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

A.S.M.M.R. KHAN¹, M.G. RABBANI²
M.A. SIDDIQUE³ AND M.A. ISI AM⁴

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 gremplam 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 transaminase 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 transaminase, 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 be 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,
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 geneplasm 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 pestle. 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 (0.1g) 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 1 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 ml 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 phosphatase, 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.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Temperature</th>
<th>Staining time</th>
<th>Others</th>
<th>Fixing agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidphosphatase (ACP)</td>
<td>37°C</td>
<td>1-12 hours</td>
<td>Continuous shaking in the dark</td>
<td>50% glycerol</td>
</tr>
<tr>
<td>Peroxidase (PER)</td>
<td>30°C</td>
<td>2-5 minutes</td>
<td>Continuous shaking in the dark</td>
<td>50% glycerol</td>
</tr>
<tr>
<td>Glutamateoxaloacetate transaminase</td>
<td>Room temperature</td>
<td>1-2 hours</td>
<td>Incubated at room temperature in the dark</td>
<td>50% glycerol</td>
</tr>
</tbody>
</table>

Isozyme banding patterns were recorded on the basis of number and the relative front (Rf) values of the bands following Mouenar 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 1 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 (A1-A8) 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 A2 was the most frequent (20.31 %) followed by A4 and A3 (17.18 %), A6 (14.06 %), and A5 (10.96 %). On the contrary, zymotypes A8 and A7 as well as A4 were the least frequent showing the presence of only 7.81 % and 6.25 % genotype, respectively. The highest number of bands was found in A7 (4 bands), while A8 was characterized by the lowest number of bands (1 band). The zymotypes A2 and A4 were comprised of three bands each. There were two bands in each zymotypes A1, A3, A5, and A6.
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 (28.12%), 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 (A₁-A₈) of acid phosphatase isozyme in pointed gourd.

Fig. 1. Zymogram of electrophoretic patterns (A₁-A₈) of acid phosphatase isozyme of pointed gourd.
Plate 2 Acid phosphatase electrophoretic banding pattern of different genotypes of pointed gourd (PG001-PG064) (arrow indicates the direction of migration). Band at Rf value 0.48 was the unique band of acid phosphates, which was common in A1, A2, and A7 Zymotypes and was distributed among 43.74%. A band with Rf value 0.71 of A2 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.

<table>
<thead>
<tr>
<th>Zymotype</th>
<th>Total number of genotypes</th>
<th>% Genotypes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A_1</td>
<td>11</td>
<td>17.18</td>
<td>PGO01, PGO02, PGO04, PGO16, PGO17, PGO18, PGO19, PGO20, PGO21, PGO22 and PGO24</td>
</tr>
<tr>
<td>A_2</td>
<td>13</td>
<td>20.31</td>
<td>PGO03, PGO05, PGO06, PGO07, PGO08, PGO09, PGO10, PGO11, PGO12, PGO13, PGO14, PGO15 and PGO23</td>
</tr>
<tr>
<td>A_3</td>
<td>11</td>
<td>17.18</td>
<td>PGO25, PGO26, PGO27, PGO28, PGO29, PGO30, PGO31, PGO32, PGO35, PGO37 and PGO39</td>
</tr>
<tr>
<td>A_4</td>
<td>4</td>
<td>6.25</td>
<td>PGO34, PGO36 and PGO40</td>
</tr>
<tr>
<td>A_5</td>
<td>7</td>
<td>10.93</td>
<td>PGO41, PGO42, PGO43, PGO44, PGO45 and PGO46</td>
</tr>
<tr>
<td>A_6</td>
<td>9</td>
<td>14.06</td>
<td>PGO47, PGO48, PGO49, PGO50, PGO51, PGO52, PGO53, PGO54 and PGO55</td>
</tr>
<tr>
<td>A_7</td>
<td>4</td>
<td>6.25</td>
<td>PGO56, PGO57, PGO63 and PGO64</td>
</tr>
<tr>
<td>A_8</td>
<td>5</td>
<td>7.81</td>
<td>PGO58, PGO59, PGO60, PGO61, and PGO62</td>
</tr>
</tbody>
</table>

Acid phosphatase enzyme zymotype analysis

Similarity coefficient between acid phosphatase pairs found in pointed gourd genotypes is presented in Table 4. The maximum similarity coefficient 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.

<table>
<thead>
<tr>
<th>Zymotypes</th>
<th>Rf value of bands</th>
<th>No. of bands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.19</td>
<td>0.24</td>
</tr>
<tr>
<td>A1</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>A2</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>A3</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>A7</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>A8</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

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 A3 and A5 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 electrophoretic 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.

<table>
<thead>
<tr>
<th>Zymotype</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>0</td>
<td>0</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>0</td>
<td>0</td>
<td>0.50</td>
<td>0.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A7</td>
<td>0</td>
<td>0.28</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.34</td>
</tr>
<tr>
<td>A8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.67</td>
</tr>
</tbody>
</table>

Peroxidase enzyme variability

Seven electrophoretic zymotypes (P1-P7) 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 band 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_3 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 electrophoretic banding 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.

<table>
<thead>
<tr>
<th>Zymotypes</th>
<th>Total number of genotypes</th>
<th>% Genotypes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_1$</td>
<td>4</td>
<td>6.25</td>
<td>PG001, PG058, PG061 and PG062</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PG002, PG003, PG004, PG005, PG056, PG057, PG059, PG060, PG063 and PG064</td>
</tr>
<tr>
<td>$P_2$</td>
<td>10</td>
<td>15.6</td>
<td>PG009, PG007, PG008, PG010, PG011, PG013, and PG54</td>
</tr>
<tr>
<td>$P_3$</td>
<td>8</td>
<td>12.5</td>
<td>PG009, PG014, PG015, PG048, PG049, PG050, PG051, PG052, PG053 and PG055</td>
</tr>
<tr>
<td>$P_4$</td>
<td>10</td>
<td>15.6</td>
<td>PG016, PG017, PG019, PG020, PG023, PG029, PG036 and PG039</td>
</tr>
<tr>
<td>$P_5$</td>
<td>8</td>
<td>12.5</td>
<td>PG018, PG021, PG022, PG024, PG025, PG026, PG027, PG028, PG030, PG31, PG032, PG033, PG034, PG035, PG037, PG038 and PG040</td>
</tr>
<tr>
<td>$P_6$</td>
<td>17</td>
<td>26.56</td>
<td>PG041, PG042, PG043, PG044, PG045, PG046 and PG047</td>
</tr>
<tr>
<td>$P_7$</td>
<td>7</td>
<td>10.93</td>
<td>PG041, PG042, PG043, PG044, PG045, PG046 and PG047</td>
</tr>
</tbody>
</table>

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 0.50 were found to be 21.38%, 15.6%, and 10.93% of the pointed gourd genotypes (Table 5). Occurrence of a wide variability in peroxides activities was found in different pointed gourd genotypes.

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

<table>
<thead>
<tr>
<th>Zymotypes</th>
<th>Rf value of bands</th>
<th>No. of bands</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_1$</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$P_2$</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>$P_3$</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$P_4$</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>$P_5$</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>$P_6$</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>$P_7$</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

| 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 P1 and P2; P3 and P4 and P5 and P6 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.

<table>
<thead>
<tr>
<th>Zymotype</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
<th>P4</th>
<th>P5</th>
<th>P6</th>
</tr>
</thead>
<tbody>
<tr>
<td>P2</td>
<td></td>
<td>0.66</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>P6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.66</td>
</tr>
<tr>
<td>P7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Glutamate oxaloacetate transaminase enzyme variability

Nine electrophoretic zymotypes (G1-G9) were observed in glutamate oxaloacetate transaminase 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 G9 was the most frequent (15.62 %) followed by G4 and G3 (14.06 %), G8 (12.5 %) and G1 (9.37 %). On the other hand, zymotype G2 was least frequent showing only 4.68 %. The zymotypes G2 and G3 had the maximum number of bands (3 bands). While G9 was characterized by the lowest number of bands (1 band). The zymotypes G1, G4, G5, G6, G7 and G8 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.

<table>
<thead>
<tr>
<th>Zymotype</th>
<th>Total number of genotype</th>
<th>% Genotypes</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>G₁</td>
<td>6</td>
<td>9.37</td>
<td>P0001, P0002, P0003, P0004, P0006 and P0007</td>
</tr>
<tr>
<td>G₂</td>
<td>3</td>
<td>4.68</td>
<td>PG005, PG008, and PG021</td>
</tr>
<tr>
<td>G₃</td>
<td>6</td>
<td>9.37</td>
<td>PG0010, P00011, P0012, P0013, PG014 and PG015</td>
</tr>
<tr>
<td>G₄</td>
<td>9</td>
<td>14.06</td>
<td>P0016, P0017, PG018, PG019, PG020, P0021, PG022, P0023 and P0024</td>
</tr>
<tr>
<td>G₅</td>
<td>9</td>
<td>14.06</td>
<td>PG025, P0026, PG027, P0028, PG029, PG030, PG031, PG032 and PG033</td>
</tr>
<tr>
<td>G₆</td>
<td>6</td>
<td>9.37</td>
<td>P0034, P0035, P0036, PG037, PG038, and P0039</td>
</tr>
<tr>
<td>G₇</td>
<td>7</td>
<td>10.93</td>
<td>P0040, P0041, PG042, P0043, P0044, PG045 and PG046</td>
</tr>
<tr>
<td>G₈</td>
<td>8</td>
<td>12.5</td>
<td>P0047, PG048, P0049, PG050, PG051, P0052, PG053 and PG054</td>
</tr>
<tr>
<td>G₉</td>
<td>10</td>
<td>15.62</td>
<td>PG055, PG056, P0057, P0058, PG059, P0060, P0061, P0062, PG063 and PG064</td>
</tr>
</tbody>
</table>

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 G₃, G₄ and G₇ 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 genotypes of pointed gourd (PG001-PG064) (arrow indicates the direction of migration of samples).

One common band was found among the zymotypes G<sub>1</sub> and G<sub>2</sub>. A band with Rf value 0.37 is the characteristics of zymotype G<sub>9</sub> was found frequent (15.62 %) of total pointed gourd genotypes. Bands at Rf value 0.88 found in the zymotypes G<sub>2</sub> 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 transaminase (GOT) bands among the zymotypes of pointed gourd genotype.**

<table>
<thead>
<tr>
<th>Zymotypes</th>
<th>RF value of bands</th>
<th>No. of bands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>G_1</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>G_2</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>G_3</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>G_4</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>G_5</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>G_6</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>G_7</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>G_8</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>G_9</td>
<td>√</td>
<td></td>
</tr>
</tbody>
</table>


**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.

<table>
<thead>
<tr>
<th>Zymotype</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>0.4</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>0</td>
<td>0</td>
<td>0.40</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G5</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6</td>
<td>0</td>
<td>0</td>
<td>0.00</td>
<td>0.00</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G7</td>
<td>0</td>
<td>0</td>
<td>0.50</td>
<td>0.50</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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 (A1-A8, P1-P7 and G1-G9) 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.

<table>
<thead>
<tr>
<th>Enzyme system</th>
<th>Zymotypes</th>
<th>Total no. of Zymotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase</td>
<td>A1-A8</td>
<td>8</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>P1-P7</td>
<td>7</td>
</tr>
<tr>
<td>Glutamate oxaloacetate transaminase</td>
<td>G1-G9</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>24</td>
</tr>
</tbody>
</table>

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.
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 of 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 germplasm. 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.

References


