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GENETIC DIVERSITY OF LEMON GENOTYPES ASSESSED BY ISOZYMES

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ABSTRACT

An experiment was carried out at the department of Horticulture, Bangladesh Agricultural University, Mymensingh to investigate genetic diversity of 73 lemon genotypes using three isozymes viz. glutamate oxaloacetate transminase (GOT), malate dehydrogenase (MDH) and peroxidase (PER) isozymes during 2006-2007. All the isozymes, used in the present study showed polymorphism for lemon. A total of 25 different electrophoretic zymotypes were observed for three isozymes studied. Eight zymotypes with GOT, 10 with MDH and 7 with PER were formed by 22, 39, 12 bands at different Rf values, respectively. A dandogram was constructed and 73 lemon genotypes were grouped into nine clusters, the genotypes collected from same location were grouped in different clusters, indicating existance of variability within single location. The result revealed that isozymes are useful genetic marker for variability analysis of lemon and other species of *Citrus*.

Keywords: Lemon, genetic diversity and isozyme

INTRODUCTION

Lemon (*Citrus limon* L.) is one of the important crop under the genus *Citrus*, a world leading tree fruit crop having wide climatic adaptability. It is assumed to be originated in north eastern India and Burma (Hodgson, 1967), has been cultivated in different regions of the world for many centuries. Genetic diversity of a large sample of lemon genotypes from a wide range of geographic locations has not been reported. Genetic erosion and habitat destruction by modern agriculture has increased the importance of germplasm characterization of plant materials.

There are many genotypes of lemon having diverse characters in different parts of Bangladesh. Those genotypes are available in the markets without any uniformity and standardization. It is impartial to rationalize conservation and use of genetic resources to guide in the establishment of strategies that ensure the maintenance of genetic variability that is essential in plant breeding. Isozymes are good biochemical markers used as powerful tool both in characterization of cultivar and in genetic and phylogenetic studies for many crop species, including Citrus (Tanksley and Orton, 1983). 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). The present investigation was aimed at elucidating genetic diversity of 73 lemon genotypes, based on the polymorphism of three enzymes.

MATERIALS AND METHODS

Plant material

Leaves of 73 lemon genotypes were used as plant materials during the investigation in 2006-2007. The plant materials were maintained in project field entitled "Collection, Evaluation, Conservation and Utilization of Land races and Wild relatives of some Important Vegetables and Fruits of Bangladesh (CVFB)", Department of Horticulture, Bangladesh Agricultural University, Mymensigh, Bangladesh.

Protein extraction

Mature leaves of lemon were taken as plant sample. Leaves were washed with tap water, dipped in distilled water and wiped dry with paper towels. Approximately 0.4g of leaf material was crushed with

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a pre chilled pestle and mortar in presence of acid washed sand and 400 ml extraction buffer: 0.1 M Tris-HCl buffer (pH 7.5) containing 20% sucrose. The sample was then poured in an Ependorf tube and was mixed by vortexing for about 30 second. The crude homogenate was centrifuged at 14000 rpm for 20 minutes at 4^0 C and supernatant was used as enzyme sample.

Electrophoresis

Vertical polyacrylamide slab gel electrophoresis (PAGE) was used for isozyme analysis. The separations were performed with 4.5% stacking gel and 9% separation gel consisting of 1.5 M Tris-HCl, pH 6.8. The gel dimension was 14 x 11 x 0.1 cm. The electrode buffer contained 0.025 M Tris and 0.19 M glycine, the pH was maintained at 8.3. Twenty microlitre of the homogenized protein sample and 5 µl of 1% bromophenol blue tracking dye was mixed thoroughly and loaded onto the gel for electrophoresis. Electrophoresis was carried out at a constant voltage (220 volts) until the tracking marker stain reaches 5 - 6 mm to the gel bottom.

Staining gels

At the end of electrophoresis, the gels were stained with three isozymes viz., glutamate oxaloacetate transaminase (GOT), malate dehydrogenase (MDH) and peroxidase (PER). Staining solution of GOT contained α-Ketogluteric acid 292 g, L- aspertic acid 1.07 g, PVP-40 (Polyvinylpyrolidine) 4.0 g, EDTA 400 mg, Sodium phosphate 11.36 g and Fast blue BB salt 50 mg. For MDH, staining solution was prepared by mixing 50 mM Tris-HCl (pH 8.5) 50 ml, Nicotinamide adenine dinucliotide 10 mg, Malic acid 1 ml, Nitro blue tetrazolium chloride 10 mg and Phenozine methosulphate 2 mg one by one. The staining solution was then poured over gel. The staining was carried out in dark for 40-60 minutes until bands appeared. For PER, at first 10 ml of POD-1 solution (3-Amino-9-ethylecarbazole 1.05 g, β-Napthol 0.725 g and Acetone 500 ml) was added to 40 ml of POD-B solution (Na-acetate buffer 1.51 g at pH 6.7, Glacial acetic acid 1.62 ml and distilled water) and was mixed gently. The mixture was then allowed for filtration in dark. The staining solution was then poured over gel. About 50 µl of 30 % H_2O_2 was added and shaken well for good mixing until bands appeared. Stained gels were washed and fixed with 50% glycerol, sealed with polythene.

Identification of band

Following Mouemar and Gasquez (1983), isozyme banding patterns were recorded. Relative mobility (Rf) values were calculated for each band based upon the migration of the band relative to the front. The Rf value of each respective band on schematic isozyme patterns was determined to allow precise comparisons among the various genotypes. The presence or absence of a certain isozymatic band was considered as a differentiating feature. Zymograms were drawn to scale and relative mobility (Rf) value for each band.

RESULTS AND DISCUSSION

A total of 25 different electrophoretic zymotypes were observed for three isozymes studied. All the isozymes, used in the present study showed polymorphism for lemon. GOT, MDH and PER analysis possessed 8, 10 and 7 zymotypes, respectively and genotypes were grouped in different electrophoretic zymotypes, which indicate the existence of higher level of genetic diversity in lemon germplasm. Polymorphism of GOT in agarose gel, which exemplifies the typical amplification.

Glutamate oxaloacetate transeminase (GOT)

Eight electrophoretic zymotypes (G_1-G_8) were observed in GOT isozyme, which formed 22 bands at different Rf values varied from 0.07 to 0.26 (Plate 1 and Fig. 1). Results of GOT isozyme revealed that zymotype G_6 was the most frequent which included 18 (24.66%) of the total genotypes. Whereas, the least frequency was observed in zymotype G_8, which included only one genotype (Table 1). The zymotypes G_1, G_3, G_4, G_5, G_6, and G_8 comprised of 3 bands each while zymotypes G_2 and G_7 produced 2 bands each (Fig. 1). However, the variation in number of zymotypes with GOT and their distribution suggested higher genetic diversity among lemon genotypes collected from different locations of the country. Protopapadakis and Papanikolaou (1999) detected genetic diversity of lemon 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. Rahman *et al.* (2001) found 10 different isozyme phenotypes with GOT in acid citrus and suggested that

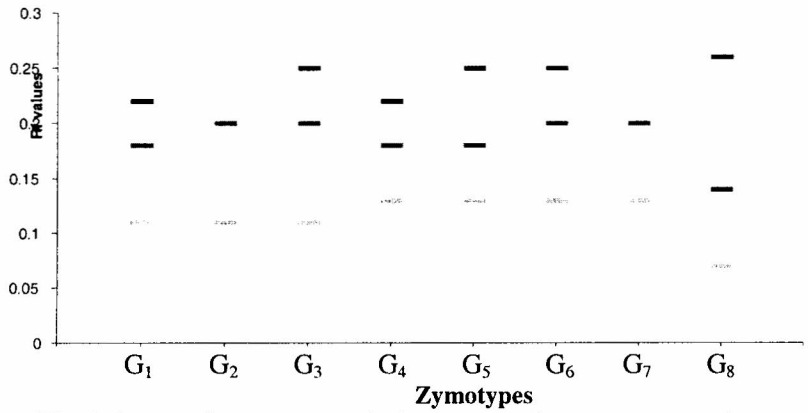

Fig. 1 Schematic zymogram of glutamate oxaloacetate transaminase (GOT) zymotypes

isozyme may provide useful markers for cultivar identification which are in agreement with present study.

Malate dehydrogenase (MDH)

There were ten electrophoretic zymotypes (M_1-M_{10}) in MDH isozyme system 39 bands were observed at different Rf values varied from 0.07 to 0.46 (Table 1 and Fig 2). Among the zymotypes of MDH isozyme, M_8 was found more frequent, included 24.66% of total genotypes of lemon (Table 1).

Table 1. Zymotypes from the electrophoresis patterns of GOT, MDH and PER isozyme in lemon

GOT			MDH			PER		
Zymotypes	Genotypes included	% Genotypes	Zymotypes	Genotypes included	% Genotypes	Zymotypes	Genotypes included	% Genotypes
G1	7	9.59	M_1	8	10.96	P_1	7	9.58
G2	9	12.33	M_2	5	6.85	P_2	6	8.22
G3	3	4.11	M_3	6	8.22	P_3	5	6.85
G4	14	19.18	M_4	3	4.11	P_4	8	10.96
G5	6	8.21	M_5	5	6.85	P_5	19	26.03
G6	18	24.66	M_6	15	20.55	P_6	27	36.99
G7	15	20.55	M_7	6	8.22	P_7	1	1.37
G8	1	1.37	M_8	18	24.66			
			M_9	6	8.22			
			M_{10}	1	1.37			

The least frequency (1.37%) was in zymotype M_{10}. The highest number of bands (6) was observed in electrophoretic zymotype M_{10} followed by zymotypes M_4 and M_7. The zymotype M_1 was noted as the lowest (Fig. 2). Bands at Rf value 0.08 and 0.44 were found as unique bands for MDH, both of which were distributed among maximum genotypes. The lowest frequency was observed at Rf values 0.39, 0.43 and 0.45, which was found only in one genotype indicating species specific band (Fig. 2). Absence of unique band in rest of the genotypes might be due to natural mutation or any other special reason. Exhibition of different types of banding pattern for MDH indicating wide variability of

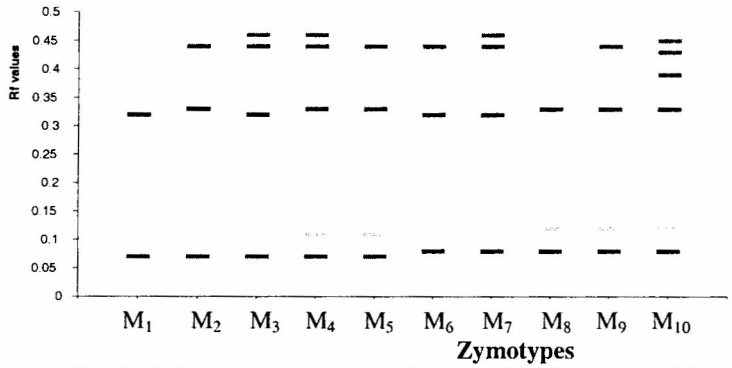

Fig. 2. Schematic zymogram of malate dehydrogenase (MDH) zymotypes

genotypes of the crop studied. Malate dehydrogenase (MDH) showed one or three bands in lemon reported by Protopapadakis and Papanikolaou (1999).

Peroxidase (PER)

In peroxidase isozyme, seven electrophoretic patterns of zymotypes (P_1 - P_7) were observed, which formed 12 bands at different Rf values varied from 0.07 to 0.26 (Fig. 3). It was revealed that zymotype P_6 was the most frequent, which possessed 36.99% of the total genotypes followed by zymotypes P_5 (26.03%). On the other hand, only one genotype was included by zymotype P_7 the lowest frequency (Table 1). Zymotypes P_2, P_3, P_5, P_6 and P_7 composed of two bands each while zymotypes P_1 and P_4 comprised of only one band each (Fig. 3).

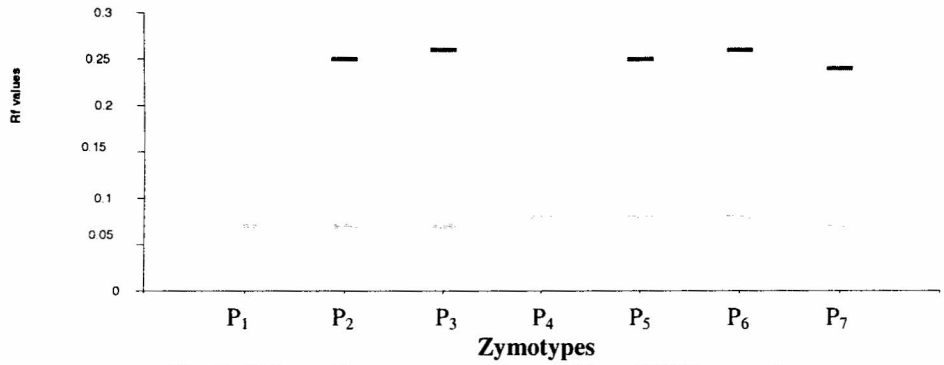

Fig. 3. Schematic zymogram of peroxidase (PER) zymotypes

Masashi and Kajiura (1987) observed one locus for peroxidase in citrus, which was controversy with this study. Bands at Rf values 0.07 and 0.08 were the unique bands for peroxidase enzyme, distributed among 73.67% and 26.03% of lemon genotypes, respectively. Bands 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 lemon genotypes.

Different electrophoretic zymotypes of three isozymes system consisted of different number of genotypes. Some of zymotypes occurred very frequently in the genotypes and some of them were rare. Frequency was very few for one zymotype in all cases of three isozymes such as, G_8, M_{10} and P_7. Generally zymotypes of higher frequency are the representative of less variation and lower frequency

of the germplasm in different zymotypes indicated random distribution of lemon genotypes through out the country.

Cluster analysis

Based on Euclidean distance, a dendogram was constructed using banding pattern of 73 lemon genotypes developed through three isozymes activities (Fig. 4).

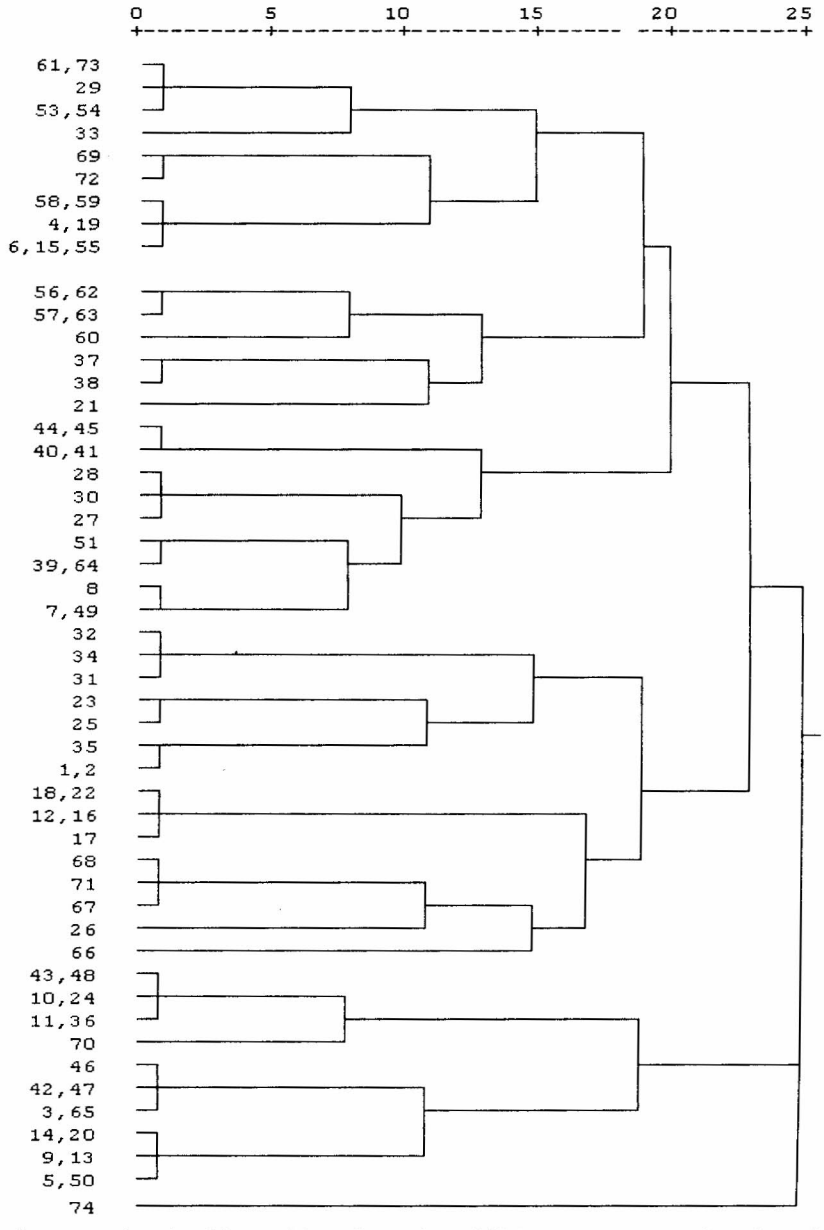

Fig. 4 Dendrogram showing hierarchical clustering of 73 lemon genotypes based on glutamate oxaloacetate transaminase, malate dedydrogenase and peroxidase isozymes

The dendogram showed nine major clusters designated as I, II, III, IV, V, VI, VII, VIII and IX. The highest number of genotypes (13) was grouped in cluster IV. The lowest number (1) of genotypes was in cluster IX. From the results, it was observed that the genotypes collected from same location were grouped into different clusters, indicating existence of genetic diversity of lemon genotypes within the location. Cluster analysis through UPGMA dendogram using isozymes electrophoretic patterns provided strong information about existence of variability among the genotypes of lemon. Grogorcena and Ortiz (1993) used dendogram analysis to observe variation among 21 sour orange. A large difference among 108 biotypes of Citrus was also observed by Fang *et al.* (1993) using UPGMA cluster analysis which was in line with the findings of this study.

In conclusion, the study provided a very simple and efficient methodology with only three isozymes for the analysis of genetic diversity of lemon. The polymorphism detected among lemon genotypes could be used in breeding programs to maximize the utilization of the genetic resources.

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