J. Bangladesh Agril. Univ. 4(1): 61–67, 2006 ISSN 1810-3030

Characterization of some mango germplasm using peroxidase isoenzyme

M.S. Biswas, M.G. Rabbani, E.H.M.S. Rahaman and E.J. Garvey[1]
Department of Horticulture, Bangladesh Agricultural University, Mymensingh

Abstract

An experiment was conducted to characterize 62 mango germplasm using peroxidase isoenzyme. The results of the experiment revealed a wide range of diversity among the germplasm based on their peroxidase isoenzyme banding patterns. In respect of isoenzyme activity, nine bands were found at different Rf values located in three loci. Band number one and two were common in all the germplasm. Third locus is the most useful isoenzyme locus, which denotes allelic polymorphism in the germplasm MI 11 MI 48 MI 52 MI 69 but other loci do not denote allelic polymorphism.

Keywords: Mango, Germplasm, Isoezyme and Polymorphism

Introduction

Mango (*Mangifera indica* L.), a predominant fruit crop under cultivation from centuries, is the most important species of Anacardiaceae. Though the center of diversity of *Mangifera* is located in South-East Asia, majority of mango cultivars are reported in India. According to Mukharjee (1997), the total number of valid species of the genus *Mangifera* stands at 39. In Bangladesh there are many genotypes of mango having diverse characters. These genotypes are available in the market under different local names without any uniformity and standardization in nomenclature. Morphological markers have certain limitations such as limited availability of easily scorable markers, difficulty in scoring homozygous from heterozygous individuals, influence of environment in equating phenotypes with genotypes. On the other hand, molecular markers have many advantages such as abundance in polymorphism, no pleiotrophic effect; less affected by environment and subjected to rapid detection Singh *et al.* (2001). Isoenzymes are widely used as molecular marker for characterization and identification of plant germplasm. Isoenzymes have been used for characterization of mango germplasm in India and other parts of the world. However, no reports are available regarding the characterization of mango germplasm using peroxidase isoenzyme in Bangladesh. The present experiment was, therefore, undertaken to characterize 62 mango germplasm collected from different parts of Bangladesh using peroxidase isoenzyme.

Materials and Methods

For isoenzyme analysis, one-month old leaf samples of the 62 mango germplasm were collected and kept under ice before use. One gram of mango leaf for each accession was weighed and ground with 0.3 ml extraction buffer. Extraction buffer consisted of 0.24% Tris (0.24 %) and 5% sucrose. Sample were centrifuged at 14,000 rpm for 15 min after finely grinding in mortar and pestle. The samples were then divided into two parts and 25µl was used immediately and rest was kept at -5^0C for periods of up to 1 week. Vertical electrophoresis unit was used to run the gel. Gels were prepared from stock solution of acrylamide (29.2 g), Bis (0.8 g), Tris (18.17 g) and ammonium per sulphate (0.1 g) by weight and was dissolved in water and pH was adjusted to 8.8 and finally the volume was made up to100 ml by adding distilled water. Electrode buffer was also used by dissolving in Tris (1.2 g)

and 5.8 g glycine in about 150 ml of distilled water, and the volume was made to 200 ml (diluted 10 times when used). Bromophenol blue (BPB) (0.1%) was dissolved in 80 ml distilled water, and the volume was made to 100 ml. The electrophoresis of the proteins of leaf samples was carried out using PAGE technique and the gels were stained for peroxidase isoenzyme. Electrophoresis was performed at 20 mA constant current per gel for 3.5 to 4 hours. The electrophoresis was stopped when tracking marker stain reached the bottom of the gel. For staining the gel, 10 ml of POD-1 (500 ml of acetone + 1.05 g of 3-Amino-9-ethylcarbazole and + 0.725 g of β-napthol) solution was added to 40 ml of B-POD (1.51 g of Tris buffer + 1.62 ml of glacial acetic acid and quantify the volume by distilled water up to 1000 ml) solution and mixed gently. The mixture was then allowed for filtration. Twenty µl of H_2O_2 was added to the filtrated solution. The gel was then incubated in the staining solution at room temperature in the dark with continuous shaking. Red bands appeared after 2-5 minutes. Gels were scored for band migration as compared to that of the tracking dye using the follow formula: Rf (relative frequency) = $\frac{X}{Y}$, X = the distance from the origin to the leading edge of the isoenzyme band and Y = the distance travelled by the tracking dye.

Results and Discussion

Based on the Rf values of peroxidase band generated in 62 mango germplasm, a zymogram was obtained (Table 1). The 62 germplasm were classified on the basis of their zymogram patterns with the Rf values. There are 9 bands of peroxidase falling into 12 zymotype classes *viz.* P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, P11 and P12 (Table 1). Band 1P lies between the Rf values 0.10 to 0.25, band 2P lies between the Rf values 0.25 to 0.40, band 3P lies between the Rf values 0.40 to 0.50, band 4P lies between the Rf values 0.50 to 0.60, band 5P lies between the Rf values 0.60 to 0.70, band 6P lies between the Rf values 0.70 to 0.72, band 7P lies between the Rf values 0.72 to 0.74, band 8P lies between the Rf values 0.74 to 0.76 and band 9P lies between the Rf values 0.76 to 0.79. Eiadthon *et al.* (1998) found polymorphism in different enzyme system examined (Isocitrate dehydrogenase, IDH and phosphoglucose isomerase, PGI) and intracultivar variation of the same cultivar collected at different locations was confirmed in banding patterns. Twelve electrophoretic zymotypes (P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, P11 and P12) were observed in peroxidase enzyme system formed by 9 bands at different Rf values varying from 0.01 to 0.79 (Plate 1 to 7). Zymotypes P2 was present in maximum germplasm (25) followed by zymotypes P1, P3, P4, P5, P6, P7, P8, P9, P10,. P11 and P12 had 6, 4, 5, 4, 5, 3, 2, 3, 2, 2 and 1 accession, respectively. Zymotype P2 was the most frequent which included 40.32% of the total germplasm followed by zymotypes P1, P3, P4, P5, P6, P7, P8, P9, P10, P11 and P12 included 9.67%, 6.45%, 8.06%, 6.45%, 8.06%, 4.84%, 3.23%, 4.84%, 3.23%, 3.23% and 1.61%, respectively. From the distribution of peroxidase bands among the zymotypes of 62 mango germplasm, it was observed that band 1 and 2 were present in all germplasm. It was observed that band 6 showed a frequency of 17.54% (Table 2) whereas the lowest band frequency (1.75%) was observed for band 9. The lowest band frequency was 1.75% at Rf value 0.76 to 0.79. Thus the germplasm had wide range of genetic variation. According to Yadav (1997), isoenzymes analyses of 200 polyembryonic mango varieties revealed that there were 34 alleles which showed monomorphism while 19 showed polymorphism. Decha (2000) studied isoenzyme characteristics of mango using PAGE technique. Enzyme from 7 month-old leaves of mango was extracted using tris-buffer (0.1 M., pH 8.2). They found that the three isoenzyme systems viz. acid phosphatase, esterase and peroxidase separately could identify 52 clones into 10, 4 and 15 groups, respectively. Using the combination of 3 isoenzyme systems, the 52 clones could be grouped into 20 clones and other 9 groups. Electrophoretic separation of isoenzymes has been widely used, both in taxonomic and genetic studies of different crops (Shannon, 1968).

Table 1. Zymogram patterns and observed number of electrophoretic zymotypes of peroxidase isoenzyme in 62 mango germplasm

Zymogram type	Description	Germplasm sharing the zymogram	Total number of germplasm	Percentage (%)
P1	Band 1,2,3,6,7 present and band 4,5,8,9 absent	MI 01 MI 16 MI 17 MI 18 MI 21 MI 61	6	9.67
P2	Band 1,2,6, present and band 3,4,5,7,8,9 absent	MI 02 MI 03 MI 04 MI 05 MI 08 MI 12 MI 13 MI 15 MI 24 MI 26 MI 29 MI 30 MI 31 MI 34 MI 42 MI 43 MI 55 MI 62 MI 66 MI 72 MI 73 MI 74 MI 75 MI 76 MI 77	25	40.32
P3	Band 1,2,3,6 present and band 4,5,7,8,9 absent	MI 09 MI 10 MI 40 MI 63	4	6.45
P4	Band 1,2,5,6 present and band 3,4,7,8,9 absent	MI 19 MI 35 MI 36 MI 37 MI 51	5	8.06
P5	Band 1,2,8 present and band 3,4,5,7,9 absent	MI 47 MI 49 MI 50 MI 71	4	6.45
P6	Band 1,2,6,7 present and band 3,4,5,6,7,8, 9 absent	MI 11 MI 27 MI 58 MI 64 MI 70	5	8.06
P7	Band 1,2 present and band 3,4,5,6,7, 8, 9 absent	MI 33 MI 38 MI 46	3	4.84
P8	Band 1,2,3,4,5, 6 present and band 7, 8, 9 absent	MI 57 MI 69	2	3.23
P9	Band 1,2,3,4,5,6, 7, 8 , 9 present	MI 07 MI 41 MI 65	3	4.84
P10	Band 1,2,3,5, 6 present and band 4, 7 absent	MI 48 MI 52	2	3.23
P11	Band 1,2,3,5, 6 present and band 4, 7, 8, 9 absent	MI 28 MI 39	2	3.23
P12	Band 1,2,3,4, 5,6, 7 present and band 8, 9 absent	MI 14	1	1.61

Table 2. Distribution of the peroxidase bands among zymotypes of in mango

Zymotype	Relative frequency of bands									Number of bands
	0.10-0.25 (1P)	0.25-0.40 (2P)	0.40-0.50 (3P)	0.50-0.60 (4P)	0.60-0.70 (5P)	0.70-0.72 (6P)	0.72-0.74 (7P)	0.74-0.76 (8P)	0.76-0.79 (9P)	
P1	√	√	√			√	√			5
P2	√	√				√				3
P3	√	√	√			√				4
P4	√	√			√	√				4
P5	√	√						√		3
P6	√	√				√	√			4
P7	√	√								2
P8	√	√	√	√	√	√				6
P9	√	√		√	√	√				5
P10	√	√	√	√	√	√	√	√	√	9
P11	√	√	√		√	√				5
P12	√	√	√	√	√	√	√			7
Total	12	12	6	4	6	10	4	2	1	57
Band frequency	21.05	21.05	10.5.	7.02	10.53	17.54	7.02	3.51	1.75	

A dendrogram representing mango germplasm was generated based on Euclidean distance (Fig. 1). Based on the polymorphic peroxidase enzyme activities, the germplasm were grouped into six major clusters designated as I, II, III, IV, V and VI. Thirty-two germplasm (Fig. 1) are found under the cluster I, which represented 51.62% of the total germplasm. Cluster IV, VI, III and II contained four, seven, eight and nine germplasm, respectively. The lowest number of germplasm (2) was found in the cluster V. The germplasm collected from the same location are grouped into different clusters. Degani *et al.* (1990) characterized 41 mango cultivars derived from self-pollinated and cross-pollinated trees using isoenzymes. They identified 6 loci with 17 allelomorphs and determine the outcross origin of some mango cultivars. Degani *et al.* (1992) also demonstrated that there were two distinct zones of PGI activity in mango (PGI 1 and PGI 2) and suggested that four alleles control the PGI 2 banding. Truscott *et al.* (1993) also characterized 88 mango cultivars using 5 isoenzymes and reported out cross origin of some of the cultivars. Torres *et al.* (1989) illustrated the use of isoenzymes for the genetic study of tree fruits were apples, pears, peaches, mulberries, figs, olives, Citrus, avocados, date palms, mangoes, cherimoyas (*Annona cherimola*).

The results of the present experiment indicate that wide variation exists among the germplasm. Also through hierarchical cluster analysis 62 mango germplasm were grouped into 6 clusters and relationship among the clusters was established which will be useful for planning future breeding programme of mango. However, in the present experiment, only peroxidase isoenzyme system was used. More isoenzyme systems as well as other molecular techniques are, therefore needed for proper characterization of mango germplasm available in the country.

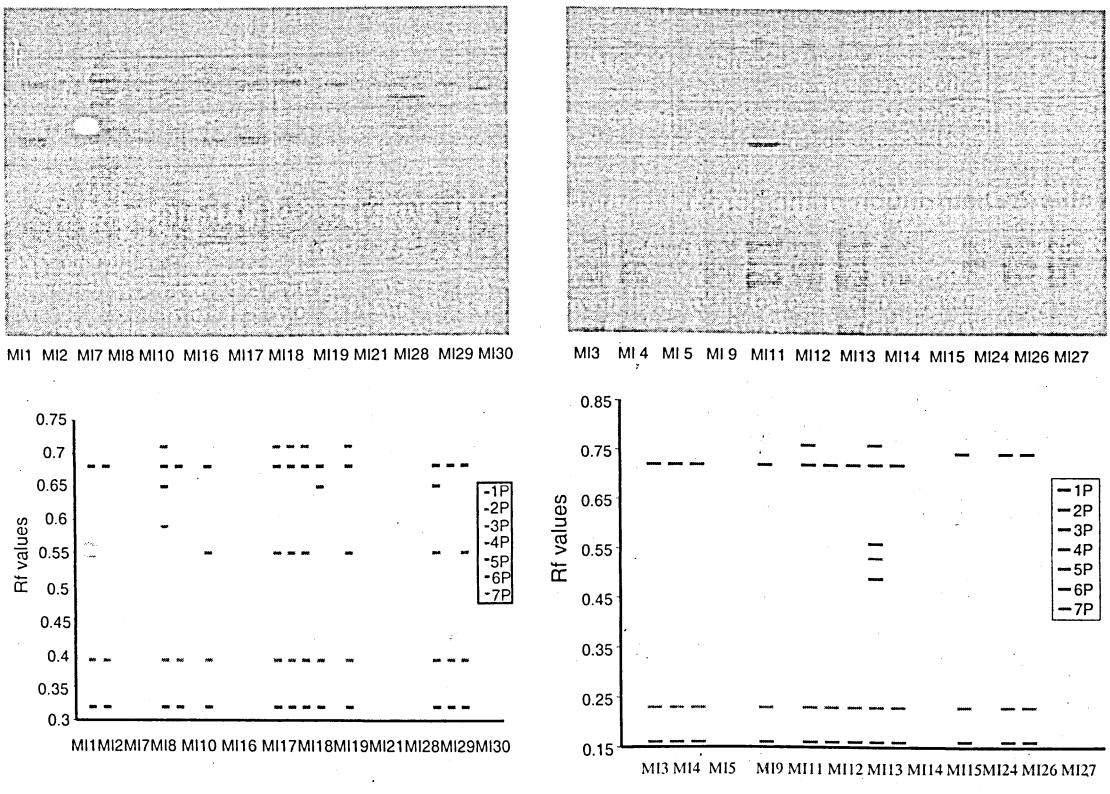
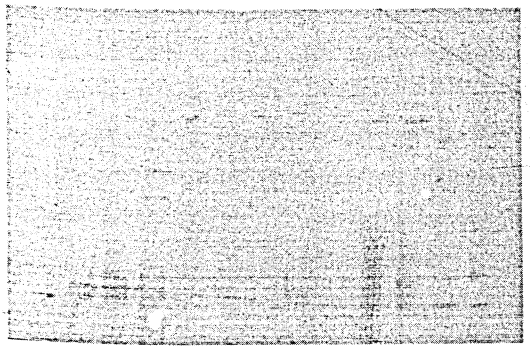
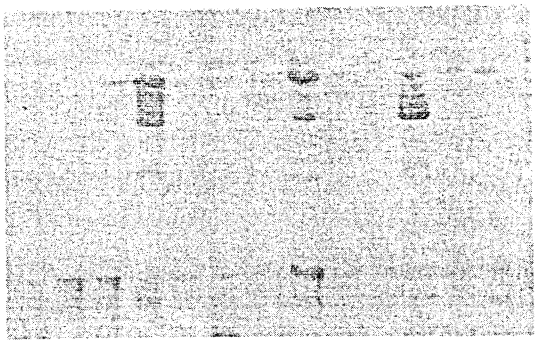
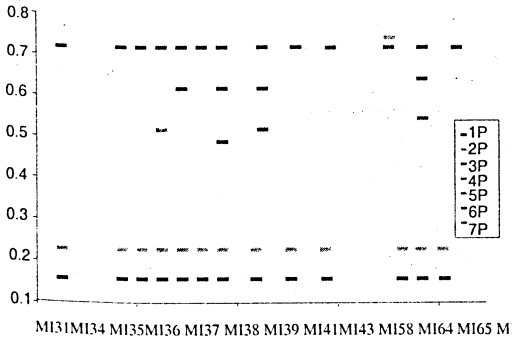
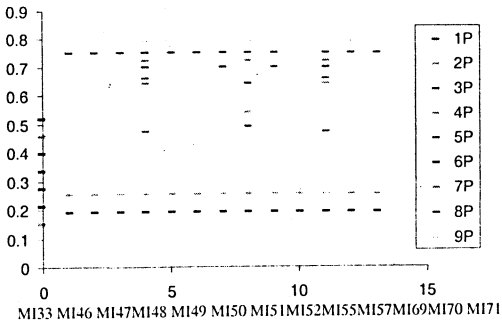


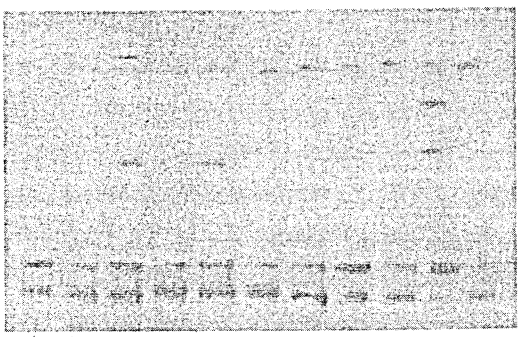
Fig. 1 Peroxidase banding pattern and zymograms of different mango germplasm











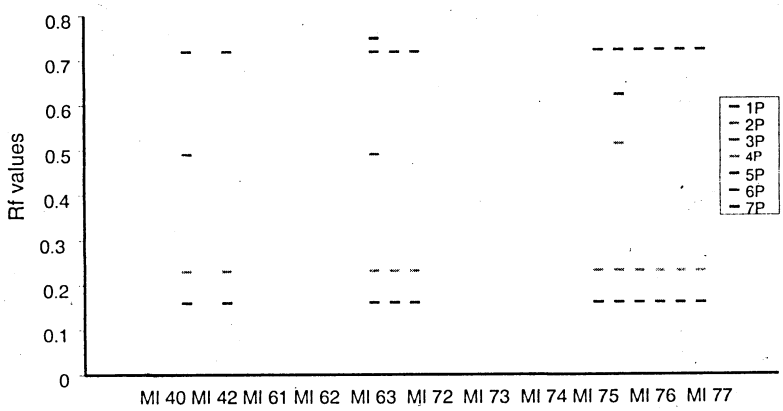


Fig. 1 (contd.) Peroxidase banding pattern and zymograms of different mango germplasm

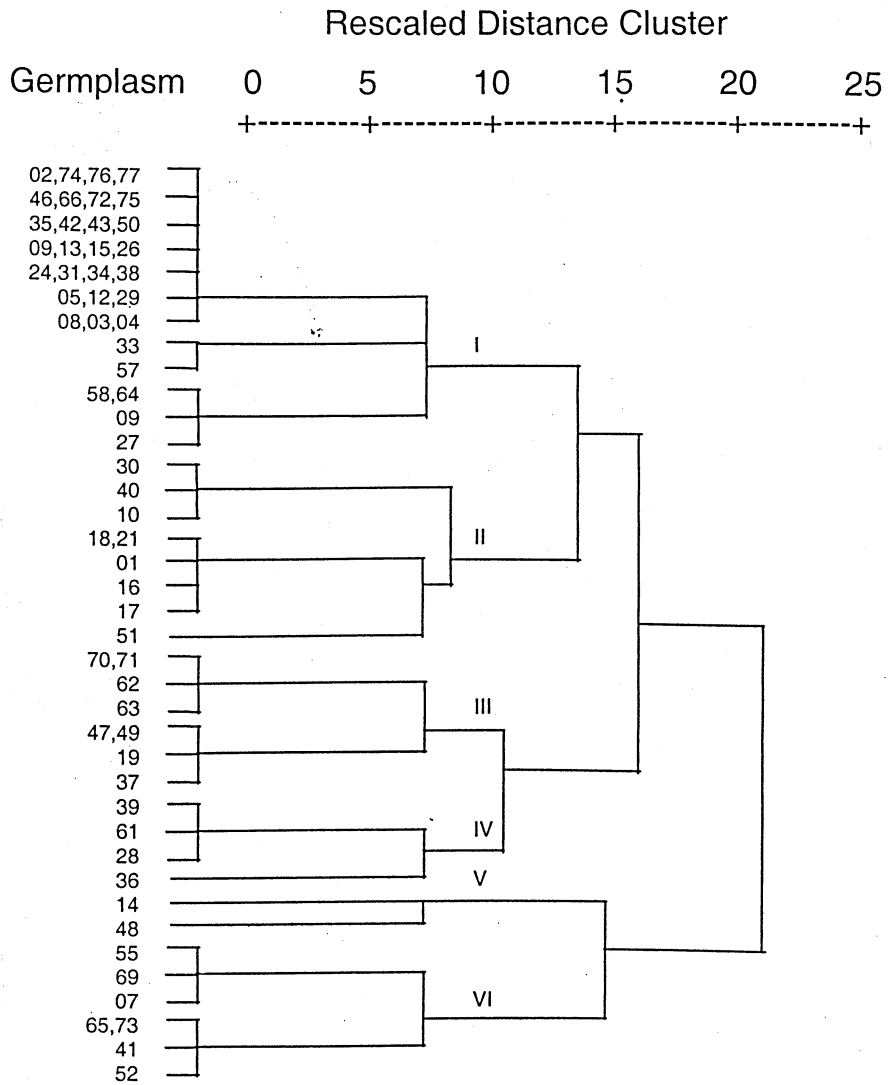


Fig. 2. Dendrogram showing hierarchical clustering of 62 mango accessions based on isozyme zymotypes of peroxidase (PRX)

Acknowledgements

The research work was funded by the United States Department of Agriculture (USDA) through a research grant BG-ARS-108.

References

Decha, P. 2000. Variation in morphological and isozyme characteristics and processing properties of selected clones of mango cv. Kaew. M.S Thesis (Horticulture), Graduate School, University of Ciang Mai, Thailand.

Degani, C., El-Batsri, R. and Gazit, S. 1990. Enzyme polymorphism in mango. J. Amer. Soc. Hort. Sci., 115:844-847.

Degani, C., Cohen, M., El-Batsri, R. and Gazit, S. 1992. PGI isozyme diversity and its genetic control in mango. Hort. Sci., 27:252-254.

Eiadthong, W. K., Yonemori, Y., Sugiura, A., Utsunomiya, N. and. Subhadrabandhu, S. 1998. Isoenzyme polymorphisms of mango cultivars in Thailand. Thai. J. Agril. Sci., 31(4): 555-568.

Mukherjee, S. K. 1997. Introduction: Botany and Importance. In: The Mango: Botany, Production and Uses. CAB International, Wallingford, U.K. pp. 1-19.

Shannon, L.M. 1968. Plant isoenzymes. Ann. Rev. Plant Physiol., 19 : 187-21.

Singh, A., Borthakur, A., and Deka, P.C. 2001. Molecular Markers In: Parthasarthy, V.A., Bose, T.K., Deka, P.C., Mitra, S.K and Mohandas, S.(Eds) Biotechnology of Horticultural Crops, Vol. 1, Naya Prokash, 206 Bidhan Sarani, Calcutta, India. pp-35-59.

Torres, A.M., Soltis, D.E., Soltis, P.S. 1989. Isoenzyme analysis of tree fruits. Isoenzyme-in-Plant- biology, 87(4): 192-205.

Truscott, M., Human, C. and Visser, G.J. 1993. Frequency of zygotic seedlings from polyembryonic mango rootstocks. J. South African Soc. Hort. Sci., 3:106-107.

Yadav, I.S. 1997. Mango Research in India. : The post 50 years. Indian Hort., 42(2) : 10-17.