African Journal of Biotechnology Vol. 11(87), pp. 15310-15323, 30 October, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2217
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Analysis of genetic diversity in mango (*Mangifera indica* L.) using isozymetic polymorphism

Majumder, D. A. N.[1]*, Hassan, L.[2], Rahim M. A.[3] and Kabir, M. A.[4]

[1]Plant Biotechnology laboratory, BRAC Agricultural Research and Development Centre, Bangladesh.
[2]Department of Genetics and Plant Breeding, Bangladesh Agricultural University (BAU), Bangladesh.
[3]Department of Horticulture, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh.
[4]Department of Horticulture, Hajee Mohammad Danesh Science and Technology University, Dinaupur-5200, Bangladesh.

Accepted 2 October, 2012

The isozymetic study was designed for assessing the genetic diversity among the selected mango cultivars/genotype available in Bangladesh. All the isozymes, used in the present study showed polymorphism for mango. A total of 25 different electrophoretic zymotypes were observed for three isozymes studied. Glutamate oxaloacetate transaminase, malate dehydrogenase and peroxidase analysis possessed 8, 10 and 7 zymotypes, respectively, and genotypes were grouped into different electrophoretic zymotypes which indicated higher level of genetic diversity of mango. For glutamate oxaloacetate transminase, G_6 was the most common zymotypes whereas G_3, G_5, and G_8 were found in few cases. Similarly, M_8 and M_6 in malate dehydrogenase as well as P_6 and P_5 in peroxidase were found more frequent while other zymotypes in both isozymes were less frequent. Frequency was very few for one zymotype in all cases of three isozymes such as, G_8, M_{10} and P_7. Cluster analysis through UPGMA dendogram using isozymes electrophoretic pattern provided strong information about existence of variability among the genotypes of mango. Based on Euclidean distance, a dendogram was constructed using banding pattern of 60 mango genotypes developed through three isozymes activities. The dendogram showed eight major clusters designated as I, II, III, IV, V, VI, VII and VIII. The results generated with isoenzyme will be helpful in improvement as well as may guide us in designing strategies that maximize the utility of genetic resources.

Key words: Polymorphism, isozyme, mango, genetic diversity, variety.

INTRODUCTION

Mango (*Mangifera indica* L.) "The King of fruits", originated in a region including the north-eastern parts of India (Assam), the western parts of Myanmar and Bangladesh (Duval et al., 2006). It is a member of the Anacardeaceae, a family of mainly tropical species with a few representatives in temperate regions. In Bangladesh, there are many genotypes of mango having diverse characters. It has been cultivated in Bangladesh for centuries. These cultivars are available in the market under different local name without any uniformity and standardization in nomenclature. Moreover, we have some exotic varieties under commercial cultivation. For the continuous improvement of mango through breeding to overcome the biotic and abiotic stress, adverse gene pool is essential. The assessment of genetic diversity of plant population in the field may be carried out in different ways such as characterization and evaluation of morphological traits or by using isozyme and DNA markers. Because of variable environmental factors in different locations, the field evaluation of morphological charac-

*Corresponding author. E-mail: dilrubamajumder@yahoo.com.

Abbreviations: GOT, Glutamate oxaloacetate transaminase; **MDH,** malate dehydrogenase; **PER,** peroxidise; **PAGE,** polyacrylamide gel electrophoresis; **APS,** ammonium per sulphate; **TEMED,** tetramethylene diamine; **AAT,** aspartate aminotransferase; **RAPD,** random amplification of polymorphic DNA.

teristics alone is not a completely reliable method. Morphological markers have certain limitations such as limited availability of easily score-able markers, difficulty in scoring homozygous from heterozygous individuals, influence of environment in equating and phenotypes with genotypes; so, the chemotaxonomic approaches have been used to confirm the characterization. For assessing diversity, protein and DNA markers have been proved to be authentic tools in characterization of many crops including mango germplasm at gene level. Moreover, electrophoretic techniques have many advantages such as abundance in polymorphism, no pleiotrophic effect; less affected by environment and subjected to rapid detection (Singh et al., 2001). Isozymes were developed as first co-dominant markers which were widely used in genetic diversity study of mango (Degani et al., 1992; Eiadthong et al., 1998). Isozymes are good biochemical markers used as a powerful tool both in characterization of cultivar and in genetic and phylogenetic studies for many crop species, including fruit crops (Tanksley and Orton, 1983).

Electrophoretic separation of enzymes has been widely used, both in taxonomic, genetic studies, and construction of genetic maps of different crops (Staub et al., 1996; Shannon, 1968). Isozyme analysis has been applied to cultivar identification and breeding (Chen et al., 1990; Sujatha et al., 1991; Sujatha and Seshadri, 1991). One use of genetic mapping is for the characterization and identification of new cultivars, which is of concern to those who desire proprietary protection for their cultivar creations. Degani et al. (1992, 1993), detected several polymorphic enzyme systems in mango and used them for the systematic characterization and percentage analysis of various cultivars. In the present study, there are three kinds of isozymes viz. glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH) and peroxidase (PER) have been used for indentifying the clustering relationships among the local and introduced cultivars of Bangladesh.

However, no reports are available regarding the characterization of mango germplasm using isoenzyme in Bangladesh. The objective of the present investigation was aimed at elucidating the phylogenetic relationship among 60 genotypes of mango, based on the polymorphism of three enzymes. Moreover, it helps to obtain genetic information in large collection of mango being maintained at the Germplasm Repository of Bangladesh Agricultural University (BAU) from isozyme analysis.

MATERIALS AND METHODS

The study was conducted with 60 mango genotypes in polyacrylamide gel electrophoresis (PAGE) techniques (Staub et al., 1997). Three isozymes *viz*., GOT, MDH and PER were used in this experiment. Isozyme analysis was done in the Molecular Biology Laboratory, Department of Genetics and Plant Breeding, Bangladesh Agricultural University, during the period from August 2007 to February 2008.

Extraction of enzyme sample

Mature leaf samples were collected, cleaned and sealed in brown paper covers from the orchard of Germplasm Repository of Bangladesh Agricultural University (BAU) and brought to the laboratory. Approximately, 0.4 g of leaf material was crushed with acid washed sand and 400 ml extraction buffer. Extraction buffer consisted of 0.1 M Tris-HCl (pH 7.5) containing 20% sucrose.

Preparation of gel for electrophoresis

Gels of different concentration *viz*., staking gel 4.5% and separation gel 9% were prepared by using the stack solutions. Ammonium per sulphate (APS) solution was prepared immediately just before use. The gel dimensions were 14 × 11 × 0.1 cm. The stock solutions for separation gel except APS and tetramethylene diamine (TEMED) were gently mixed in a 100 ml beaker. Gel solution was poured into glass plate to the level of about 5 mm lower from the comb to be placed.

Electrophoresis

Electrophoresis means migration of suspended electrically charged particles such as protein macromolecules under the influence of an electric field. Vertical polyacrylamide slab gel electrophoresis (PAGE) was used for isozyme analysis. About 500 ml of the electrode buffer (Tris 0.025 M and Glycine 0.190 M) was poured into the electrophoresis chamber and 300 ml of buffer was poured on to the upper portion of the electrode. The electrode assembly was connected with the power supply unit (120 volts) at constant voltages and electrophoresis was carried out for 3.5 to 4 h.

Staining of isozyme

The electrophoresis of the proteins of leaf samples was carried out using PAGE technique and the gels were stained for GOT, MDH and PER were used for staining gel.

Staining solution of GOT was prepared by aspartate aminotransferase (AAT) substrate solution (pH 7.4) α-Ketogluteric acid 292 mg, L- aspartic acid 1.07 g, PVP-40 (Polyvinylpyrolidine) 4.0 g, ethylenediaminetetraacetic acid (EDTA) 400 mg and sodium phosphate, dibasic 11.36 g were mixed with 800 ml distilled water. Then, 50 mg of fast blue BB salt was added to the AAT substrate solution and poured over the gel. For malate dehydrogenase, staining solution was prepared by mixing 50 mN Tris-HCl (pH 8.5) 50 ml, nicotinamide adenine dinucliotide (NAD) 10 mg, malic acid 1 ml (after neutralized with NaOH), nitro blue tetrazolium chloride (NBT) 10 mg and phenozine methosulphate (PMS) 2 mg.

In case of peroxidase, 10 ml of POD-1 (500 ml of acetone + 1.05 g of 3-amino-9-ethylcarbazole and + 0.725 g of B-napthol) solution was added to 40 ml of POD-B (1.51 g of Tris buffer + 1.62 ml of glacial acetic acid) solution and mixed gently. About 50 µl of 30 % H_2O_2 was added and the gel was then incubated at room temperature in the dark and continuously shaken until the bands appeared. The formation of bands was completed within an hour.

Identification of band

Following Mouemar and Gasquez (1983), isozyme banding patterns were recorded on the basis of number and the relative front (Rf) values of the bands. The Rf value of each respective band on schematic isozyme patterns was determined to allow precise comparisons among the various genotypes. The Rf value is the

Table 1. Zymotypes from the electrophoresis patterns of glutamate oxaloacetate transminase (GOT) isozyme in 60 mango genotypes.

Zymotype	Total number of genotype	Genotypes sharing the zymogram	Genotype (%)
G1	7	MI01, MI-23, MI50, MI75, MI81, MI82, MI86	11.67
G2	8	MI04, MI16,MI19, MI24, MI26, MI27, MI94, MI95	13.33
G3	4	MI09, MI70, MI92, MI097	6.67
G4	10	MI12, MI22, MI33, MI39, MI41, MI52, MI60, MI61, MI80, MI91	16.66
G5	5	MI02, MI25, MI90, MI93, MI98	8.33
G6	13	MI20, MI44, MI46, MI47, MI51, MI54, M64, MI74, MI77, MI84, MI88, MI96, MI97	21.67
G7	9	MI03, MI29, MI38, MI40, MI43, MI45, MI58, MI83, MI85	15.11
G8	4	MI08, MI21, MI28, MI49	6.67

Table 2. Distribution of glutamate oxaloacetate transminase (GOT) bands among the zymotypes of 60 mango genotypes.

Zymotype	RF Value							Number of band
	0.11	0.13	0.14	0.18	0.20	0.22	0.25	
G1	√			√		√		3
G2	√				√			2
G3	√				√		√	3
G4		√		√		√		3
G5		√		√			√	3
G6		√			√		√	3
G7		√			√			2
G8			√					1
Band frequency (%)	21.66	68.33	11.67	36.67	63.33	26.67	36.33	

mobility of each isozyme band that traveled from the origin divided by the distance traveled by the front tracking dye. The presence or absence of a certain isozymatic band was considered as a differentiating feature. Zymograms were drawn to scale and relative mobility values were calculated for each band.

Analysis of similarity coefficient

Similarity coefficients of two zymotypes from electrophoretic banding pattern were calculated using Nei and Li's (1979) index of genetic similarity comparisons (S_{xy}), which are: $S_{xy} = 2n_{xy}/(n_x+n_y)$; Where, n_{xy} = Number of shared bands; n_x = number of bands in electrophoretic patterns of x zymotype; and n_y = number of bands and electrophoretic patterns of y zymotype.

Cluster analysis

Cluster analysis was performed by un-weighted pair group method using arithmetic average (UPGMA) according to Nei (1978). Similarity coefficient (S_{xy}) values of electrophoretic phenotypes were subjected to cluster analysis for each isozyme. For the analysis of overall electrophoretic patterns, the value 1 was put for the presence of the band and value 0 was used against the absence of the band for each genotype. Zymotypes were used for clustering and the Euclidean distance method was used for the dissimilarity (Nourish, 1993; SPSS, 2003). The original data was transformed to Z-scores prior to cluster analysis (Anderburg, 1973; Romeshurg, 1984).

RESULTS

Based on the Rf values of the three kinds of isozymes band generated in 60 mango germplasm, three types of zymotypes were obtained which are discussed below and showed some representative gel plate.

Glutamate oxaloacetate transeminase (GOT) isozyme variability

Eight electrophoretic zymotypes (G_1-G_8) were observed in glutamate oxaloacetate transminase isozyme, which formed 20 bands at different Rf values varied from 0.11 to 0.25 (Table 2). Glutamate oxaloacetate transminase isozyme zymotypes are presented in Tables 1, 2 and 3, Plate 1 and Figure 1.

Results of glutamate oxaloacetate transminase isozyme revealed that zymotype G_6 was the most frequent, which included 21.67% of the total genotypes. The second frequent zymotypes was G_4 which covered 16.66% followed by G_7 (15.11%), G_2 (13.33%) and G_1 (11.67%); whereas, the least frequency was observed in zymotype G_3 and G_8 which included 6.67% of total genotypes of mango.

Enzymatic activity of glutamate oxaloacetate transmi-

Table 3. Similarity co-efficient values of eight glutamate oxaloacetate transminase (GOT) banding pattern as observed in 60 mango genotypes.

Zymotype	G_1	G_2	G_3	G_4	G_5	G_6	G_7
G_2	0.40						
G_3	0.33	0.80					
G_4	0.66	0	0				
G_5	0.33	0	0.33	0.66			
G_6	0	0.40	0.66	0.33	0.66		
G_7	0	0.50	0.40	0.40	0.40	0.80	
G_8	0	0	0	0	0	0	0

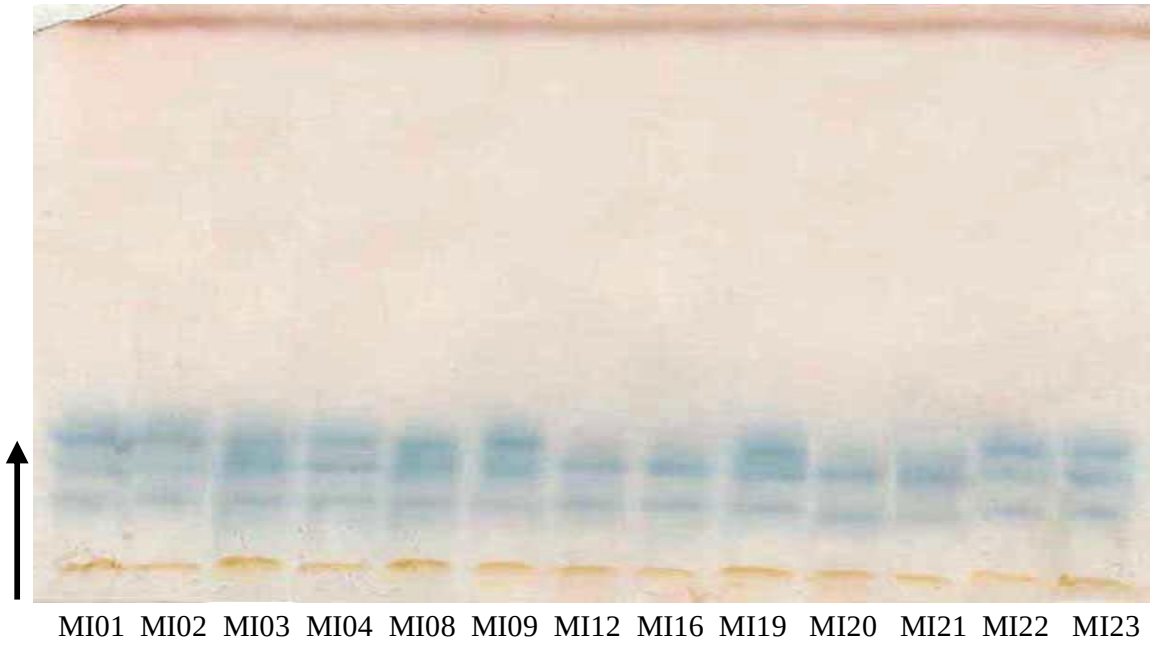

Plate 1. Variability in glutamate oxaloacetate transminase (GOT) banding patterns of different mango genotypes (MI01-MI23) (arrows indicate the direction of migration of sample).

nase was found to be polymorphic. The zymotypes G_1, G_3, G_4, G_5, and G_6, comprised of 3 bands each while zymotypes G_2 and G_7 produced 2 bands each but G_8 comprised of only one. Band of Rf value 0.13 was the unique band for glutamate oxaloacetate transminase which were common in 68.33% of total population of mango. Bands at Rf value 0.20 was the second frequent band, which was common in zymotypes G_2, G_3, G_6 and G_7 and covered 63.33% of total population of mango.

Zymotypes G_1 and G_4 exhibited a common band at Rf values 0.22, which was distributed among 26.67% of total populations. There was another unique band in zymotypes G_1, G_2 and G_3 at Rf value 0.11, frequency of which was 21.66%. The bands with Rf value 0.14 represent characteristic of the zymotype G_8 and frequent in 11.67% of total genotypes under investigation noticed species specificity. It was observed that Rf value of 0.13 showed the highest frequency (68.33%) whereas the lowest band frequency (11.67%) was observed for the Rf value of 0.14. Thus, it confirmed the wide range of genetic variation among the genotypes.

Glutamate oxaloacetate transeminase (GOT) isozyme zymotype analysis

The wide range of similarity coefficient (0 to 80%) was observed between the pairs of zymotypes of glutamate oxaloacetate transminase banding pattern. The maximum similarity (80%) of electrophoretic banding pattern was estimated between the pairs of zymotypes G_2 and G_3 and zymotypes G_6 and G_7 indicated strong association among the genotypes under those zymotypes. The second highest similarity coefficient (66%) of electrophoretic banding

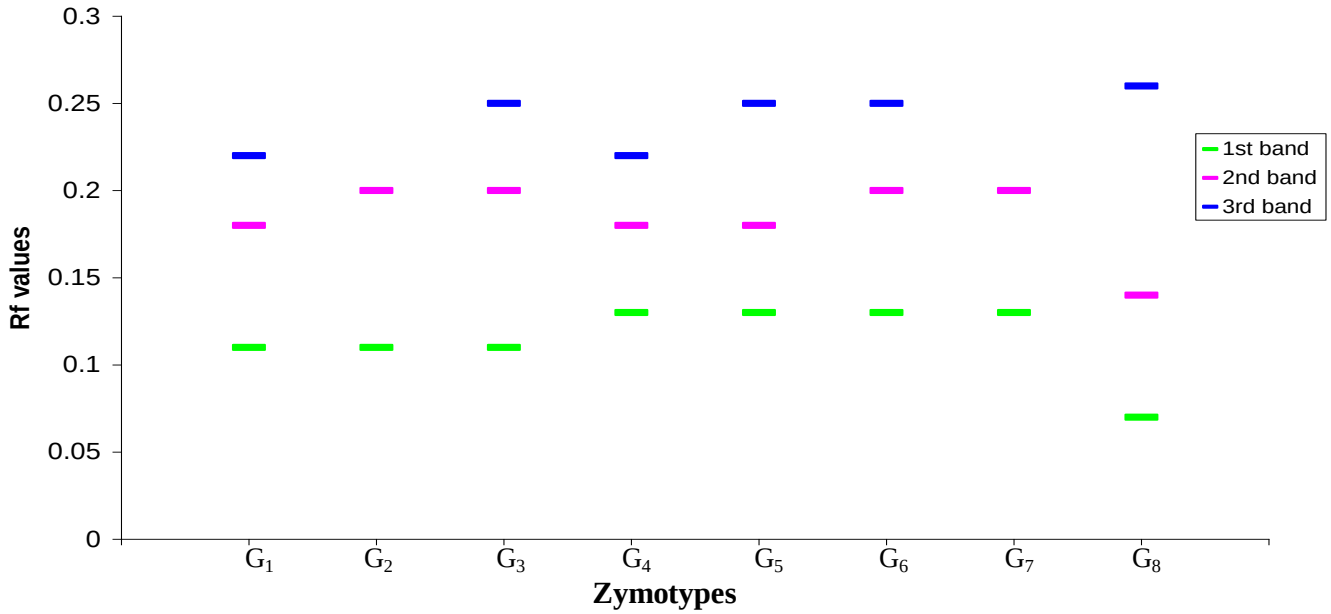

Figure 1. Schematic zymogram of glutamate oxaloacetate transminase (GOT) isozyme zymotypes.

Table 4. Zymotypes from the electrophoresis patterns of malate dehydrogenase (MDH) isozyme in 60 mango genotypes.

Zymotype	Total number of genotype	Genotypes sharing the zymogram	Genotype (%)
M_1	6	MI01, MI02,MI24, MI49, MI50, MI52	10.96
M_2	4	M04, MI25, MI29, MI40	6.85
M_3	5	MI60, MI61, MI93, MI94, MI97	8.22
M_4	3	MI12, MI16, MI54	4.11
M_5	4	MI22, MI26, MI27, MI28	6.85
M_6	12	MI47, MI51, MI70, MI74, MI80, MI81, MI88, MI90, MI91, MI95, MI96, MI98	20.55
M_7	5	MI23, MI41, MI58, MI75, MI77	8.22
M_8	15	MI03, MI08, MI09, MI14, MI20, MI21, MI33, MI38, MI39, MI64, MI82, MI83, MI84, MI86, MI92	24.66
M_9	5	MI23, MI41, MI58, MI75, MI77	8.22
M_{10}	1	MI85	1.67

pattern was found between the pairs of zymotypes G_1 and G_4, G_3 and G_6, G_4 and G_5, and between G_5 and G_6. The zymotypes pair of G_2 and G_7 showed 50% similarity coefficient, while pairs between zymotypes G_1 and G_2, G_2 and G_6, G_3 and G_7, G_4 and G_7 and between G_5 and G_7 showed 40%. The minimum similarity coefficient (33%) was observed between G_1 and G_3, G_1 and G_5, G_3 and G_5 and between G_4 and G_6, while the rest of the pairs showed no similarity.

Malate dehydrogenase (MDH) isozyme variability

The 60 germplasm were classified on the basis of their zymogram patterns with the Rf values. There were 11 bands of malate dehydrogenase isozyme system falling into ten electrophoretic zymotypes (M_1-M_{10}). Thirty-nine bands were observed at different Rf values which varied from 0.07 to 0.46. Banding patterns of mango genotypes and zymotypes for malate dehydrogenase isozyme are presented in Tables 4, 5, 6, Plate 2 and Figure 2.

Among the zymotypes of MDH isozyme, M_8 was found more frequent, included 24.66% of total genotypes of mango. Zymotypes M_6 was found to be the next frequent which included 20.55% of total genotypes followed by zymotypes M_1 (10.96%). The same frequencies (8.22%) were recorded in electrophoretic zymotypes M_3, M_7 and M_9 (Table 4). Zymotypes M_2 and M_5 were found to be less frequent and distributed among 6.85% of total genotypes of mango. The least frequency (1.67%) was in

Table 5. Distribution of malate dehydrogenase (MDH) bands among the zymotypes of 60 mango genotypes.

Zymotype	RF value											Number of band
	0.07	0.08	0.11	0.12	0.32	0.33	0.39	0.43	0.44	0.45	0.46	
M_1	√				√							2
M_2	√					√			√			3
M_3	√				√				√		√	4
M_4	√		√			√			√		√	5
M_5	√		√			√			√			4
M_6		√			√				√			3
M_7		√		√	√				√		√	5
M_8		√		√		√						3
M_9		√		√		√			√			4
M_{10}		√		√		√	√	√		√		6
Band frequency (%)	36.99	63.01	10.96	42.47	47.95	52.05	1.67	1.67	63.01	1.37	20.55	

Table 6. Similarity co-efficient values of eight malate dehydrogenase (MDH) banding pattern as observed in 60 mango genotypes.

Zymotype	M_1	M_2	M_3	M_4	M_5	M_6	M_7	M_8	M_9
M_2	0.40								
M_3	0.67	0.57							
M_4	0.29	0.75	0.67						
M_5	0.33	0.86	0.50	0.89					
M_6	0.40	0.33	0.57	0.25	0.29				
M_7	0.29	0.25	0.67	0.40	0.22	0.75			
M_8	0	0.33	0	0.25	0.29	0.33	0.50		
M_9	0	0.57	0.25	0.44	0.50	0.57	0.67	0.86	
M_{10}	0	0.22	0	0.18	0.20	0.22	0.36	0.67	0.60

zymotype M_{10}. The highest number of bands (6) was observed in electrophoretic zymotype M_{10} followed by zymotypes M_4 (5) and M_7 (6). On the contrary, zymotypes M_3, M_5, M_9 and M_2, M_6, M_8 were characterized with 4 and 3 bands, respectively.

Bands at Rf value 0.08 and 0.44 were found as unique bands for malate dehydrogenase, both of which were distributed among 63.01% of total genotypes. Absence of unique band in the rest of the genotypes may be due to natural mutation or any other special reason. Bands at Rf value of 0.33 was the third frequent (52.05%) in mango genotypes representing M_2, M_4, M_5, M_8, M_9 and M_{10} zymotypes followed by bands at Rf value of 0.32 (47.95%) which represents M_1, M_3, M_6 and M_7 zymotypes; Rf value 0.12 (42.47%) represents M_7, M_8, M_9 and M_{10} zymotypes; Rf value 0.07 (36.99%) represents M_1, M_2, M_3, M_4 and M_5 zymotypes, respectively. The lowest frequency (1.67%) was observed at Rf values 0.39, 0.43 and 0.45, which was found only in one genotype indicating species specific band. Exhibition of different types of banding pattern for malate dehydrogenase indicate wide variability of genotypes of mango.

Malate dehydrogenase (MDH) isozyme zymotype analysis

Similarity coefficients between the pairs of genotypes for MDH isozyme were computed and presented in Table 6. The maximum similarity coefficient (89 %) observed between M_4 and M_5 zymotypes indicated strong association among genotypes of these zymotypes followed by the pairs of zymotypes M_2 and M_5 and zymotypes M_8 and M_9, both of which showed 86% similarity coefficient. Moderate similarity coefficients were observed between the pairs of zymotypes M_9 and M_{10} (60 %) followed by the pair of zymotypes M_2 and M_3 (57%), zymotypes M_2 and M_9, zymotypes M_3 and M_6 and zymotypes M_6 and M_9. The lowest was between M_4 and M_{10}. The range of similarity coefficient was from 0 to 89%, which was very wide noticed considering genetic diversity among the genotypes of mango.

Peroxidase (PER) isozyme variability

In peroxidase isozyme, seven electrophoretic patterns of

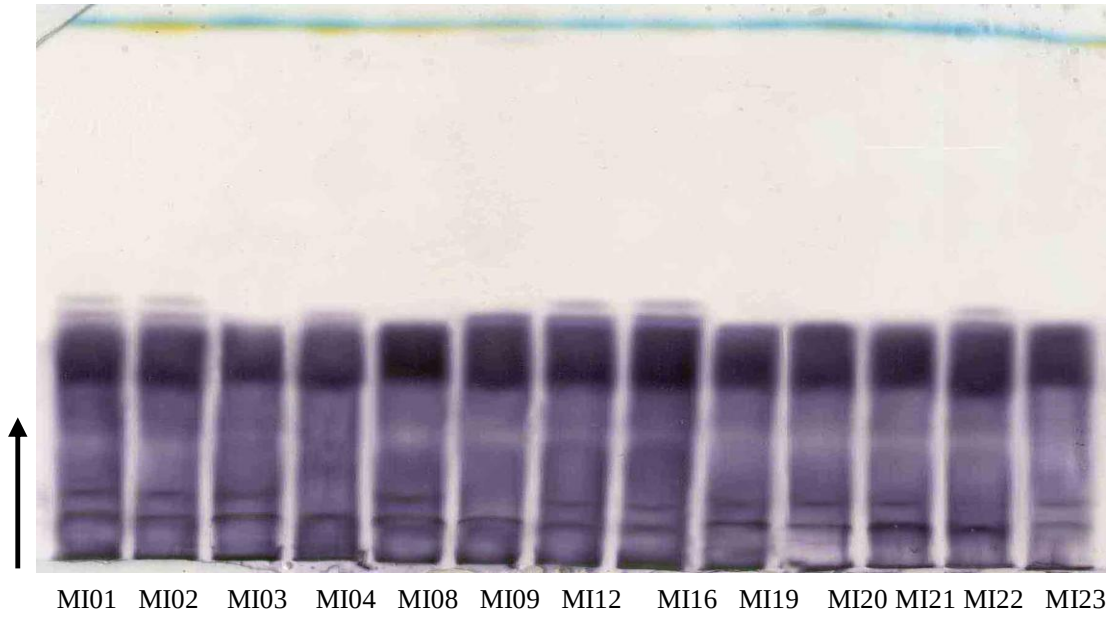

Plate 2. Variability in malate dehydrogenase (MDH) banding patterns of different mango genotypes (MI01-MI23) (arrows indicate the direction of migration of sample).

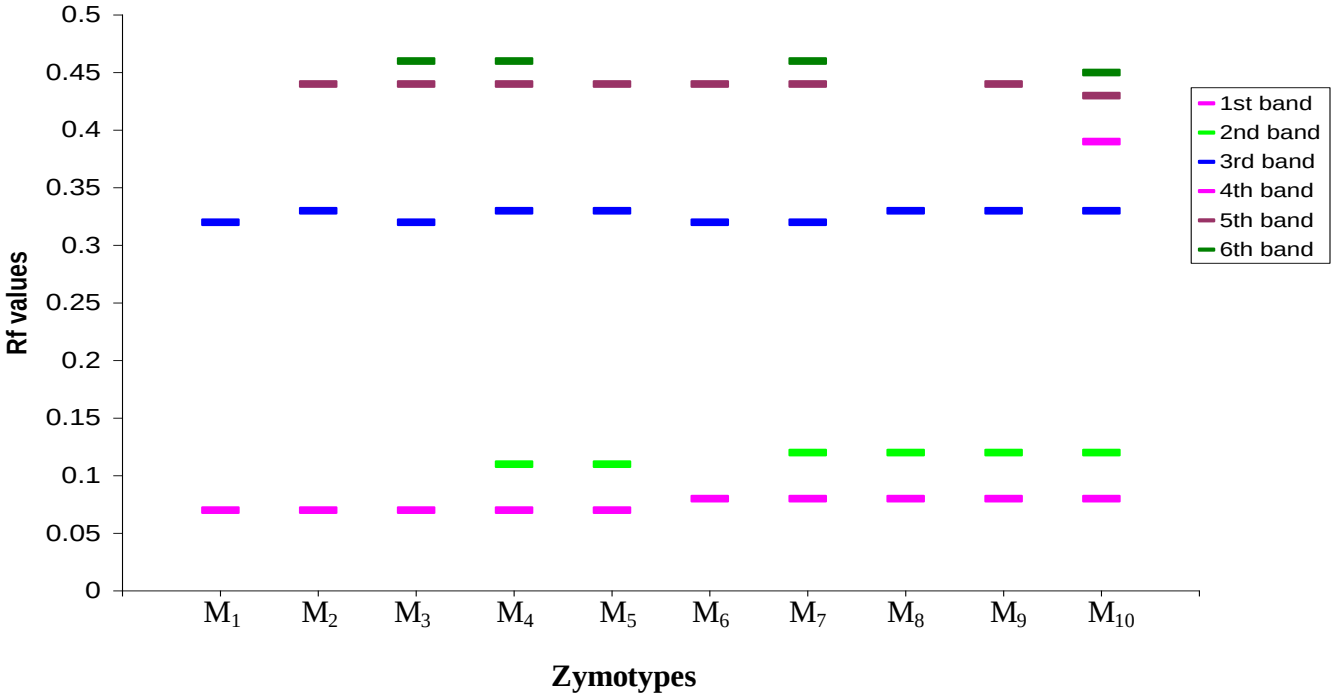

Figure 2. Schematic zymogram of malate dehydrogenase (MDH) isozyme zymotype.

zymotypes (P_1 to P_7) were observed which formed 12 bands at different Rf values varying from 0.07 to 0.26 (Tables 7, 8. 9, Plate 3 and Figure 3). It was revealed that zymotype P_6 was the most frequent which possessed 36.67% of the total genotypes followed by zymotypes P_5 (21.67%). Zymotype P_4 included 7 genotypes, which covered 11.67% of total populations. On the other hand, only one genotype was included by zymotype P_7 and showed the lowest frequency (1.67%) of total genotypes. Zymotypes P_2, P_3, P_5, P_6 and P_7 composed of two bands

Table 7. Zymotypes from the electrophoretic patterns of peroxidase isozyme in 60 mango genotypes.

Zymotype	Total number of genotype	Genotypes sharing the zymogram	Genotype (%)
P_1	6	MI20, MI21, MI41, MI58, MI77, MI84	10.00
P_2	6	MI3, MI8, MI19, MI23, MI24, MI33	10.00
P_3	5	MI5, MI7, MI82, MI83, MI86	8.33
P_4	7	MI12, MI16, MI45, MI46, MI48, MI85, MI88	11.67
P_5	13	MI1, MI2, MI22, MI26, MI27, MI28, MI39, MI40, MI43, MI44, MI49, MI50, MI52	21.67
P_6	22	MI4, MI9, MI25, MI29, MI38, MI47, MI51, MI60, MI61, MI70, MI74, MI80, MI81, MI90, MI91, MI92, MI93, MI94, MI95, MI96, MI97, MI98	36.67
P_7	1	MI64	1.67

Table 8. Distribution of peroxidase bands among the zymotypes of 60 mango genotypes.

Zymotype	RF value					Number of band
	0.07	0.08	0.24	0.25	0.26	
P_1	√					1
P_2	√			√		2
P_3	√				√	2
P_4		√				1
P_5		√		√		2
P_6		√			√	2
P_7	√		√			2
Band frequency (%)	30.00	70.00	1.67	31.67	36.67	

Table 9. Similarity co-efficient values of seven peroxidase banding pattern as observed in 60 mango genotypes.

Zymotype	P_1	P_2	P_3	P_4	P_5	P_6
P_2	0.67					
P_3	0.67	0.50				
P_4	0	0	0			
P_5	0	0.50	0	0.67		
P_6	0	0	0.50	0.67	0.50	
P_7	0.67	0.50	0.50	0	0	0

each while zymotypes P_1 and P_4 comprised of only one band each (Table 7). Bands at Rf values 0.07 and 0.08 were the unique bands for peroxidase enzyme, distributed among 30.0 and 70.0% of mango genotypes, respectively. Bands at Rf value 0.26 was found common in electrophoretic zymotypes of P_3 and P_6, distributed among 36.67% of genotypes. The band at Rf value 0.24 was observed in only one genotype. Occurrence of different types of zymotypes and banding pattern for peroxidase enzyme revealed remarkable variability of mango genotypes.

Peroxidase (PER) isozyme zymotypes analysis

Peroxidase isozyme differentiated seven zymotypes from which pair wise similarity coefficient was calculated (Table 9). The highest similarity coefficient (67%) was estimated between the pairs of zymotype P_1 and P_2, P_1 and P_3, P_1 and P_7, P_4 and P_5 and zymotype P_4 and P_6 indicating strong association of genotypes under those zymotypes. The moderate similarity coefficient (50%) were observed between the pairs of zymotype P_2 and P_3, P_2 and P_5, P_2 and P_7, P_3 and P_6, P_3 and P_7 and zymotype

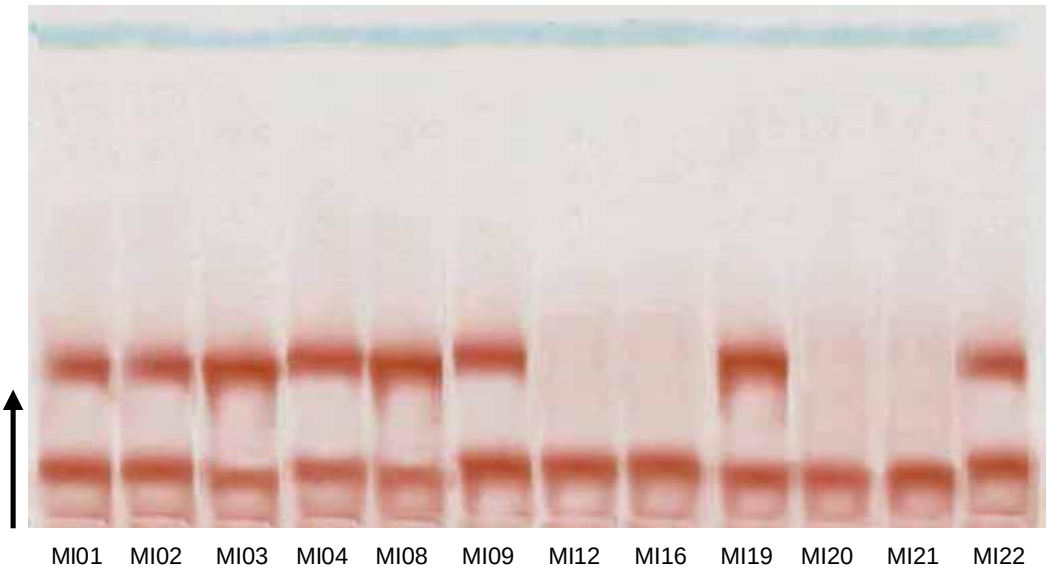

Plate 3. Variability in peroxidase (PER) banding patterns of different mango genotypes (MI01-MI21). Arrows indicate the direction of migration of sample).

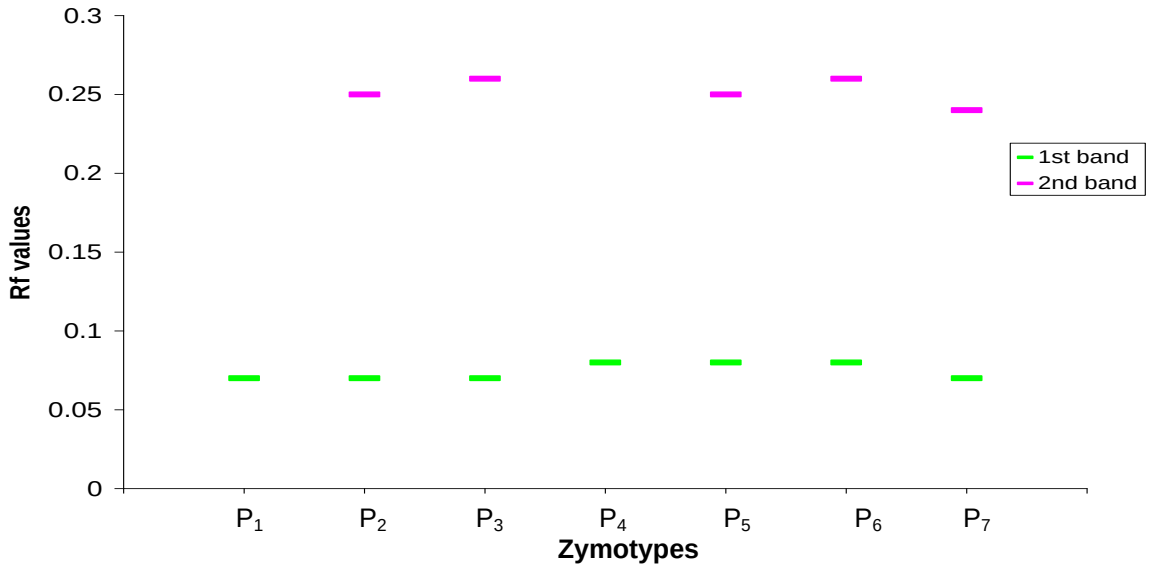

Figure 3. Schematic zymogram of peroxidase (PER) isozyme zymotypes.

P_5 and P_6. From the results, it was revealed that most of the pairs of genotypes showed zero percent similarity and point out very wide range of genetic divergence of mango genotypes for peroxidase enzyme activities.

Cluster analysis

A dendogram representing mango germplasm was generated based on Euclidean distance (Figure 4). Based on three kinds of polymorphic isoenzyne activities, the germplasm were grouped into eight major clusters designated as I, II, III, IV, V, VI, VII and VIII. The distributions of genotypes in different clusters are presented in Table 10.

Twenty germplasm were found under cluster I which represented 20.00% of the total germplasm. Cluster II, VIII and VI contained eleven, ten and nine germplams, respectively. Cluster VI and cluster IV consisted of 6 (10.00%) in each; cluster V consisted of 5 (8.33%). The lowest number (1) of genotypes was in cluster III representing only 1.67% of total genotypes of mango. It

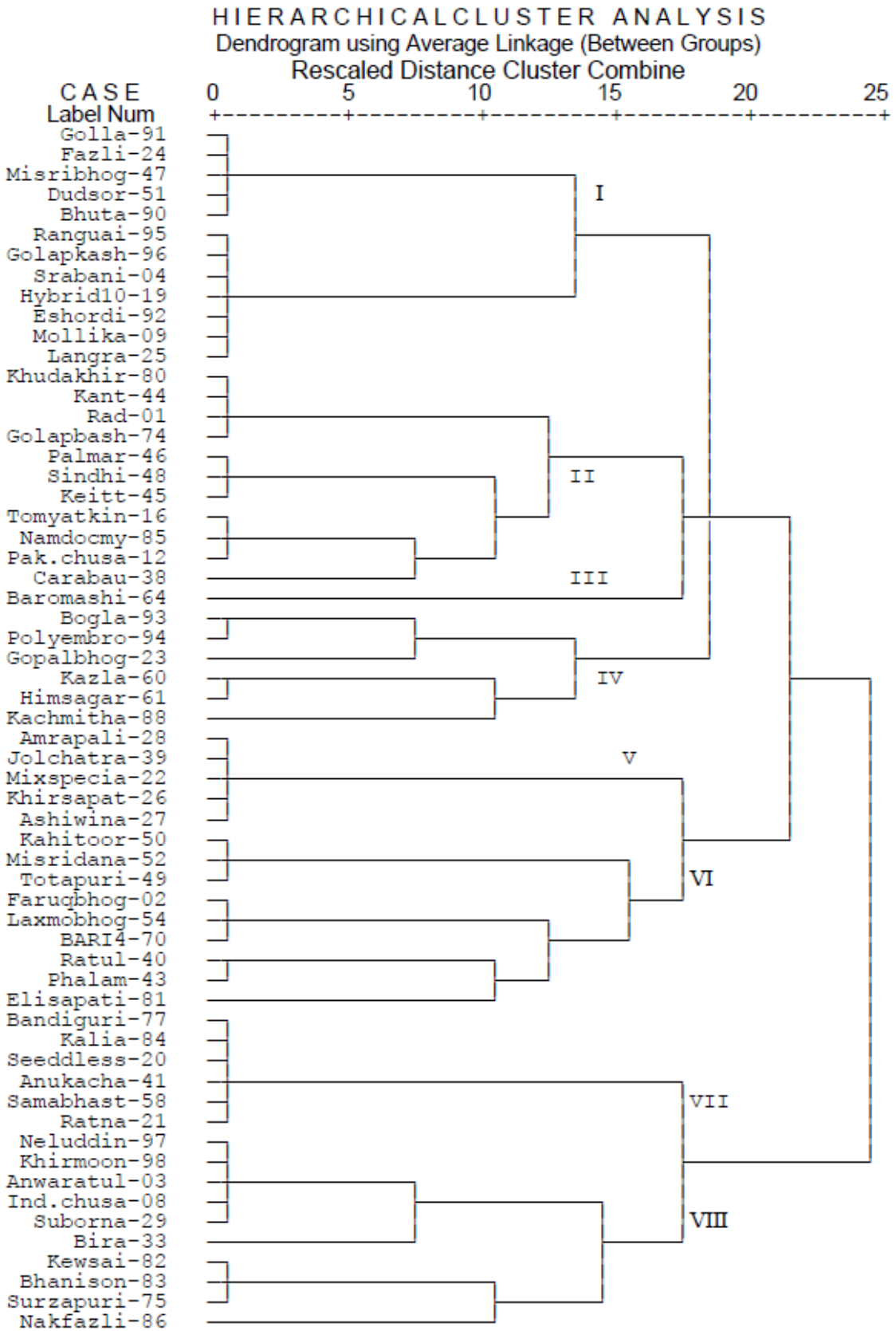

Figure 4. Dendrogram showing hierarchical clustering of mango genotypes based on isozyme type of glutamate oxaloacetate transminase (GOT), malate dedydrogenase (MDH) and peroxidase.

Table 10. Distribution of 60 mango genotypes under different cluster based on nonspecific isozyme type of glutamate oxaloacetate transminase (GOT), malate dedydrogenase (MDH) and peroxidase (PER).

Cluster	Genotypes included (% of total genotypes)	Genotype
I	12 (20)	MI09, MI14, MI24, MI25, MI47, MI51, MI90, MI91, MI92, MI95, MI96, MI98
II	11 (18.33)	MI01, MI12, MI16, MI38, MI44, MI45,MI46, MI48, MI74, MI80, MI85
III	1 (1.67)	MI64
IV	6 (10.00)	MI23, MI60, MI61, MI88, MI93, MI94
V	5 (8.33)	MI22, MI26, MI27, MI28, MI39
VI	9 (15.00)	MI02, MI40, MI43, MI49. MI50, MI52, MI54, MI81, MI70
VII	6 (10.00)	MI20, MI21, MI41, MI58, MI77, MI84
VIII	10 (16.67)	MI03, MI08, MI29, MI33, MI75, MI82, MI83, MI86, MI197, MI98

was observed that the genotypes collected from same location were grouped into different clusters indicating existence of genetic diversity of mango genotypes within the location.

DISCUSSION

Protein electrophoresis is the migration of protein under the influences of an electric field (Messeguer et al., 1987; Sambrook et al., 1989). Isozymes are all functionally similar forms of enzymes including all polymers of sub-units produced by different gene loci or by different alleles at the same locus. Their electrophoretic mobilities are the result of different size and shapes of enzyme molecules and their variation is a good indicator of genetic diversity (Shannon, 1968). So, electrophoresis separation of isozymes has been widely used both in taxonomic and genetic studies of different crops. The present study reported 25 different electrophoretic zymotypes for three studied isozymes studied. All the isozymes used in the present study showed polymorphism for mango. Glutamate oxaloacetate transminase, malate dehydrogenase and peroxidase analysis possessed 8, 10 and 7 zymotypes, respectively and genotypes were grouped in different electrophoretic zymotypes which indicated higher level of genetic diversity among the cultivars and exotic germplasm (Table 10).

According to Yadav (1997), isoenzyme analyses of 200 polyembryonic mango varieties revealed that there were 34 alleles which showed mono-morphism while 19 showed polymorphism. Decha (2000) studied isoenzyme characteristic of mango using tris-buffer (0.1M., pH 8.2). They found that the 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. So many scientists determined some polymorphic enzyme systems in mango from different experimental observation; these isozyme systems enabled the identification of zygotic seedlings in the polyembryonic cultivars (Degani et al., 1993; Schnell and Knight, 1992; Truscott, 1992).

Different electrophoretic zymotypes of three isozymes system consisted of different number of genotypes. Some zymotypes occurred very frequently in the genotypes and some of them were rare. For glutamate oxaloacetate transminase, G_6 was the most common zymotypes, whereas G_3, G_5, and G_8 were found in few cases (Table 1). Similarly, M_8 and M_6 in malate dehydrogenase (Table 4) as well as P_6 and P_5 in peroxidase (Table 7) were found more frequent, while other zymotypes in both isozymes were less frequent. Frequency was very few for one zymotype in all cases of three isozymes such as, G_8, M_{10} and P_7. However, the variation in number of zymotypes and their distribution suggested higher genetic diversity among cultivars and exotic mango varieties of Bangladesh. Eiadthong et al. (1998) found polymorphism in different enzyme system examined (Isocitrate dehydrogenase (IDH) and phosphoglucose isomerase (PGI)) and intra-cultivar variation of the same cultivar collected at different locations was confirmed in banding patterns.

Generally, zymotypes of higher frequency are representative of less variation and lower frequency of the germplasm in different zymotypes indicated random distribution of mango genotypes. Isozymes have been used to distinguish cultivars in several perennial fruit crops, including mango (Hirano, 1977; Torres et al., 1978; Pontikis et al., 1980; Mielke and Wolfe, 1982). They are useful as genetic markers because of their co-dominant inheritance (Brown et al., 1975; Torres and Tisserate, 1980; Arulsekar et al., 1981). Ben-Hayyim et al. (1982) stated that isozyme analysis has proved useful to detect differences in gene expression in several organs of same plant, or to distinguish between closely related cultivars. In case of perennial fruit species, genotypes may be identified directly from zymograms, because isozymes are free from epistacis or environmental factor effects (Zhong, 1993; Rahman et al., 2001). Isozyme expressions are almost exclusively of the

genetic make up of the plant and therefore, independent of environmental conditions (Lee and Ronalds, 1967; Schwartz, 1960).

Eight electrophoretic zymotypes (G_1-G_8) were observed in glutamate oxaloacetate transminase isozyme at different Rf values ranging from 0.11to 0.25 that formed 20 bands. Band of Rf value 0.13 was the unique band for glutamate oxaloacetate transminase which were common in 68.33% of total population of mango; and bands at Rf value 0.20 was the second frequent band, which was common in zymotypes G_2, G_3, G_6 and G_7 and was covered 63.33% of total population. Some commo isozyme bands of glutamate oxaloacetate transminase (EC 2.6.1; GOT) were observed among 'true citrus fruit trees' to indicated their phylogenetic relationship (Rahman and Nito, 1994). Three kinds of GOT isozymes loci, *Got-1, Got-2, Got-3* were obtained from the progenies of inter-generic crosses between *Citrus* and *Microcitrus* species which followed a simple Mendelian inheritance, suggesting that *Citrus* and *Microcitrus* species have similar genetic systems and the species of these two genera are closely related to each other. They also mentioned common alleles at the three loci controlling GOT isozymes. So, these results coincide with the present study.

In this research, we recognized some frequent zymotypes G_6, M_8, M_6, P_6 and P_5 for three kinds of isozymes GOT, MDH and PER between the cultivars and exotic germplasm, suggesting that a certain degree of genetic identity is maintained among them. Yamamoto et al. (1998) described the methods to determine isozymes and leaf genotype and subsequent classification into species. Isozyme analysis consisted of GOT and peroxidase (Px) which was related to sample genotype. Protopapadakis and Papanikolaou (1999) detected genetic diversity of citrus at the species level and found adequate enzyme differences. They also observed GOT zymograms consisted of bands in two zones and stated that zymograms are useful as diagnostic tool for cultivar identification. A great range of variability was observed with GOT by Chen et al. (1991) in *Aurantae genera*.

Cluster analysis through UPGMA dendogram using isozymes electrophoretic pattern provided strong information about existence of variability among the genotypes of mango. The dendrobram (Figure 4) showed eight major clusters named as I, II, III, IV, V, VI, VII and VIII (Table 10). The first cluster (I) comprised 12 released varieties with oblong type fruit shape and more or less similar total soluble solids (TSS) percentage. The group contained eleven exotic genotypes; they had ovate and medium size fruit, lighter stone, dwarf and bushy type plant. They were all distantly related with local cultivars. This is in agreement with the fact that Tommyatkin (Florida) and local cultivars of Pakistan were grouped in different clusters (Ahmad et al., 2008); cluster-III contained only one local variety. But cluster-IV was made up with land races cultivars: Bogla, Polyembroni, together with Gopalbhog, Kazla, Himsagar and Kachamitha. Cluster–V comprised with local cultivars Khirsapat, Ashiwina, Mixed Special, Jolchatra together with one hybrid genotype; Amrapali. In a marker study with random amplification of polymorphic DNA (RAPD), Karihaloo et al. (2003) found the three local cultivars of India clustering together. Genotypes Kohitoor, Misridana, Totapuri, Lashambhog, BARI-4, Ellishpatti, Phalam, Ratul and Faraquebhog were assigned to cluster VI. This cluster was made up of indigenous variety. Cluster –VII was assigned with the local variety Bandiguri, Kaila, Seedless, Anukachamitha, Summarbahast and Ratna. All the genotypes had roundish shape fruit, heavier stone size and medium percentage of non-edible portion. Cluster VIII consisted with exotic and local late varieties. Bhenison, Kewsai, Indian Chusa and Anwar ratul were exotic late varieties belonging to cluster VIII. The dendrogram results indicated that the exotic and local varieties existed within the same group. This is expected in a highly cross-pollinated plant like mango where most cultivars have arisen through selection of desirable types among naturally produced seedling. These findings agreed with Karihaloo et al. (2003); they also concluded that landrace diversity existed within the southern region of India. Gogorcena et al. (1993) used dendogram analysis to observe variation among 21 sour orange. A large difference among 108 biotypes of citrus was observed by Fang et al. (1993) using UPGMA cluster analysis. Degani et al. (1990) characterized 41 mango cultivars derived from self- pollinated and cross-pollinated trees using isoenzymes. They identified 6 loci with 17 allelo-morphos and determine the outcross origin of some mango cultivars. They also demonstrated that there were two distinct zones of phosphoglucose isomerase (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 to be apples, pears, peaches, mulberries, figs, olives, citrus, avocados, date palms, mango, cherimoyas (*Annona cherimola*).

The specific co-dominant makers detected in the present study are to be applied in our mango breeding programme and also, the extension of the method applied may be useful for mango taxonomy studies and true-to-type verification of cultivars.

Conclusion

The isozymetic study is suitable for investigating mango diversity and rendered huge information for further studies. It would be interesting to introduce more accessions from the land races and diversity to infer the relationships and to evaluate the genetic difference that

has occurred in Bangladesh. Isozymetic study revealed a high degree of genetic diversity among the cultivars examined in the study, which can contribute to the improvement of fruit crops like mango. Furthermore, these electrophoretic zymotypes should supplement conventional breeding programmes aimed at developing elite mango cultivars possessing larger fruit size, smaller stone size and greater yield stability over biotic and aboitic tress. This study would also help for choosing diverse parents in mango breeding programme and would help to confirm the genetic purity of elite mango cultivars. To the best of my knowledge, this is the first reports on isozymatic clustering on mango of Bangladesh.

ACKNOWLEDGEMENTS

The author wishes to express gratitude to the Prime-Minister's Advanced Studies and Research Scholarships from the Prime-Minister Office, Government of the People's Republic of Bangladesh for providing the financial support to conduct the research work and to Bangladesh Agricultural University (BAU) for capacity building.

REFERENCES

Ahmad I, Malik AU, Malik SA, Tabassam N, Rahaman M, Zafar Y (2008). Application of DNA fingerprinting technology to estimate genetic divergence among mango cultivars-genotypes. Acta Hort. 773:127-133.

Anderburg MR (1973). Cluster Analysis for Application. Academic Press. New York pp.24-39.

Arulsekar L, Bringhurst RS, Voth V (1981). Inheritance of PGI and LAP isozymes in octoploid cultivated strawberries. J. Am. Soc. Hort. Sci. 106:679-683.

Ben-Hayyim G, Shani A, Vardi A (1982). Evaluation of isozyme system in *Citrus* to facilitate identification of fusion products. Theor. Appl. Genet. 64:1-5.

Brown AHD, Matheson AC, Eldrige KG (1975). Estimation of the mating system of *Eucalyptus obliua.* L. Herit using allozyme polymorphisms. Aust. J. Bot. 23:931-949.

Chen FC, Hsiao CH, Chang YM, Li WH. (1990). Isozyme variation in *Cucumis melo* L. I. Peroxidase and shikimate dehydrogenase variation if four melo varieties and its application for F_1 hybrid production. J. Agric. Res. China 39:182-189.

Chen LG, Omura M, Hidaka T (1991). A study on the taxonomy of citrus with GOT isozymes. Acta Hort. 18(1):27-32.

Decha P (2000). Variation in morphological and isozyme characteristics and processing properties of selection clones of mango cv. Kaew. MS Thesis (Horticulture) Graduate School Univ. Chiang Mai, Thailand.

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.

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

Degani C, Cohen M, Reuveni O, El-Batsri R, Gazti S (1993). Frequency and characteristics of zygotic seedlings from polyembryonic mango cultivars, determined using isozymes as genetic markers. Acta Hort. 341:78-81.

Duval MF, Bunel J, Sitbon C, Risterucci AM, Calabre C, Fabrice LB (2006). Genetic Diversity of Caribbean mangoes (*Mangifera indica* L.) using microsatellite markers. 8th international Mango Symposium, Sun city, South Africa.

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

Fang DQ, Zhang WC, Xiao SY (1993). A study on the evolution and taxonomy of *Citrus* and its related genera using isoenzyme analysis. Acta Phytotaxono. Sini. 31(4):329-352 [Cited from Hort. Abst. 55(3):782 (1993].

Gogorcena Y, Zubrzycki H, Ortiz JM (1993). Identification of mandarin hybrids with the aid of isozymes from different organs. Scientia Hort. 41:285-291.

Hirano H (1977). Varietal affinities in mulberry (*Morus* spp.) assessed by peroxidase isozymes. Jpn. J. Breed. 27:350-358.

Karihaloo JL, Dwivedi YK, Archak S, Gaikwai AB (2003). Analysis of genetic diversity of Indian mango cultivars using RAPD markers. J. Hort. Sci. Biotech. 78(6):285-289.

Lee JW, Ronalds JA (1967). Effect of environment on wheat. Nature 213:844-846.

Messeguer R, Arus P, Carrera M (1987). Identification of peach cultivars with pollen isozymes. Scientia Hort. 31:107-117.

Mielke EA, Wolfe WH (1982). Identification of pecan cultivars with pollen isozymes. Hort .Sci. 17:382-383.

Mouemar AAL, Gasquez J (1983). Environmental conditions and isozyme polymorphism in *Chenopodium album* L. Weed Res. 23:241-249.

Nei M (1978). Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nei M, Li WH (1979). Mathematical models for studying genetic variation in terms of endonucleases. Proc. Nat. Acad. Sci. 76:5269-5273.

Nourish MJ (1993). SPSS for windows professional statistics, Release 6.0 SPSS Inc. 444N. Michigan Avenue, Chicago, Illinoise p.6061.

Pontikis CA, Looukas M, Kousounis G (1980). The use of biochemical markers to distinguish olive cultivars. J. Hort. Sci. 55:333-343.

Protopapadakis E, Papanikolaou X (1999). Use of four isozymatic system in lemon and lemon-like citrus cultivars to detect their genetic diversity. J. Hort. Sci. Biotech. 74 (1):26-29.

Rahman MM, Nito R (1994). Phylogenetic Relationships among the "True Citrus Fruit Trees" by Glutamate Oxaloacetate Trasaminase Isozymes Analysis. J. Jpn. Soc. Hort. Sci. 62(4):755-760.

Rahman MM, Nito N, Isshiki S (2001). Cultivar identification of 'Yuzu' (*Citrus junos* Sieb. Ex Tanaka) and related acid citrus by leaf isozymes. Scientia Hort. 87(3):191-198.

Romeshurg HC (1984). Cluster analysis researches. Belmont, Calif, Lifetime learning publication, Belmont, California, USA.

Sambrook J, Frist FF, Maniatis T (1989). Molecular Cloning – A Laboratory Manual, 2nd ed., Cold Spring, harbour, New York.

Schnell RJ and Knight RJ. 1992. Frequency of zygotic seedling from five polyembryonic mango rootstocks. HortSci. 27:174-176.

Schwartz D (1960). Genetic studies on mutant enzymes in maize: synthesis of hybrid enzymes by heterozygotes. Proc. Nat. Acad. Sci. 46:1210-1215.

Shannon LM (1968). Plant isozymes. Ann. Rev. Plant Physiol. 19:187-210.

Singh A, Borthakur A, Deka PC (2001). Molecular Markers In: Parthasarthy VA, BoseTK, Deka PC, Mitra SK and Mohandas S (Eds), Biotechnol. Hort. Crops, Vol. 1, Naya Prokash, 206 Bidhan Sarani, Calcutta, India pp.35-59.

SPSS (2003). SPSS 12.0 for Windows. In SPSS(R) 12.0 Developer's Guide. SPSS Inc 233 South Wacker Drive, 11th floor, Chicago, USA.

Staub JE, Box J, Meglic V, Horejsi TF, McCreight JD (1997). Comparison of isozyme and random amplified polymorphic DNA data for determining inter-specific variation in *Cucumis*. Gent. Resour. Crop Evol. 44:257-269.

Staub JE, Serquen F, Gupta RK (1996). Genetic markers, map construction and their application in plant breeding. Hort. Sci. 31:729-741.

Sujatha VS, Seshadri VS (1991). Genetics of isozyme varation in *Cucumis melo* L. Indian J. Genet. 51:445-447.

Sujatha VS, Seshadri VS, Srivastava KN, More TA (1991). Isozyme variation in muskmelon (*Cucumis melo* L.). Indian J. Genet. 51:438-444.

Tanksley SD, Orton TJ (1983). Isozyme in Plant Genetics and Breeding.

Part B Elesvier Amstardam p.472 .
Torres AM, Tisserat B (1980). Leaf isozymes as genetic markers in date palms. Am. J. Bot. 67:162-167.
Torres AM, Soltis DE, Soltis PS (1989). Isozyme analysis of tree fruits. Isozyme-in-Plant-biology. Hort. Sci. 87(4):192-205.
Torres AM, Soost RK, Diedenhofen U. (1978). Leaf isozyme as genetic markers in *Citrus*. Am. J. Bot. 65(8): 869-881.
Truscott M (1992). Biochemical screening of polyploidy mango seedlings. CSFRI Info.Bull. 23:17-18.
Truscott M, Human C, Visser GJ (1993). Frequency of zygotic seedlings from polyembryonic mango rootstock. J. South African Soc. Hort. Sci. 3:106-107.
Yadav IS (1997). Mango Research in India: The post 50 years. Indian Hort. 42(2):10-17.
Yamamoto M, Matsuo Y, Kuniga T, Matsumoto R, Yamada Y (1998). Isozyme and RAPD analysis of shiikuwashas (*Citrus depressa* Hoyata). Bull. Natl. Inst. Fruit Tree 30(31):39-51.
Zhong GY, Ye YM, Chen ZS, Wu YL (1993). Glutamate oxaloacetate transeminase isoenzymes of Citrus germplasm resources. Acta Phytotaxono. Sin. 31(2):162-169.