0.54	PRX 6^2	-	+	-	-	-	-	-
0.58	PRX 6^3	+	-	-	+	-	-	-
0.64	PRX 7^1	+	+	-	-	-	-	-

Table 2: MW- Rf values and protein banding profile of maize

MW-RF	Band Positions	Age of the seedlings in Days						
		3	7	11	15	19	23	27
0.01	PP 1^1	-	-	-	-	+	-	-
0.03	PP 1^2	+	+	-	-	-	+	-
0.09	PP 1^3	+	-	-	-	-	-	+
0.12	PP 2^1	-	-	-	-	-	+	-
0.18	PP 2^2	+	-	-	-	-	-	-
0.20	PP 2^1	-	+	+	-	+	-	+
0.21	PP 3^1	-	-	-	-	-	+	-
0.23	PP 3^2	-	-	-	+	+	-	-
0.30	PP 3^3	-	-	-	-	-	+	+
0.32	PP 4^1	+	-	+	-	-	-	-
0.34	PP 4^2	-	-	-	-	+	-	+
0.36	PP 4^3	-	+	-	-	-	+	-
0.38	PP 4^4	-	-	+	+	-	-	-
0.40	PP 4^5	-	-	-	-	+	-	-
0.41	PP 5^1	+	-	-	-	-	-	+
0.45	PP 5^2	-	-	+	-	-	-	+
0.49	PP 5^3	-	-	-	-	-	+	-
0.50	PP 5^4	-	+	-	-	+	-	+
0.52	PP 6^1	-	-	-	+	-	+	-
0.54	PP 6^2	-	-	-	-	-	-	+
0.56	PP 6^3	-	-	-	-	+	+	-
0.58	PP 6^4	+	-	-	-	-	-	+
0.61	PP 7^1	-	+	+	+	+	+	+
0.65	PP 7^2	+	-	-	-	-	+	-
0.67	PP 7^3	-	-	-	-	+	-	+
0.69	PP 7^4	-	+	-	-	-	-	-
0.70	PP 7^5	-	-	-	-	-	+	-
0.72	PP 8^1	-	+	+	-	+	-	-
0.74	PP 8^2	+	-	-	+	-	-	-
0.80	PP 8^3	-	-	-	-	-	-	+
0.83	PP 9^1	-	-	-	-	-	+	-
0.87	PP 9^2	-	+	+	+	-	+	+
0.92	PP 10^1	-	-	-	-	-	+	-
0.94	PP 10^2	-	+	-	-	-	-	-
0.96	PP 10^3	-	-	+	+	-	-	-
0.98	PP 10^4	-	-	+	-	-	-	-

DISCUSSION

The morphological variation, a product of genotype and the environment is an important parameter, but much diversity which remains unexpressed morphologically can be revealed by biochemical methods (Smila *et al.,* 2007). Growth of any organ is associated with an additional synthesis of proteins which are building blocks of protoplasm and are again the resultant on inter-mediatory metabolism. Study of biochemical / protein (total protein and isozymes) variation are important and powerful procedure that has often been employed for this purpose. The general pattern of appearance and disappearance of bands can be explained on the

basis of gradual shifts of isozyme patterns in samples taken in the course of development due to differential activation of genes involved in synthesis of these enzymes at different stages of development. Scandalios (1969) has listed 46 isozyme systems, in which the pattern of gene expression varies with the developmental condition. Similar to the previous observation, in the present study also we observed different isoform and protein expression varies with the developmental stages (3-27 d). The changing pattern of isozymes and proteins during development may be interpreted as evidence for differential timing of gene expression correlated with the physiological changes (Johnson *et al.,* 1973; Rao *et al*., 1992). Mehta and Ali (1996) and Smila *et al*., (2007) observed the polymorphism and genetic expression at different developmental stages in *Lens culinaris* and *Pennisetum glaucum* using the isoenzymes. Similarly in the present study, polymorphism was observed in the different developmental stages of maize using protein and isoperoxidase. Rao *et al*., (1992) and Smila *et al*., (2007) observed zymogram variation at the developmental stages of pearl millet and *P. glaucum*. A similar observation was also reported in the present study. The presence of common banding profile suggests that protein shares similar functional properties. Expression of common protein banding profiles in different parts of seedling has been shown in different varieties of *Brassica juncea* (Sharma and Deswal, 2004; Deswal *et al.,* 2004; Gasic and Korban, 2005). The results of the present study suggest that the stable expression of several proteins and isoperoxidase as well as up and down regulation of several genes coding for different polypeptides in *Z. mays* upon its gradual development. Indeed, the results on electrophoretic characterization are the first report on the comparison of protein and isoperoxidase profile of *Z. mays* at its different growth stages to the best of our knowledge. These findings can be further explored for precise identification of the major proteins present at different developmental stages of *Z. mays* with 2D-gel electrophoresis and various molecular marker techniques.

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