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STUDIES ON THE GENETIC DIVERSITY OF POINTED GOURD USING BIOCHEMICAL METHODS (ISOZYME ANALYSIS)

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Abstract

Biochemical characterizations of 64 pointedgourds were done using three isozyme viz., acid phosphatase, peroxidase and glutamate oxaloacetate transaminase. A wide range of diversity among the gremplasm based on their acid phosphatase, peroxidase and glutamate oxaloacetate transaminase isoenzyme banding patterns were observed. In respect of isoenzyme activity; 8 acid phosphatase, 7 peroxidase and 9 glutamate oxaloacetate transminase electrophoretic zymotypes were formed by 19, 11, and 19 bands at different Rf values varying from 0.19 to 0.82, 0.38 to 0.69 and 0.15 to 0.95, respectively. The wide range of similarity co-efficient of 0.0-80.0, 0.0-66.0, and 0.0-80.0 as found among the electrophoretic patterns in acid phosphatase, peroxidase, and glutamate oxaloacetate transminase, respectively, indicating wide genetic diversity among the accessions. Based on the polymorphic activity of these three enzymes, 27 combinations of electrophoretic zymotypes were identified, each of which can he equated to genotypes. Each of the groups consisted of one to eight genotypes. Sixty four accessions of pointed gourd were grouped into 12 clusters. The genotypes collected from the same location were grouped into different clusters.

Key Words: Genetic diversity, pointed gourd, biochemical methods.

Introduction

Pointed gourd *(Trichosanthes dioica* Roxb.) is one of the popular cucurbitaceous vegetable crops cultivated in Bangladesh. The Bengal-Assam area is the primary centre of origin of pointed gourd (Nath and Subramanyam, 1972). It is a dioecious crop having perennial habits. In Bangladesh, there are many genotypes of pointed gourd having diverse characters. Morphological markers have certain limitation, such as limited availability of easily scrabble markers and phenotypic expression of the morphological traits modified by environmental conditions. On the other hand, isozyme is closely related to gene products (Soost and Lorrcs, 1981) and useful to detect differences in gene expression in several organs of the same plant, or to distinguish between closely related cultivars (Ben Hayyim *et al.,* 1982). Since their codominant expression and stability over environment,

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isozymes have been successfully used in hybrid confirmation (Isshiki, 1993) and identification of cultivar (Tuwafe *et al*., 1988). Nevertheless, no report on isozyme variation in pointed gourd is available. Hence, it became worthy to know the amount of isozyme variation in pointed gourd and their usefulness to crop improvement programme. Considering the above view in mind, the present investigation was undertaken.

Materials and Method

The experiment was conducted with pointed gourd genotypes in polyacrylamide gel system using acid phosphatase, peroxidase, and glutamate oxaloacetate transaminase enzymes. Isozyme analysis was done in the Isozyme laboratory of Horticulture Research Centre, BARI, Joydeppur during the period from January to December 2004. For isoenzyme analysis, young leaf sample of the 64 pointed gourd gremplasm were collected and kept under ice before use. One gram of pointed gourd leaf for each genotype was weighted and grounded with small amount of sea sand as a crushing agent with one ml extraction buffer in mortar with pstle. Extraction buffer consisted of 0.24% Tris and 5% sucrose. The crude homogenate was centrifuged at 14,000 rpm at 4°C for 20 minutes. The samples were then divided into two parts and 25 µl was used immediately and rest was kept at -5°C in a refrigerator until use. Vertical electrophoresis unit was used to run the gel. Gels were prepared from stock solution of Acrylamide (29.2 g). Bis (0.8g), and Tris (18.17 g), and ammonium per sulphate (0.lg) by weight and were dissolved in water and pH was adjusted to 8.8 and finally the volume was made upto 100 ml by adding distilled water. An amount of 0.1g ammonium per sulfate was dissolved in I ml of distilled water. This solution was prepared just before using. Electrode buffer was also used by dissolving in Tris (1.2g) and 5.8 g glycine in about 150 ml distilled water and the volume was made to 200 m1 and diluted 10 times when used. An amount of 100 mg of Bromophenole Blue (BPB) was dissolved in 80 ml distilled water and the volume was made to 100 ml. Electrophoresis of the protein of leaf sample was carried out using PAGE technique and the gel were stained for phosphatase, peroxidase, and glutamate oxaloacetate transaminase isoenzymes. The electrophoresis was carried out at a constant current of 220 volt. 15 Amp per gel until the BPB dye began to run off the gel in approximately 4.5 hrs. The electrode buffer level was monitored carefully, when the level of the buffer was low, the filling up was done with additional buffer. The gel was stained for acid phospatage, peroxidase and glutamate oxaloacetate transaminase following the procedure (Table 1). After staining, the gel was washed with distilled water gently. Then the gel was preserved and scanned by *hp* scanner using a computer. Banding patterns were recorded on graph paper and zymograms were drawn to scale.

Table 1. Staining conditions of enzymes with fixing agents.

Enzyme	Temperature	Staining time	Others	Fixing agents
Acidphosphatase (ACP)	37^0C	1-12 hours	Continuous shaking in the dark	50% glycerol
Peroxidase (PER)	30^0C	2-5 minutes	Continuous shaking in the dark	50% glycerol
Glutamateoxaloacetate transaminase	Room temperature	1-2 hours	Incubated at room temperature in the dark	50% glycerol

Isozyme banding patterns were recorded on the basis of number and the relative front (Rf) values of the bands following Mouemar and Gasquez (1983). In zymogram analysis. Rf values were used. Rf values were calculated for each band based upon the migration of the band relative to the front.

$$\text{Rf values} = \frac{\text{Distance traveled by the band from the tip of the running gel}}{\text{Distance traveled by the tracking dye}}$$

Similarity coefficients were calculated using Nei and Li's (1979) index. Cluster analysis was done employing the unweighted pair group method using arithmetic averages (UPGMA). For cluster analysis of overall isozyme electrophoretic patterns, the value I was put for the presence of the electrophoretic pattern and value 0 was used against the absence of the pattern for each genotype. Zymotypes were used for clustering and the Euclidean distance method was used for the dissimilarity (Nourish, 1993). The original data was transformed to Z-scores prior to cluster analysis (Anderburg. 1973: Romesburg, 1984). Differences in mobility of enzyme bands were used for zymogram analyses and to find out genetic variability, genetic distance and genetic differentiation of genotype.

Results and Discussion

Acid phosphatase enzyme variability

Eight electrophoretic patterns (A_1-A_8) were observed in this enzyme system formed by 19 bands at different Rf values varying from 0.19 to 0.82 (Plate 1, Plate 2 & Fig. 1). Genotypes under each acid phosphatase zymotypes are listed in Table 2. It appears that the electrophoretic zymotype A_2 was the most frequent (20.31 %) followed by A_l and A_3 (17.18 %), A6 (14.06 %), and A_5 (10.96 %). On the contrary, zymotypes A_8 and A_7 as well as A_4 were the least frequent showing the presence of only 7.81 % and 6.25 % genotype, respectively. The highest number of bands was found in A_7 (4 bands), while A_8 was characterized by the lowest number of hands (1 band). The zymotypes A_2 and A_4 were comprised of three bands each. There were two bands in each zymotypes A_l, A_3, A_5, and A_6.

The highest frequency of bands was observed at Rf value 0.48. Bands at Rf value 0.31 was second frequent (34.49%) in the pointed gourd accession followed by the bands at Rf value 0.19 (34.36%), Rf value 0.24 (28.l2%i, Rf value 0.41 (23.43%), Rf value 0.71 (20.31%), Rf value 0.60 (17.18%), and Rf value 0.70 (14.06%) (Table 3).

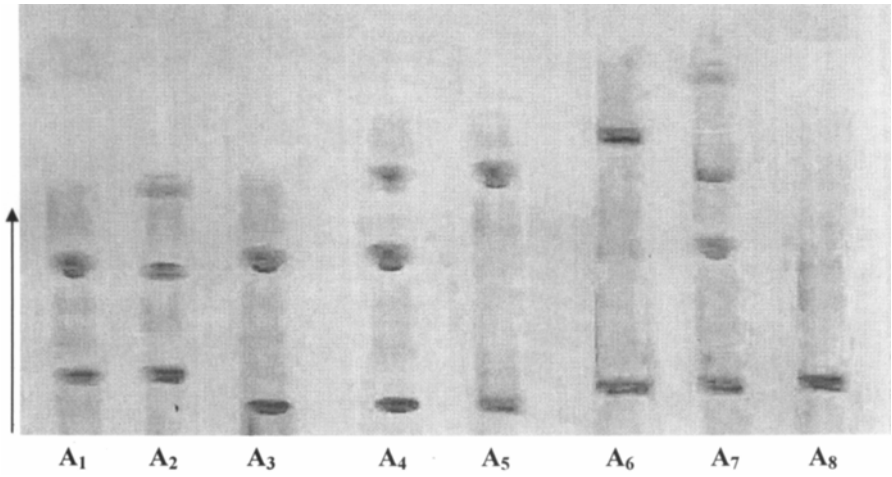

Plate 1. Electrophoretic patterns (A_1-A_8) of acid phosphatase isozyme in pointed gourd.

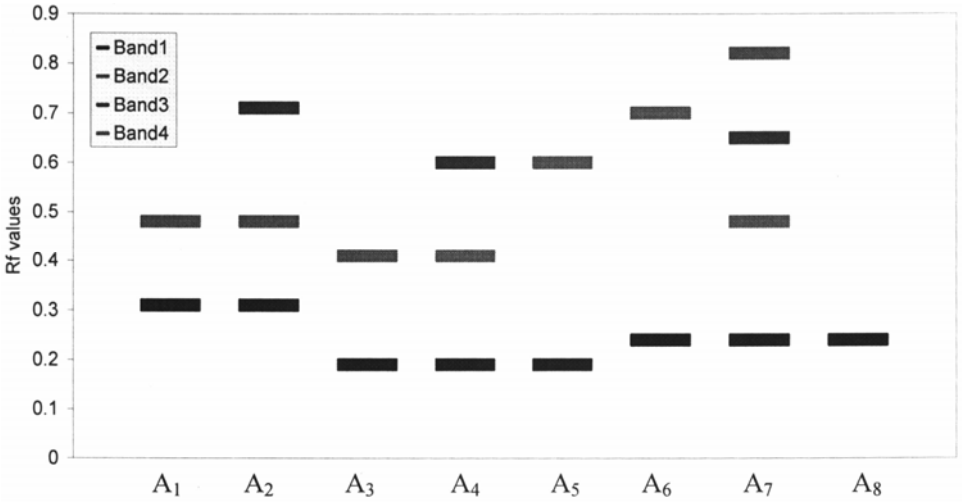

Fig. 1. Zymogram of electrophoretic patterns (A_1-A_8) of acid phosphatase isozyme of pointed gourd.

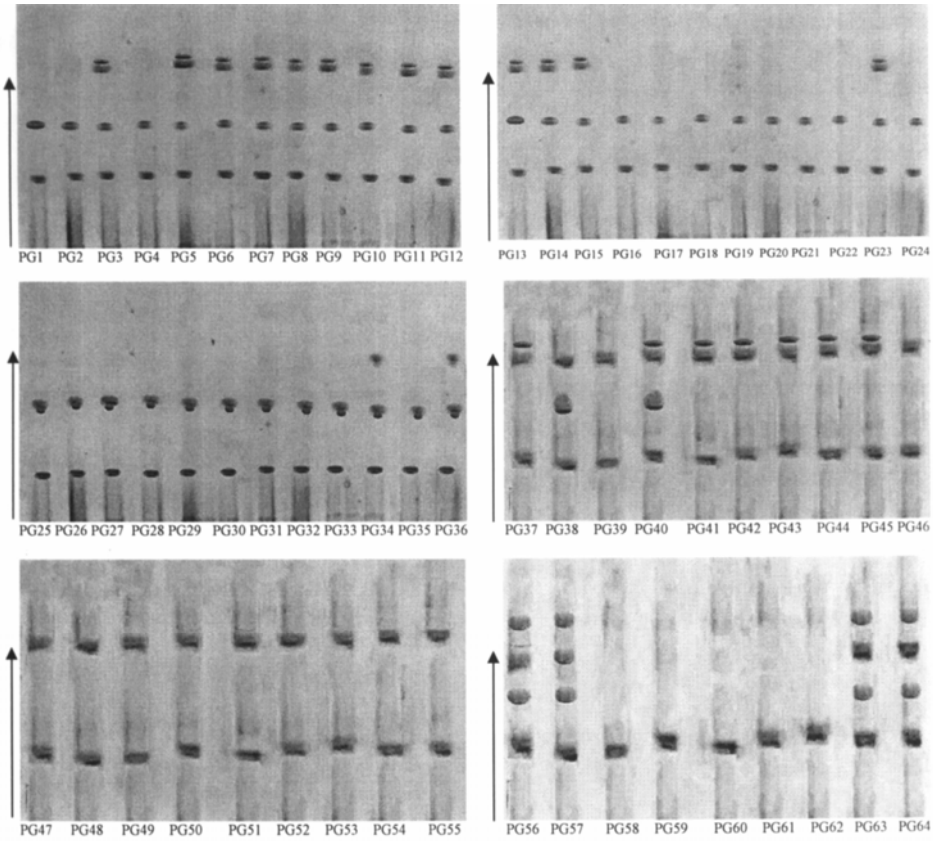

Plate 2 Acid phosphatase electrophoretic banding pattern of different genotypes of pointed gourd (PGOO1-PG064) (arrow indicates the direction of migration).

Band at Rf value 0.48 was the unique band of acid phosphates, which was common in A_1, A_2, and A_7 Zymotypes and was distributed among 43.74 %. A band with Rf value 0.71 of A_2 was found in 20.31 % of the total pointed gourd genotypes. Bands at Rf values 0.65 and 0.82 were found to be least frequent (6.25 %). Occurrence of wide variability in acid phosphatase activities was found in different pointed gourd genotypes.

Table 2. Zymotypes from the electrophoretic patterns of acid phosphatase isozyme in pointed gourd.

Zymotype	Total number of genotypes	% Genotypes	Genotypes
A_1	11	17.18	PGO0I, PG002, PG004, PGOI6, PGO17, PGOI8, PGO19, PGO2O PG021, PG022 and PG024
A_2	13	20.31	PGOO3, PG005, PGOO6, PG006, PGOO7, PGOO8, PGOO9, PGO1O, PGO1 1, PGOI2, PGO13, PGOI4, PGOI5 and PG023
A_3	11	17.18	PG025, PG026, PG027, PG028, PG029, PG030, PGO31, PG032, PG035, PG037 and PG039
A_4	4	6.25	PG034 , PG036 and PGO4O
A_5	7	10.93	PGO4I, PG042, PG043, PG044, PG045 and PG046
A_6	9	14.06	PG047, PG048, PG049, PGO5O, PGO51, PG052, PG053, PG054 and PG055
A_7	4	6.25	PG056, PG057, PG063 and PG064
A_8	5	7.81	PG058, PG059, PG060, PG061, and PG062

Acid phosphatase enzyme zymotype analysis

Similarity coefficient between acid phosphatase pairs found in pointed gourd genotypes is presented in Table 4. The maximum similarity co-efficient of 80 % was found between A_1 and A_2; A_3 and A_4; A_4 and A_5 electrophoretic patterns showing strong associations followed by 67% similarity between A_6 and A_8.

Table 3 Distribution of acid phosphatase bands among the zymotypes of pointed gourd genotypes.

Zyrnotypes	Rf value of bands										No. of bands
	0.19	0.24	0.31	0.41	0.48	0.60	0.65	0.70	0.71	0.82	
Al			√		√						2
A2			√		√				√		3
A3	√			√							2
A4	√			√		√					3
A5	√					√					2
A6		√						√			2
A7		√			√		√			√	4
A8		√									1
Bands frequency(%)	34.36	28.12	34.49	23.43	43.74	17.18	6.25	14.06	0.31	6.25	
Total bands											19

Electrophoretic zymotypes A_3 and A_5 showed only 50% genotype, while rest of the pairs of the electrophoretic zymotypes showed 0 to 40% similarity. Such similarity among the electrophoretic patterns indicates a wide genetic diversity among the genotypes of pointed gourd. Gorman and Kiang (1977) also observed distinct variety- specific electrophorelic zymograms for acid phosphatase (ACP) in commercial varieties of soybean.

Table 4 Similarity coefficient values of eight acid phosphatase banding pattern as observed in different pointed gourd genotypes.

Zynotype	A_1	A_2	A_3	A_4	A_5	A_6	A_7
A_2	0.80						
A_3	0	0					
A_4	0	0	0.80				
A_5	0	0	0.50	0.80			
A_6	0	0	0	0	0		
A_7	0	0.28	0	0	0	0.34	
A_8	0	0	0	0	0	0.67	0.40

Peroxidase enzyme variability

Seven electrophoretic zymotypes (P_1-P_7) were observed in peroxidase enzyme system formed by 11 bands at different Rf values varying from 0.38 to 0.69 (Plate 3, Plate 4 & Fig.2). The genotypes under each peroxidase zymotypes are

listed in Table 5. The zymotype P_6 was the most frequent which included 26.56 % of the total genotypes of pointed gourd. The zymotypes P_2 and P_4 were found to be the next frequent of 15.6 %. On the contrary, the zymotype P_1 was the least frequent showing presence of only 6.25 %. The zymotypes P_2, P_4, P_6 and P_7 comprised two bands each, while the genotypes P_1, P_3 and P_5 had one hand each (Table 5). Bands at Rf value 0.40 and 0.69 were found to be most frequent and were the unique bands for peroxidase, which was common in P_5 and P_6 and P_6 and P_7 zymotypes and was distributed among 39.06 % and 34.49 % of the pointed gourd genotypes, respectively. Absence of unique band in rest of the population may be due to natural mutation. Bands at Rf value 0.45 was found in P_3 and P_4 pattern, which existed of 28.1% of the total genotypes.

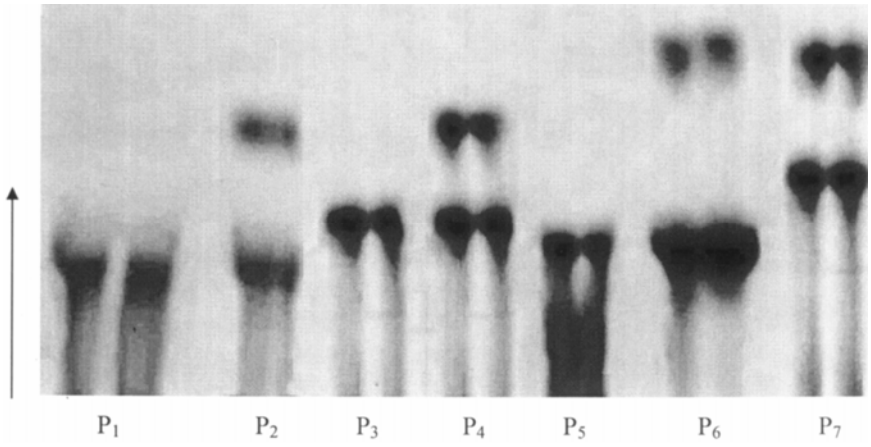

Plate 3. Electrophoretic patterns (P_1-P_7) of peroxidase isozyme in pointed gourd.

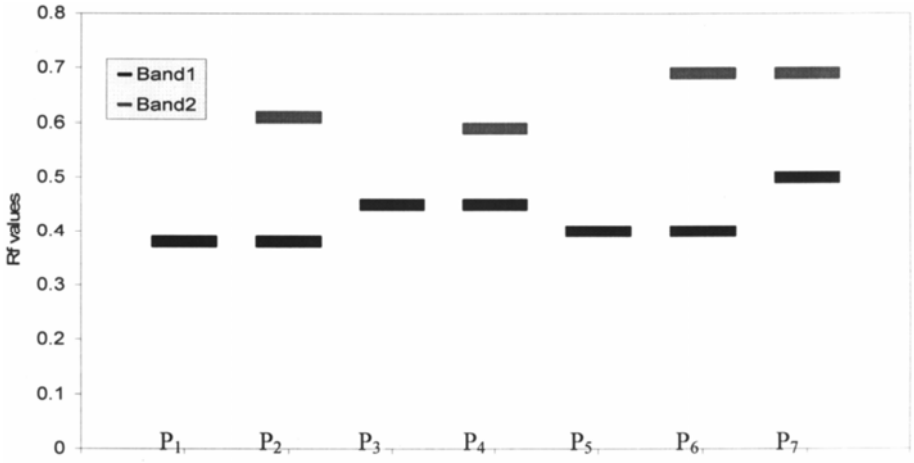

Fig. 2. Zymogram of electrophoretic patterns (P_1-P_7) of peroxidase isozyme of pointed.

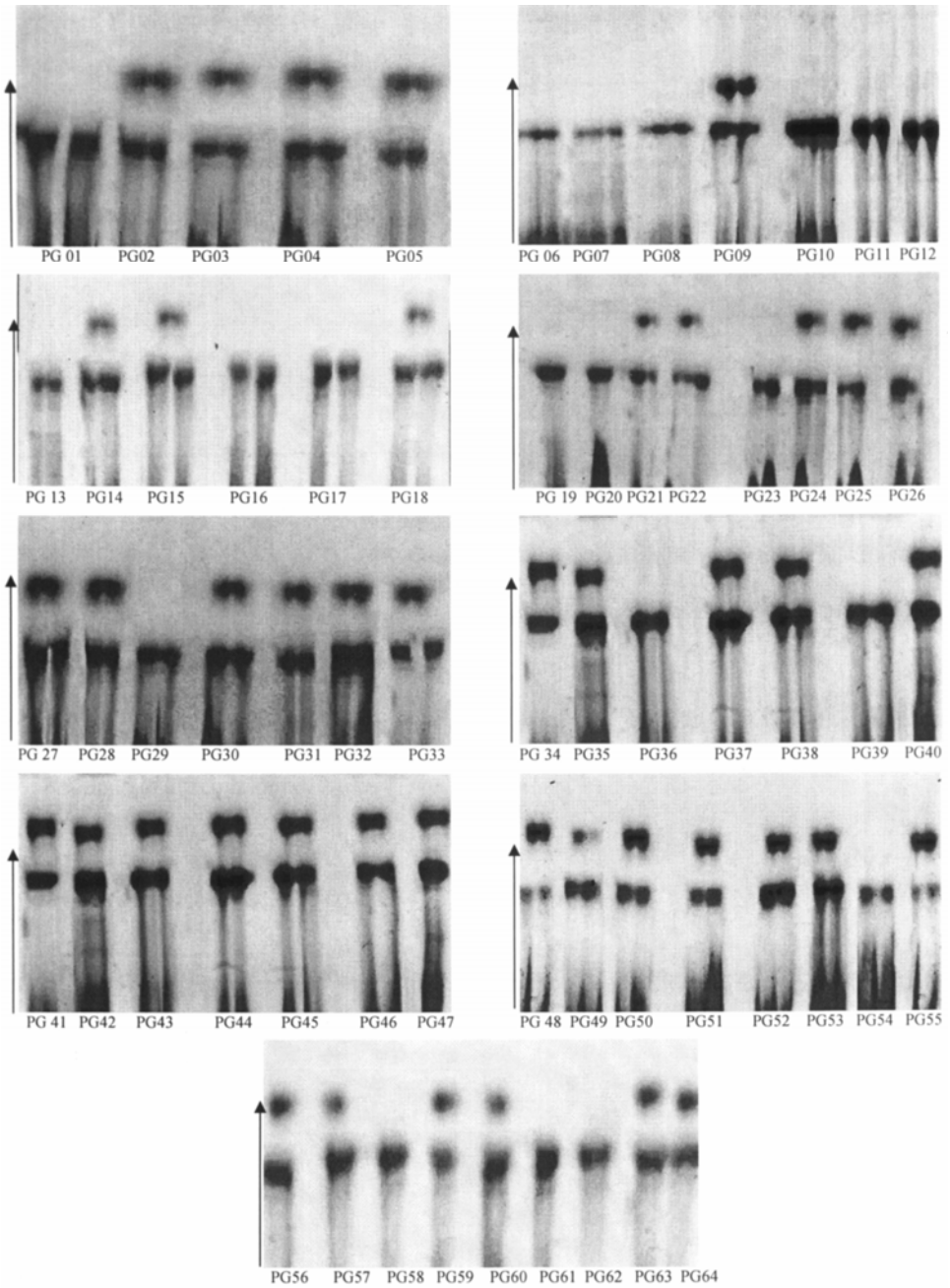

Plate 4. Peroxidase electrophoreticbanding pattern of different genotypes of pointed gourd (PG001-PG064) (arrow indicates the direction of migration of samples.

Table 5. Zymotypes from the electrophoretic patterns of peroxidase isozyme in pointed gourd.

Zyrnotypes	Total number of genotypes	% Genotypes	Genotypes
P_1	4	6.25	PGOO1, PG058, PGO61 and PG062
P_2	10	15.6	PGOO2, PGOO3, PGOO4, PGOO5, PG056, PG057, PG059, PGO6O, PG063 and PG064
P_3	8	12.5	PG009, PG007, PG008, PG010, PG011, PG013, and PG54
P_4	10	15.6	PGOO9, PG014, PGOI5, PG048, PG049, PGO5O, PGO5I, PG052, PG053 and PG055
P_5	8	12.5	PGOI6, PGOI7, PGOI9, PGO2O, PG023, PG029, PGO 36 and PG039
P_6	17	26.56	PGOI8, PGO2I, PG022, PG024, PG025, PG026, PG027, PG028, PGO3O, PGO 31, PG032, PGO 33, PG034, PG035, PG037, PG038 and PGO4O
P_7	7	10.93	PG041, PG042, PG043, PG044, PG045, PG046 and, PG047

Those three bands discussed above constituted the electrophoretic pattern of P_3, P_4, P_5, P_6, and P_7 and covered most of the genotypes, which might be the original zymotypes. Bands at Rf value 0.38, 0.59, and 0.61 and.50 were found to be 21.38%, 15.6%, and 10.93% 01 of the pointed gourd genotypes (Table 5). Occurrence of a wide variability in pcroxides activities was found in different pointed gourd genotypes.

Table 6 Distribution of peroxidase bands among the zymotypes of pointed gourd accession.

Zyrnotypes	Rf value of bands							No. of bands
	0.38	0.40	0.45	0.50	0.59	0.61	0.69	
P_1	√							1
P_2	√					√		2
P_3			√					1
P_4			√		√			2
P_5		√						1
P_6		√					√	2
P_7				√			√	2
Bands frequency (%)	21.85	39.06	28.1	10.93	15.6	15.6	34.49	
Total bands								11

Peroxidase enzyme zymotype analysis

Similarity co-efficient between peroxidase pairs observed in pointed gourd genotypes are presented in Table 7. The maximum similarity co-efficient of 66 % was found between P_1 and P_2; P_3 and P_4 and P_5 and P_6 electrophoratic pattern showing strong association. The rest of the pairs showed no similarity at all. The wide range of 0-66 % similarity co-efficient among the electrophoretic patterns is the indication of wide genetic diversity among the 64 genotypes of pointed gourd. Tuwafe *et al.* (1988) reported one isozyme banding zone for peroxidase, while studying with chickpea germplasm electrophoretically. Dvorak and Cernohorska (1967), Loy (1972) and Denna and Alexander (1975) examined the peroxidase enzyme in *C. pepo.* They observed polymorphism in this isozyme system, which indicated that *C. pepo,* possesses significant level of inherent allozymic variation. Dane (1976) and Esquinas-Alcazar (1977) also reported dissimilar results. They did not found any difference in peroxidase banding pattern in *C. sativus.*

Table 7. Similarity coefficient values of seven peroxidase banding patterns as observed in different pointed gourd genotypes.

Zymotype	P_1	P_2	P_3	P_4	P_5	P_6
P_2	0.66					
P_3	0	0				
P_4	0	0	0.66			
P_5	0	0	0	0		
P_6	0	0	0	0	0.66	
P_7	0	0	0	0	0.00	0

Glutamate oxaloacetate transaminase enzyme variability

Nine electrophoretic zymotypes (G_1-G_9) were observed in glutamate oxaloacetate transaminase enzyme system formed by 19 bands at different Rf values varying from 0.15 to 0.95 (Plate 5, Plate 6 & Fig 3). The genotypes under each glutamate oxaloacetate transaminase zymotypes are listed in Table 8. It appears that electrophoretic zymotype G_9 was the most frequent (15.62 %) followed by G_4 and G_5 (14.06 %), G_8 (12.5 %) and (ii and G_4 (9.37 %). On the other hand, zymotype G_2 was least frequent showing only 4.68 %. The zymotypes G_2 and G_3 had the maximum number of bands (3 bands). While G_9 was characterized by the lowest number of bands (1 band). The zymotypes G_1, G_4, G_5, G_6, G_7 and G_8 were comprised of two bands in each (Table 9). Common band was found in all the zymotypes.

Table 8. Zymotypes from the electrophoretic patterns of glutamate oxaloacetate transaminase isozyme in pointed gourd.

Zyrnotype	Total number of genotype	% Genotypes	Genotypes
G_1	6	9.37	P0001, P0002, P0003 P0004, P0006 and P0007
G_2	3	4.68	PG005, PG008, and PG021
G_3	6	9.37	PG0010, P00011, P0012, P0013, PG014 and PG0l5
G_4	9	14.06	P0016, P0017, PG018, PG019, PG020, P0021, PG022, P0023 and P0024
G_5	9	14.06	PG025, P0026, PG027, P0028, P0029, PG030, PG0 31, PG032 and PG033
G_6	6	9.37	P0034, P0035, P0036, PG037, PG038, and P0039
G_7	7	10.93	P0040, P0041, PG042, P0043, P0044, PG045 and PG046
G_8	8	12.5	P0047, PG048, P0049, PGO5O, PG051, P0052, PG053 and PG054
G_9	10	15.62	PG055, PG056, P0057, P0058, PG059, P0060, P0061, P0062, PG063 and PG064

These results are in agreement with the findings of Rahman and Nito (1994). They observed that enzymatic activity of glutamate oxaloacetate transaminase was found to be polymorphic and controlled by three zone and displayed five banding pattern in the species of Kumquat *(Fortunella).* Bands at Rf value 0.22 was the unique band for glutamate oxaloacetate transaminase which was common in G_3, G_4 and G_7 zymotypes and was distributed among 34.36 % pointed gourd genotype (Table 9). Band at Rf value 0.54 was the second frequent band (28.12 %) in the pointed gourd genotype followed by the bands at Rf value 0.60 (23.4 %), Rf value 0.37 *(15.62* %), Rf value 0.15 (14.06 %), Rf value 0.29 (14.05 %), Rf value 0.51 as well as 0.22 *(12.5* %), Rf value 0.61 (10.93), and Rf value 0.41, as well as 0.73 and 0.95 (9.37 %).

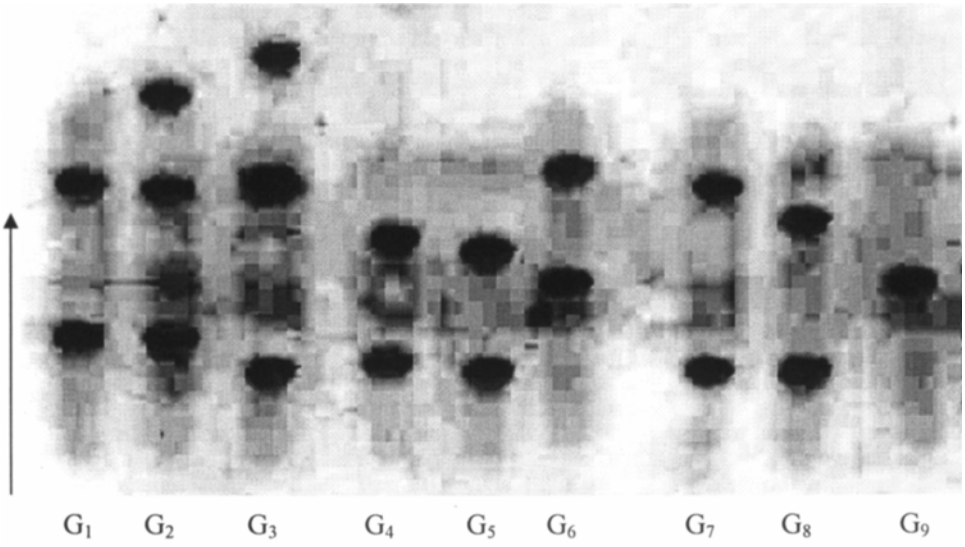

Plate 5. Electrophoretic patterns (G1-G9) of glutamate oxaloacetate transaminase isozyme in pointed gourd.

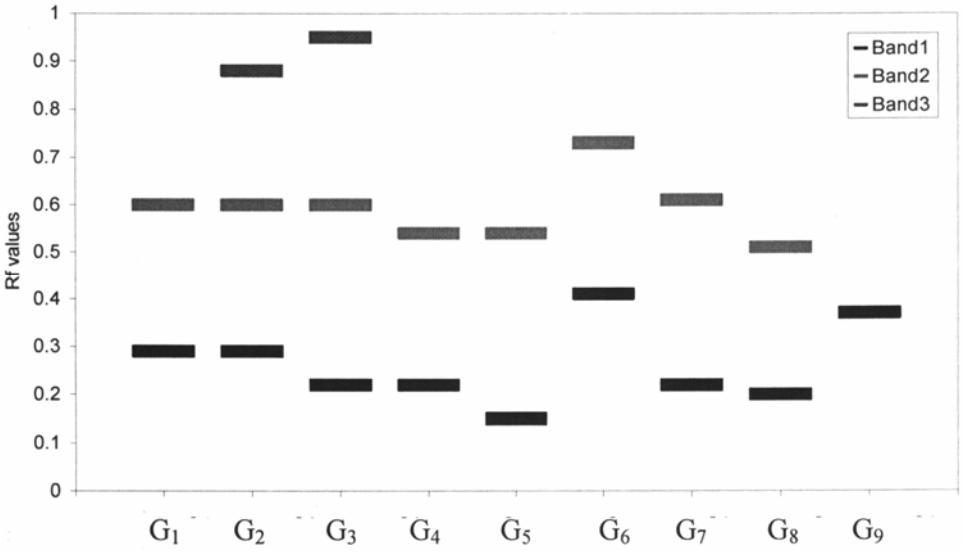

Fig. 3. Zymogram of electrophoretic (G1-G9) of glutamate oxaloacetate transminase isozyme in pointed gourd.

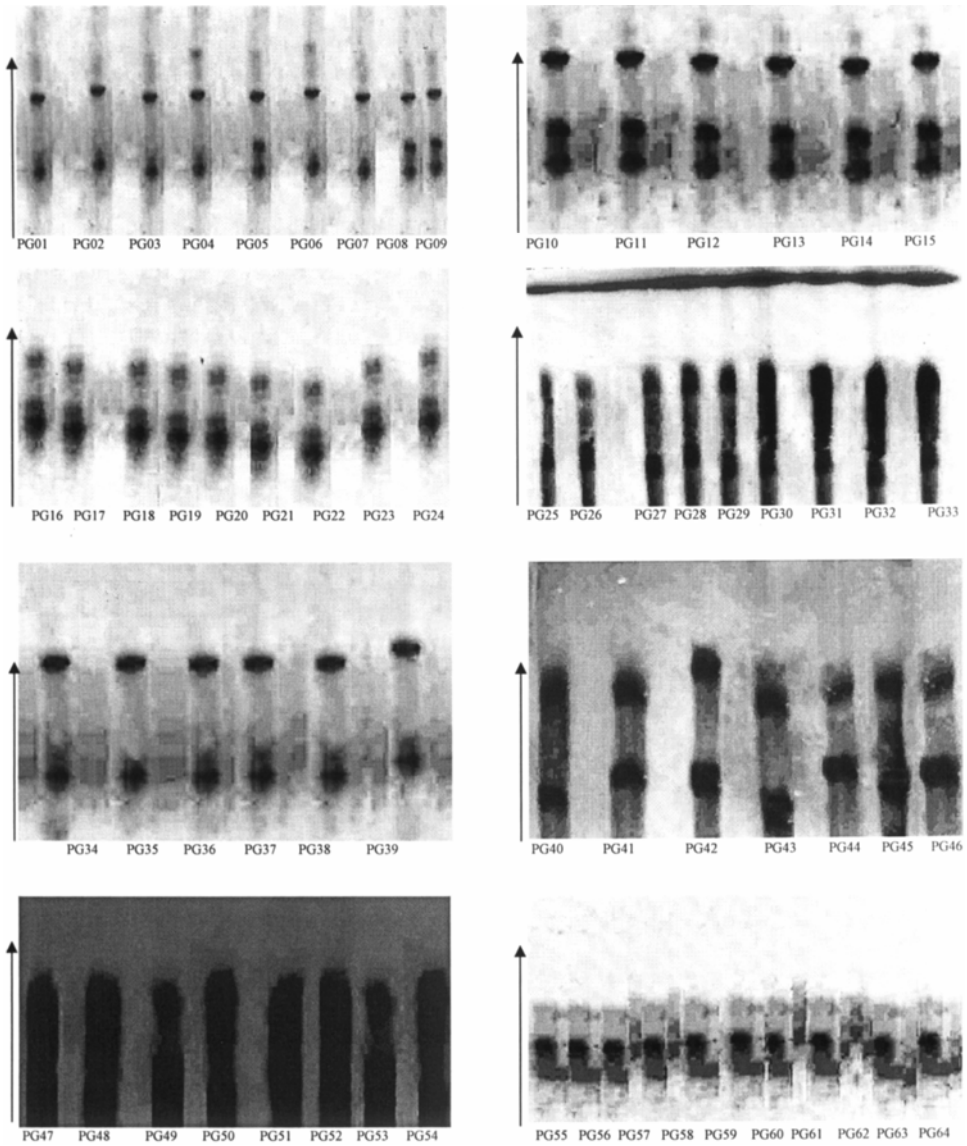

Plate 6. Glutamate oxaloacetate transaminase electrophoretic banding pattern of different genotympes of pointed gourd (PG001-PG064) (arrow indicates the direction of migration of samples).

One common band was found among the zymotypes G_1, and G_2. A band with Rf value 0.37 is the characteristics of zymotype G_9 was found frequent (15.62 %) of total pointed gourd genotypes. Bands at Rf value 0.88 found in the zymotypes G_2 was least frequent 4.68 % (Table 8). Occurrence of a wide variability in

glutamate oxaloacetate transaminase activities was found in different pointed gourd genotypes.

Table 9. Distribution of glutamate oxaloacetate transminase (GOT) bands among the zymotypes of pointed gourd genotype.

Zymotypes	RF value of bands													No. of bands
	0.15	0.20	0.22	0.29	0.37	0.41	0.51	0.54	0.60	0.61	0.73	0.88	0.95	
G_1				√					√	√				2
G_2				√					√			√		3
G_3			√						√				√	3
G_4			√					√						2
G_5	√							√						2
G_6						√					√			2
G_7			√							√				2
G_8		√					√							2
G_9					√									1
Bands frequency (%)	14.06	12.5	34.36	14.05	15.62	9.37	12.5	28.12	23.42	10.93	9.37	4.67	9.37	
Total bands														19

Glutamate oxaloacetate transaminase enzyme zymotype analysis

Similarity co-efficient between glutamate oxaloacetate transaminase zymotype pairs found in pointed gourd is presented in Table 10. The highest similarity co-efficient of 80% was found between G_1 and G_2, showing strong association followed by 50% similarity between G_4 and G_5 and G_4 and G_7. Electrophoretic zymotypes G_1 and G_3 as well as G_3 and G_4 showed 40% genotypes, while rest of the pairs of the electrophoretic zymotypes showed no similarity. Such variation among the electrophoretic patterns indicates a wide genetic diversity among the genotypes. Rahman and Nito (1994) investigated electrophoretic isozyme technique and concluded that Glutamate oxaloacetate transaminase was suitable for cultivar identification within most commercial classes in Kumquat *(Fortunella).*

Table 10. Similarity coefficient values of nine glutamate oxaloacetate transaminase (GOT) banding patterns as observed in different pointed gourd genotype.

Zymotype	G_1	G_2	G_3	G_4	G_5	G_6	G_7	G_8
G_2	0.8							
G_3	0.4	0.33						
G_4	0	0	0.40					
G_5	0	0	0.00	0.50				
G_6	0	0	0.00	0.00	0			
G_7	0	0	0.50	0.50	0	0		
G_8	0	0	0	0	0	0	0	
G_9	0	0	0	0	0	0	0	0

Overall isozymes variability

A total of 24 different electrophoretic zymotypes were observed for three isozymes studied in pointed gourd (Table 11). The genotypes were grouped in different electrophoretic zymotypes (A_1-A_8, P_1-P_7 and G_1-G_9) indicating considerable level of genetic diversity in the genotypes of pointed gourd collected from different parts of Bangladesh. Acid phosphatase, peroxidase, and glutamate oxaloacetate transaminase analysis showed eight, seven, and nine electrophoretic zymotypes (Table 11). The results also indicate that higher level of genetic diversity in pointed gourd population was associated with glutamate oxaloacetate transaminase as it demonstrated the highest (9) number of electrophoretic zymotypes than acid phosphatase and peroxidase (Table 11). Azad (1999) and Paudyal (1999) used the same technique in case of *Artocarpus heteraphyus* (L.) and *Citrus grandis* (L.), respectively.

Table 11. Number of isozyme zymotypes of acid phosphatase, peroxidase and glutamate oxaloacetate transaminase in pointed gourd genotype.

Enzyme system	Zymotypes	Total no. of Zymotype
Acid phosphatase	A_1-A_8	8
Peroxidase	P_1-P_7	7
Glutamate oxaloacetate transaminase	G_1-G_9	9
Total		24

A dendrogram genotypes of 64 pointed gourd genotypes was generated based on Euclidean distance (Fig. 4). The dendrogram showed 12 major groups designated as I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and XII.

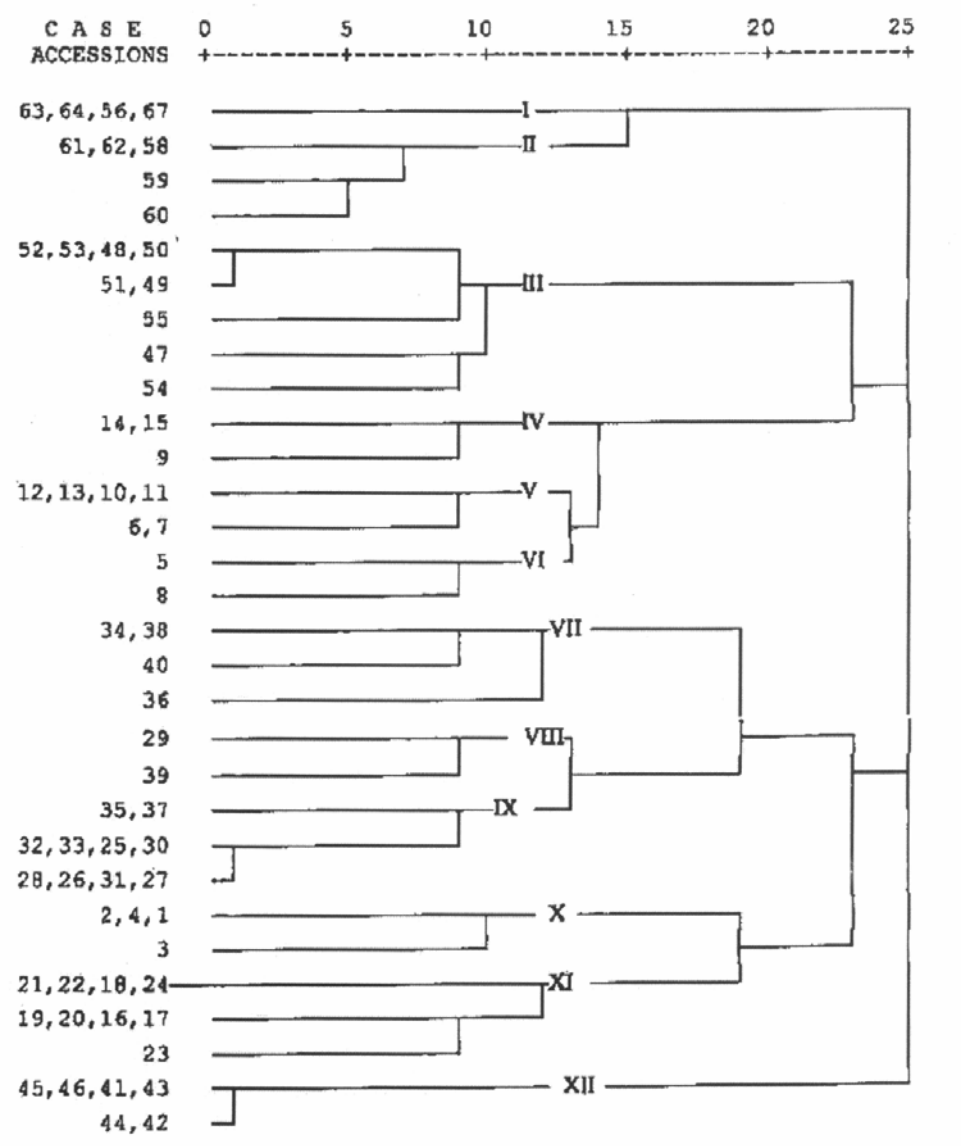

Fig. 4. Dendrogram showing hierarchical clustering of 64 pointed gourd genotypes based on isozymes type of acid phosphatase, peroxidase and GOT.

Based on the three polymorphic enzyme activities, the genotypes were grouped into twelve major clusters designated as I, II, III, IV, V, VI, VII, VIII, IX, X, XI, and XII. Ten genotypes were found under the cluster number IX,

which represented 15.62% of the total genotypes. Cluster III and cluster XI contained nine genotypes. Six accessions were grouped in cluster V and XII, followed by 5 genotypes in cluster II, 4 genotypes each of cluster I, VII, and cluster X (4). The lowest number of genotypes (2) was found in cluster VI and VIII. The genotypes collected from the same location were grouped into different clusters. Different electrophoretic zymotypes of different isozyme consisted of different number of genotype. Some of the zymotypes occurred very frequently in the genotypes and some ol them were rarely found. However, the variations in the number of zymotypes in different locations suggest higher genetic diversity in some locations and lower in other locations. The results of the present experiment indicated that wide variation exists among the gremplasm. Also through hierarchical cluster analysis, 64 pointed gourd were grouped into 12 clusters and relationship among the clusters was established which will be useful for planning future programme of pointed gourd. However, in this study only three isoenzyme systems were used. Therefore, more isoenzyme systems should be needed to proper characterization of pointed gourd accessions.

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