Polymorphism Assessment of Six Lentil (*Lens culinaris* Medik.) Genotypes Using Isozyme

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Abstract

*Lentil* (*Lens culinaris* Medik.) is one of the important legumes and cheaper source of protein in Bangladesh that displays great biological diversity. Isozyme, one of the most important protein markers to detect genetic polymorphism in lentil, whereas we considered thirteen- isozyme in six varieties viz., BARI masur-1, BARI masur-2, BARI masur-3, BARI masur-4, BARI masur-5 and BARI masur-6. The highest polymorphism was found in tyrosinase isozyme system. UPGMA analysis revealed that the highest similarity between BARI masur-5 and BARI masur-6 whereas, the highest genetic distance between BARI masur-1 and BARI masur-5 reflecting higher intervarietal variation. Principal component analysis (PCA) also revealed the similar results that of unweighted pair group method with arithmetic mean (UPGMA). The first, second and third PCs contributed 81.58%, 11.19% and 4.94% variation respectively, with cumulative variation of the first three PCs was 75.45%. Consequently, Isozyme could clearly assed the genetic diversity at intervarietal levels and these two varieties can be considered as valuable gene resources for future breeding and conservation programs.

Key words: *Lens culinaris* Medik, Genetic Polymorphism, Lentil, Isozyme.

1. Introduction

*Lentil* is one of the oldest food crops of mankind that researchers have traced back to 7000 - 8000 BC and probably originated from fertile crescent from where it spread to adjacent regions of west Asia and Africa and later to Europe and North America[1,2]. Worldwide, lentil is grown on total area of 1.8 million ha, of which 60% is in the South Asian region, which includes the lentil producing countries of Bangladesh, Burma, India, Nepal and Pakistan[3]. It is an annual, herbaceous diploid (2n = 2x = 14) and autogamous species that has the ability to fix atmospheric nitrogen symbiotically with certain bacteria (such as *Rhizobium leguminosarum*) and thus contributes greatly to soil fertility[4,5]. Lentils are a good source of proteins and other important nutrient elements. 100 g of dried seeds contain 340-346 g calories, 12% moisture, 20.2 g protein, 0.6 g fat, 65.0 g total carbohydrate, about 4 g fiber, 2.1 g ash, 68 mg Ca, 325 mg P, 7.0 mg Fe, 29 mg Na, 780 mg K, 0.46 mg thiamine, 0.33 mg riboflavin, 1.3 mg niacin[6,7]. Lentil shows high genetic variation both in morphological and molecular level. Many researchers have reported on genetic variation in lentil about morphological variation; isozyme variation and seed storage protein level variation as well[8-11]. Traditional breeding is very much important for crop improvement but they proved to be slow in targeting complex trait like grain yield, grain quality, and drought or salinity tolerance. To meet the great need to increase food production necessitates by increasing population growth, biotechnology offers novel and powerful tools to assist and complement the breeding efforts[12]. Molecular marker Isozyme is one of the most significant protein marker which has proven to be a powerful tool for estimating genetic diversity in germplasm collections, identifying cultivars and selections, studying the inheritance of qualitative and quantitative traits, and determining linkage relationships to aid in the construction of genetic maps[13]. For better management of genebank, a precise comprehensive knowledge of

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agricultural and biochemical data (protein and DNA) is essential. No single method is adequate for assessing genetic variation because the different methods sample genetic variation at different levels and differ in their power of genetic resolution as well as in the quality of information content. The present study was undertaken to characterize and molecular divergence analysis of some important lentil varieties of Bangladesh using isozyme markers, as a first step in their use for future lentil breeding.

2. Experimental Section

2.1. Plant Materials

All the experiments were carried out in Professor Joarder DNA and Chromosome Research Laboratory, Department of Genetic Engineering and Biotechnology, University of Rajshahi, Bangladesh. Experimental materials such as BARI masur-1, BARI masur-2, BARI masur-3, BARI masur-4, BARI masur-5, and BARI masur-6 were collected from Bangladesh Agricultural Research Institute (BARI). At first, leaves (2 g) from young plants of six lentil varieties were randomly collected from 10 plants and stored at -80°C refrigerator in pre chilled mortar with pestle separately. Enzyme extracts were prepared by homogenizing the samples in ice along with 1% polyvinylpyrrolidone and 2 mL of chilled extraction buffer containing 0.1 M tris base, 0.25 M sucrose, 0.1% ascorbic acid, 1% cystein HCl, 1 mM EDTA, 1% MgCl₂ prior to centrifuge at 15000 rpm for 15 min at 4°C and supernatants were stored at -20°C. Protein concentration was determined by Lowry method with bovine serum albumin as standard using UV spectrophotometer (T80 UV/VIS Spectrometer, PG Instruments Ltd). Native-PAGE was performed with vertical gel electrophoresis unit (SLAB GEL SYSTEM, BIOTECH, YERCAUD-636601) using 5% stacking gel and 10% resolving gel. Equal amount (15 µL) of protein from each sample mixed with sample loading buffer (2X) and loaded for electrophoresis.

2.2. Electrophoresis Analysis (Native-PAGE)

The electrophoresis was carried out at 12 v/cm for stacking gel and 15 v/cm for resolving gel at 4°C and electrophoresis was stopped when the distance of staining dye to bottom was 1 cm. After completion electrophoresis, gels were removed, washed and stained with enzyme specific chemicals for different isozyme tests for band development.

2.3. Enzyme Assay

For peroxidase isozyme (PRX; E.C. 1.11.1.7) test, the gel was separated and placed in 50 mL 0.1 M sodium phosphate buffer, pH 6.0 for 30 min at 4°C and then the gel was incubated in staining solution containing 30% H₂O₂; 30 µL, 20 mM guaiacol for band formation and in esterase (EST; E.C. 3.1.1) isozyme activity test, same staining process was followed as in peroxidase test.

In acid phosphatase (ACP; E.C. 3.1.3.2) test, first the gel was incubated in 50 mL 0.15 M acetate buffer (pH 5.0) for 1 hour at 4°C and then the gel was immersed in staining solution which containing following chemicals 0.15 M acetate buffer α-naphthyl acid phosphate sodium salt 0.125 g, fast garnet GBC salt 0.125 g at 4°C overnight for band formation.

Malate dehydrogenase (NAD⁺) (MDH; E.C. 1.1.1.37) test, was performed by the gel incubating in 200 mL 0.1 M malate buffer, pH 7.0 at 4°C for 30 min and after that the gel was placed in staining solution which containing following chemicals MTT 60 mg, NAD 60 mg, PMS 4 mg at 4°C for band formation.

In β-glucosidase (GLU; E.C. 3.2.1.21) test, the gel was immersed with 50 mL 0.1 M sodium phosphate buffer, pH 6.5 at 4°C for 30 min and transfer the gel in staining solution which contain 50 mg 6 bromo-2 napthyl β-D-glucopyraniside, 50 mg fast blue BB salt and 1.0 g PVP-40.

For β-Galactosidase (GAL; E.C. 3.2.1.23) the gel was immersed with 50 mL 0.1 M sodium phosphate buffer, pH 6.0 at 4°C for 30 min and placed the gel in staining solution which containing 25 mg β-naphthyl-β-D-galactopyraniside, 50 mg fast blue BB salt.

Malate dehydrogenase (NADP⁺) (MDH; E.C. 1.1.1.40) isozyme test was done by incubating the gel in 50 mM tris buffer (pH 8.0) 50 mL at 4°C for 30 min and the gel was transfer in staining solution which contain following chemicals MgCl₂; 50 mg, MTT 60 mg, NADP 60 mg, PMS 4 mg at 4°C for band formation.

Sodium oxide dismutase (SOD; E.C. 1.15.1.1) enzyme activity was performed by incubating the gel in 50 mL 0.2 M Tris buffer (pH 8.0) at room temperature for 30 min and then the gel was transfer in light box containing 50 mL buffer and 2 mg riboflavin, 1 mg EDTA.
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Tyrosinase isozyme (TYR; E.C. 1.14.18.1) test was done by incubating the gel in 0.1 M sodium phosphate buffer (pH 6.0) 50 mL at room temperature for 30 min and the gel was incubated in staining solution which contain 4 mg L-DOPA in 37°C.

Glucose-6-phosphate isomerase (GPI; E.C. 5.3.1.9) test was followed by incubating of tris base and HCL (1N) and immersed by Fructose 6-phosphate Na₂ salt, Glucose 6-phosphate, MTT and PMS staining solution.

Alkaline phosphatase (ALP; E.C. 3.1.3.1) was performed by incubating the gel in tris buffer (pH 8.7) 50 mL at 4°C for 1 hour and changed the buffer every 15 minutes interval. Then the gel was immersed in 50 mL tris buffer containing staining chemicals α-naphthyl acid phosphate 50 mg, fast blue BB salt 50 mg, MgCl₂ 60 mg, MnCl₂ (0.25M) 50 mg at 4°C up to band formation.

Aspartate amino transferase (AAT; E.C. 2.6.1.1) test, the gel was incubated in 50 mL buffer containing α-ketoglutaric acid 0.02 g, L-aspertic acid 0.05 g, PVP 0.2 g, EDTA 0.02 g and sodium phosphate 0.57 g then for staining 50 mg BB salt was added in buffer.

Alcohol dehydrogenase (ADH; E.C. 1.1.1.1) was performed by incubating the gel in 50 mL tris buffer (pH 8.0), and then for staining the gel NAD 10 mg, ethanol 0.2 mL, MTT 10 mg, 2 mg PMS was added in 10 mg NAD, 20 mg fructose 6-phophatase Na₂ salt, glucose-6-phosphate dehydrogenase, MTT, PMS.

### 2.4. Relative Mobility Value Analysis

Photographs were taken of gels and zymograms were drawn manually by relative mobility (Rm) of each of the band. Relative mobility (Rm) was measured by the following formula.

Relative mobility (Rm) = \[ \frac{\text{Migration distance of band}}{\text{Migration distance of dye front}} \]

### 2.5. Band Scoring and Data Analysis

The bands were visually scored as present (1) or absent (0) separately for each individual isozyme and used for the analysis. Jaccard Genetic Similarities Matrix was calculated and used to build an unweighted pair-group method with arithmetic means (UPGMA) clustering were used to prepare dendrogram for estimating isoenzymatic variation among the varieties \[^{[14]}\]. NTSYS-pc version 2.1 was used for genetic similarity computing, dendrogram construction and Principal Component Analysis (PCA).

### 3. Results and Discussion

Isozyme provides useful evidence in the study of variation of varieties in terms of intensity and width of common bands, presence or absence of the bands, percentage of polymorphisms and the rate of time of band formation of different isozymes in varieties. This observation has been proved that isozyme markers are very effective for identification of genetic divergence of lentil varieties. Many researchers have used isozymes to study for genetic diversity and plant systemic in many crop plants\[^{[15,16]}\].

We examined thirteen isozyme markers in six lentil varieties where eight (viz. PRX, EST, ACP, MAD (NAD+), β-GLU, MAD (NADP+), SOD, TRX) showed polymorphism and one isozyme (GAL) exhibited identical banding pattern in all the varieties, since three of them such as AAT, ADH and GPI showed no activity in banding pattern. In peroxidase isozyme test, total seven banding patterns (PRX-1 to PRX-7 with Rm value 1.9 to 6.9) were observed and two of them (PRX-5 and PRX-6) at the position of Rm-6.2 and 6.5 were monomorphic whereas PRX-1, PRX-2, PRX-3, PRX-4, PRX-7 at the position of Rm-1.9, 2.6, 3.0, 3.7, and 6.9 were polymorphic (Fig. 1a, Fig. 2a and Fig. 3) and exhibited 71% polymorphism (Fig. 4). BARI masur-5 showed all peroxidase banding patterns (Fig. 2a). On contrast, BARI masur-3, BARI masur-4 and BARI masur-6 exhibited 4 bands each in the same intensity but differing in its position and width along with high level of morphological similarity (Fig. 2a).

In esterase isozyme system, total five banding patterns (EST-1 to EST-5) were exhibited between positions of Rm-1.2 to 4.2 The EST-1, EST-2, EST-3 and EST-4 bands were polymorphic (Fig. 1a, Fig. 2b, and Fig. 3), representing 80% polymorphism (Fig. 4), BARI masur-3 and BARI masur-4 showed all banding patterns except PRX-4 (Fig. 2a).

In esterase isozyme system, total five banding patterns (EST-1 to EST-5) were exhibited between positions of Rm-1.2 to 4.2 The EST-1, EST-2, EST-3 and EST-4 bands were polymorphic (Fig. 1a, Fig. 2b, and Fig. 3), representing 80% polymorphism (Fig. 4), BARI masur-3 and BARI masur-4 showed all the banding patterns with different intensity. On the other part, BARI masur-1, BARI masur-2, BARI masur-5, and BARI masur-6 exhibited 4 bands each in the same intensity but differing in its position and width along with high level of morphological similarity (Fig. 2b).
Acid phosphatase isozyme showed total three banding patterns where 1st (ACP-1) and 2nd (ACP-2) were polymorphic and produced 66% polymorphism at the position of Rm-1.1 and 1.6. BARI masur-1 and BARI masur-2 exhibited all banding patterns (Fig. 1c, Fig. 2c, Fig. 3, and Fig. 4). BARI masur-3 and BARI masur-4 showed 2 banding patterns i.e. ACP-1 to ACP-3 with same intensity, and position (Fig. 2c).
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Malate dehydrogenase (NAD$^+$) discriminated total three banding patterns (MDH-1 to MDH-3) at the positions of Rm-1.2, 1.5 and 1.9 from which two were polymorphic (Fig. 1d, Fig. 2d, and Fig. 3) and produced 66% polymorphism (Fig. 4). BARI masur-2, BARI masur-3 and BARI masur-4 showed three banding patterns with same position and intensity BARI masur-3 and BARI masur-4 also exhibited the highest possible

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similarity in banding pattern and position for peroxidase, esterase, and acid phosphatase (Fig. 2d).

For β-galactosidase, all the varieties showed a common banding pattern at position 0.22 i.e. there is no variation within them (Fig. 1e, Fig. 2e, Fig. 3, and Fig. 4). Total three zones (Glu-1 to Glu-3) of activity appeared in β-glucosidase. Two of them at position Rm-0.10 and 0.24 were monomorphic whereas the band position at Rm 0.40 was polymorphic (Fig. 1f, Fig. 2f, and Fig. 3) which showing 50% polymorphism (Fig. 4). BARI masur-2, BARI masur-3 and BARI masur-4 showed all banding pattern with same position and intensity. On the other part, BARI masur-1, BARI masur-5, and BARI masur-6 exhibited two bands each in the same intensity but differing in its position along with high level of morphological similarity (Fig. 2f).

In the malate dehydrogenase (NADP+) isozyme test system, total four banding patterns i.e. MDH-1 to MDH-4 were distributed between positions of Rm 0.11 to 0.85 (Fig. 1g, Fig. 2g, and Fig. 3), from which three were polymorphic showing 75% polymorphism (Fig. 4). The third band at position Rm 0.57 was monomorphic. BARI masur-1, BARI masur-4 and BARI masur-6 exhibited four bands (1, 2, 3, and 4) each with similar in banding pattern and position. BARI masur-2 and BARI masur-5 showed 3 banding pattern with same intensity but differing in its position (Fig. 2g).

Sodium oxide dismutase (SOD) isozyme test discriminated total four banding patterns at position Rm 0.29, 0.47, 0.64, and 0.85. The bands at position 0.29, 0.47, and 0.64 were polymorphic (Fig. 1h, Fig. 2h, and Fig. 3), which produced 75% effective polymorphism (Fig. 4). The third band at position 0.64 was monomorphic. BARI masur-1 and BARI masur-5 exhibited two bands each with similar in banding patterns and position. BARI masur-3 and BARI masur-6 showed two banding pattern i.e. SOD-1 to SOD-2 with same intensity and position (Fig. 2h). Finally the tyrosinase isozyme system revealed three banding patterns (TYR-1 to TYR-3) at positions 0.08, 0.42, and 0.77 (Fig. 1i and Fig. 2i). The entire banding patterns were polymorphic (Fig. 3) that means which produced 100% polymorphism (Fig. 4). BARI masur-1, BARI masur-3, BARI masur-4 and BARI masur-5 exhibited 2 bands each with similar in pattern but different in position (Fig. 2i). BARI masur-2 and BARI masur-6 showed 3 banding pattern i.e. TYR-1 to TYR-3 with same intensity, and position.

These results have helped in developing a dendrogram on the basis of relatedness of the varieties. According to the Jaccard’s similarity coefficient matrix (Table 1), BARI masur-5 was noted to be closely related with BARI masur-6 with the highest similarity coefficient 0.87 and the same time BARI masur-4 Vs BARI masur-6 showed 0.84 , whereas BARI masur-3 Vs
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Table 1. Jaccard’s similarity coefficient among six lentil varieties based on nine isozymes

|                | BARI masur-1 | BARI masur-2 | BARI masur-3 | BARI masur-4 | BARI masur-5 | BARI masur-6 |
|----------------|--------------|--------------|--------------|--------------|--------------|--------------|
| BARI masur-1   | 1.00         |              |              |              |              |              |
| BARI masur-2   | 0.72         | 1.00         |              |              |              |              |
| BARI masur-3   | 0.51         | 0.72         | 1.00         |              |              |              |
| BARI masur-4   | 0.60         | 0.75         | 0.78         | 1.00         |              |              |
| BARI masur-5   | 0.45         | 0.66         | 0.75         | 0.72         | 1.00         |              |
| BARI masur-6   | 0.57         | 0.72         | 0.75         | 0.84         | 0.87         | 1.00         |

Fig. 5. Dendrogram of six lentil varieties based on isozyme banding pattern. Using similarity coefficient and UPGMA method Dandrogram was made through NTPsys-pc software version 2.1.

Fig. 6. 2D distribution of six lentil varieties revealed by first two principal components (PC1 and PC2) based on Isozyme markers data. NTPsys-pc software version 2.1 was used. PC1: Principle component 1; PC2: principle component 2.

BARI masur-4 showed 0.78. Same similarity coefficient (0.72) was found between BARI masur-1 Vs BARI masur-2, BARI masur-2 Vs BARI masur-3, BARI masur-4 Vs BARI masur-5 and BARI masur-2 Vs BARI masur-6. On the other part, BARI masur-1 Vs BARI masur-4 showed 0.60 and BARI masur-2 Vs BARI masur-4 exhibited 0.66 similarity coefficients. On the hand the lowest similarity coefficient was found in
BARI masur-1 vs BARI masur-5. The relationship among the varieties needs to be confirming through Dendrogram analysis. Dendrogram of six lentil varieties based on isozyme banding pattern for PRX, EST, ACP, MDH (NAD+), GLU, MDH (NADP+), SOD and TYR using similarity percentage is represented in Fig. 5. A dendrogram based on Jacard (1908) similarity using unwighted pair group method of arithmetic mean (UPGMA), indicates segregation of six varieties of lentil into two main clusters; BARI masur-1 and BARI masur-2 were grouped in cluster I and BARI masur-3, BARI masur-4, BARI masur-5 and BARI masur-6 were grouped in cluster II where Cluster II form two sub clusters. Thus, constituent members of two sub clusters are- sub cluster I - consist of BARI masur-5 and BARI masur-6 and sub cluster II - Consist of BARI masur-3 and BARI masur-4. The results of the principal component analysis (PCA) are shown in Fig. 6 and Fig. 7. Three principal components (PCA) was obserbed viz. PC1, PC2 and PC3. The first three principal components from PCA accounted for 81.58% of the total variation among varieties. The proportions of the principal components one (PC1), two (PC2) and three (PC3) were 75.45%, 11.19% and 4.94%, respectively. Two dimensional (Fig. 6) and three dimensional (Fig. 7) plots were prepared by using the first two and first three PCs, respectively. From two dimensional plot, PC1 revealed that BARI masur-1 and BARI masur-2 positively distributed but BARI masur-4, BARI masur-3, BARI masur-6 and BARI masur-5 distributed negatively, whereas in PC2 BARI masur-1, BARI masur-5 and BARI masur-6 positively distributed and BARI masur-2, BARI masur-4 and BARI masur-3 distributed negatively. These two plots clearly revealed that the highest distance between BARI masur-1 and BARI masur-5.

4. Conclusion

Genetic variation between and within populations of crop species is useful for analyzing and monitoring germplasm during the maintenance phase and predicting potential genetic gain in a breeding programme[17]. In order to maintain, evaluate and utilize germplasm effectivity, it is important to investigate the extent of genetic diversity avaiable. The Search of protein markers of specieis, varieties and genotypes is an urgent problem in modern breeding. Isoenzymatic electrophororosis has been shown to be applicable to lentil cultivar identification. Several isoymes have been found to detect useful amount of variation in lentil. Earlier studies investigated the genetic variability within and among different lentil populations using isozymes and tested eight isozyme systems such as IDH, MDH, SOD, ME, 6-PGD, PGM, LAP and GOT in wild lentils and also reported inheritance of three isozyme viz., Leucine aminopeptidase-1, Phosphoglucoisomerase-1 and Phosphoglucomutase-2 in lentil. From our investigation, eight isozyme systems showed polymorphism[18]. In the peroxidase isozyme test, five were polymorphic among seven banding patterns with 71 % polymorphism. Similar result in rice and tested peroxidase in pumpkin and showed 58.33% polymorphism[18,19]. Two polymorphic band of PRX was reported to detect genetic diversity in lentil[19]. BARI masur-3, BARI masur-4 and BARI masur-6 showed all banding pattern of peroxidase except PRX-4. Esterase isozyme showed four polymorphic banding patterns with 80% effective polymorphism. Same number of banding pattern was accounted; where two were polymorphic in rice and garlic clones[20,21], while monomorphic band was reported for esterase in the all accession of chickpea[22]. 75 % polymorphic statement was found in pumpkin for ester-
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Highest genetic diversity was reported in Grasspea and *Fusarium oxysporum* through esterase test. Two polymorphic banding patterns were identified and exhibited 66% polymorphism, in where BARI masur-1 and BARI masur-2 confirmed all banding patterns for acid phosphatase isozyme test. Only one polymorphic band (Acp-3) was reported using acid phosphatase in Barley landraces. BARI masur-3 and BARI masur-4 showed 2 banding pattern i.e. ACP-1 to ACP-3 with same intensity, and position. These two varieties also exhibited the highest possible similarity in banding pattern and position for peroxidase and esterase profiles along with high level of morphological similarity, which indicate that they were basically of the same genetic source, but may have adapted to dissimilar agro-climatic condition through its cultivations and for several years and thus acquired minor modification of its morphological behavior. In malate dehydrogenase (NAD+) test, two polymorphic banding patterns were observed and produced 66% polymorphism. Same number banding pattern also reported for malate dehydrogenase (NADH) in hazelnut from which Mdh-1 was polymorphic but Mdh-2 and Mdh-3 were monomorphic and Mdh-1 was monomorphic in all population of *Lambertia orbifolia*. In case of malate dehydrogenase (NADP+), showed three polymorphic banding patterns which with 75% polymorphism. Four banding patterns viz. Mdh-1, Mdh-2, Mdh-3 and Mdh-4 observed in *Pinus Contora* with all polymorphic.

In another investigation, β-galactosidase did not show any polymorphism but in β-glucosidase, total 3 banding patterns Glu-1 to Glu-3 were observed with 50% polymorphism. Gal-1 and Gal-2 were identified in lentil. Sodium oxide dismutase isozyme test discriminated two banding pattern were polymorphic which exhibited 75% polymorphism. Tyrosinase isozyme test represented 100% effective polymorphism. Tyrosinase isozyme test represented 100% effective polymorphism. According to the similarity coefficient matrix, the highest similarity was observed between BARI Masur-5 and BARI masur-6. On the other hand, highest distant relation was noticed between BARI masur-1 Vs BARI masur-5 proceeded by BARI masur-1 Vs BARI masur-3. According to the similarity percentage, the highest similarity was observed between BARI Masur-5 and BARI masur-6. On the other hand, highest distant relation was noticed between BARI masur-1 Vs BARI masur-5 proceeded by BARI masur-1 Vs BARI masur-3. According to the similarity percentage, the highest similarity was observed between BARI Masur-5 and BARI masur-6.

Principal component analysis (PCA) demonstrated that the first three principal components accounted for 81.58% of the total variation. The result of the PCA was similar to that of the UPGMA analysis. The highest genetic distance observed between BARI-1 and BARI-5. In our previous work already have proven similar result using morphological and molecular marker. So crosses could be made among these varieties for lentil crop improvement in lentil breeding program.

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| AAT          | Aspartate aminotransferase |
| ACP          | Acid Phosphatase |
| ADH          | Alcohol dehydrogenase |
| ALP          | Alkaline phosphatase |
| BARI         | Bangladesh Agricultural Research Institute |
| DNA          | Deoxyribonucleic acid |
| EDTA         | Ethylenediaminetetraacetic acid |
| EST          | Esterase |
| GAL          | β-galactosidase |
| GPI          | Glucose phosphate isomerase |
| IDH          | Lactate dehydrogenase |
| L-DOPA       | L-3,4-dihydroxyphenylalanine |
| LAP          | Aminopeptidase |
| MAD          | Malate dehydrogenase |
| ME           | Malic enzyme |
| MTT          | Dimethyl tetrazolium thiazolyl |
| NAD          | Nicotinamide dinucleotide |
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