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Original Article

Determination of genetic diversity in *Acacia modesta* germplasm using SDS-PAGE

Determinação da diversidade genética em germoplasma de *Acacia modesta* usando SDS-PAGE

M. Zahoor[a]*, M. Nisar[b], A. Ur Rahman[a] and W. Ul Bari[c]

[a]University of Malakand, Department of Biochemistry, Chakdara, Dir Lower, Pakistan
[b]University of Malakand, Department of Botany, Chakdara, Dir Lower, Pakistan
[c]University of Chitral, Department of Chemistry, Seenlasht Chitral, Pakistan

Abstract

Biochemical markers such as protein are very important to determine genetic diversity among plant species in a given population which in turn is very important for breeders and farmers as they can then easily select the most appropriate variety to grow in a given locality. In this connection, the present study is aimed to evaluate genetic diversity in *Acacia modesta* germplasm through Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) technique. About 40 genotypes were subjected to SDS-PAGE analysis where a total of 12 polypeptide bands were observed in electrophoretogram. Out of which 16.67% were monomorphic while the remaining 83.33% were polymorphic. Variation found in B-2, 4, 5, 6, 7, 8, 9, 10, 11 and 12, were 20, 22.50, 32.50, 10, 2.50, 22.50, 15, 5, 2.50 and 75% respectively. Locus contribution toward genetic disagreement was 83.33%. Cluster analysis sorted all the genotypes into 9 clusters. The genotypes in one cluster were identical regarding protein profiling and showed less intra-specific genetic variation whereas differences were find from other genotypes.

Keywords: genetic diversity, cluster analysis, locus variation, germplasms.

Resumo

Marcadores bioquímicos, como proteínas, são muito importantes para determinar a diversidade genética entre espécies de plantas em determinada população, o que, por sua vez, é muito importante para criadores e agricultores, pois eles podem selecionar facilmente a variedade mais adequada para crescer em certa localidade. Nesse sentido, o presente estudo tem como objetivo avaliar a diversidade genética em germoplasma de *Acacia modesta* por meio da técnica de Eletroforese em Gel de Poliacrilamida com Dodecil Sulfato de Sódio (SDS-PAGE). Cerca de 40 genótipos foram submetidos à análise SDS-PAGE, em que foi observado um total de 12 bandas polipeptídicas no eletroforetograma. Destes, 16,67% eram monomórficos, enquanto os 83,33% restantes eram polimórficos. As variações encontradas em B-2, 4, 5, 6, 7, 8, 9, 10, 11 e 12 foram de 20, 22,50, 32,50, 10, 2,50, 22,50, 15, 5, 2,50 e 75%, respectivamente. A contribuição do lócus para a discordância genética foi de 83,33%. A análise de agrupamento classificou todos os genótipos em 9 agrupamentos. Os genótipos em um cluster foram idênticos em relação ao perfil de proteínas e apresentaram menor variação genética intraespecífica, enquanto diferenças foram encontradas em outros genótipos.

Palavras-chave: diversidade genética, análise de agrupamento, variação de lócus, germoplasmas.

1. Introduction

Genetic diversity deals with the variances of hereditary characteristics amongst the alleles of genes in individuals of populations; that plays a significant role in the development of species to adjust with a fresh environment (Kremer et al., 1998; Jan et al., 2016a). Genetic variation amongst the individuals of a population can be identified through various approaches. The simplest approach is to identify genetic variation through phenotypic observations of a particular character. Examination of the variations at enzyme level through electrophoresis is another approach. The final and ultimate approach is the determination of variation in the order of bases in the nucleotides of genes. The critical cause of genetic diversity is gene alteration that is alteration in the DNA chain; shaped and molded by selection, gene flow, recombination, genetic waft and movement in varied environments in time and space (Hartl and Clark, 1997; Zahoor et al., 2015; Jan et al., 2017a). The natural selection chooses the best fit within and among

*e-mail: mohammadzahoorus@yahoo.com
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a population, because without genetic diversity there will be no adaptive evolution (Ayana, 2001; Jan et al., 2017b).

Genetic diversity plays a key role to improve many crops and plants. Generally the plants or crops with better genetic makeup are well adopted to fluctuation in climate thus plant breeders first determine the genetic diversity amongst the individuals of a population and then select the best one in cultivar development. Genetic similarities amongst genotypes would be helpful in the selection of parents to be breed on larger scale (Jan et al., 2016b, 2017c; Petrovic et al., 2017; Qadir et al., 2017a). The crops/plants varieties developed with wider genetic base would be helpful in increasing their yield under varied agro-climatic conditions of environment (Jan et al., 2016c; Qadir et al., 2017b). The diverse genetic base is helpful in controlling the spread of diseases amongst the individuals of a population (Jan et al., 2016d; Qadir et al., 2017c). Genetic variation enables a population of the crop species to settle in new conditions, survive and develop to produce fresh genetic variants. Out of which few will be fit variants that would be durable to cope with changes in the surroundings (Jan et al., 2016a, 2017b; Petrovic et al., 2017). The most important point of research in the field of genetics is to find out the amount of genetic difference in both domestic and natural populations and finding out the probable mechanisms responsible for such changes that make them well suited to cope with the ecological challenges (Jan et al., 2016a, b, c). Genetic diversity among individuals arises due to changes in genotype and environment. Increasing the genetic diversity, improving the ability of a population to adapt a new environmental conditions (Jan et al., 2017a, b; Petrovic et al., 2017).

Without genetic diversity there will be no adaptive evolution and natural choice chooses the best fit within and among a population (Petrovic et al., 2017; Qadir et al., 2017c). Genetic variation is an important raw material that enables a population of the crop species to settle in new conditions (Saroei et al., 2017). Genetic diversity is very important in plant propagation for growing fresh and high yielding varieties of cereal crops that are enable to defend them against diseases and to tolerate abiotic stresses (Yadegari and Ansari, 2017). In the evolutionary prospective of a species the determination of the genetic diversity is a key factor and is the most important point in research in the field of genetics that enable us to find out difference both domestic and natural populations and to find out the probable mechanisms of variations amongst the individuals of a population (Petrovic et al., 2017; Yadegari and Ansari, 2017).

The information about genetic diversity is useful in the gene-bank organization as well as breeding experiments. Second purpose of the information of genetic diversity is its use in categorization of population for genome mapping experiments. Description of germplasm by using biochemical markers have got distinct attention due to its wide use in crop development and the assortment of desired genotypes for crop/plant breeding. To resolve the evolutionary and taxonomic complications of various crop/plants, the genetic marker and protein sketching has been effectively used (Petrovic et al., 2017; Yadegari and Ansari, 2017; Saroei et al., 2017).

The most successful technique for determining the genetic diversities is the use of molecular markers which are not practically influenced by environment, abundant and do not require previous pedigree information (Qadir et al., 2017a, b). Amongst them the biochemical markers like sodium dodecyl sulphate polyacrylamide gel electrophoresis has been widely used due to its simplicity and effectiveness for estimating genetic diversities amongst individuals of a population. The molecular markers are helpful tools of phylogenetic analysis and identification of diverse plant cultivars and species. A biochemical marker involves the investigation of seed storage proteins as well as isozyme. This method is a relatively not expensive and is powerful way of measuring allele frequencies of a particular gene. Seed storage protein patterns observed in electrophoretogram developed for various plant seeds have been widely used for assessing genetic diversity (Petrovic et al., 2017; Yadegari and Ansari, 2017; Saroei et al., 2017).

A. modesta belong to the sub family mimosoideae/ mimosaceae. Mimosaceae is a sub-family of Fabaceae consisting of about 56 genera and 2800 species. The Acacia genus is the second most important genus of this family, with about 1411 species recorded currently that are familiar in Australia, America, Africa and Asia.

The small young shoots of the *A. modesta* are from glabrous to sub glabrous. Bark of the tree is greenish grey or brownish and irregular. The prickles are below the petiole, and in pairs. They are dark brown; compressed, shining, recurved, 3-5 mm long while sometimes prickles are not present. Rachis is 1.2-5 cm long, having small gland close to the base and sometimes one between the upper most pair of pinnae. Pinnae are generally 2-3 pairs and rarely 1. It is 1.2-2.5 cm long. The leaflets are in 3-5 pairs with prominent veins. Inflorescence is pedunculate spike ranging in length from 3.7-7.5 cm. Calyx is 1-1.5 mm long, mostly campanulate and glabrous. Corolla is 2-2.5 mm long. Stamens are indefinite. Seeds are 3-5 in number. Flowering time is from March-May (Farrukh et al., 2006; Sarwar, 2016; Massey et al., 2017). The wood of *A. modesta* is hard and durable. *A. modesta* is used traditionally for various purposes. To relieve the body weakness of women after childbirth, the gum obtained from the bark is mixed with butter, almond and wheat flour and fed to women as tonic. One teaspoonful of gum, dissolved in a glass of water, called Zhuble sharbat, is used as a health tonic. The ash from the wood or branches is used as narcotic ingredient, used as tooth brush (locally called Miswak) and cure of cough. Gum is restorative and used as tonic for backache and sex. *A. modesta* extract has significant antibacterial efficacy against Lactobacillus (gram positive) strain of bacteria which cause dental carrier (Asghar et al., 2003; Farrukh et al., 2006; Qazi et al., 2006).

Owing to the importance of genetic diversity in propagation of plant species and use of biomarkers in determining the genetic diversities, this study aimed to determine the genetic diversity in *A. modesta.*

modesta germplasm collected from selected districts of Khyber Pakhtunkhwa, Pakistan, using SDS-PAGE

2. Material and Methods

In year 2014-2015 different exploratory trips were arranged to 40 different ecological zones of Khyber Pakhtunkhwa, Pakistan. Forty seeds samples of *A. modesta* from different localities of Dir (Upper), Dir (Lower), Bajawar and Malakand District were collected. The samples were dried in shade and then subjected to total seed storage protein extraction. The dried seed samples were grounded to fine powder with mortar and pestle, and were stored in Eppendorf tubes till further use. For the extraction of proteins, 400 µL of protein extraction buffer (PEB) was added to 0.01 gm of seed fine flour and vortexed (using Gyromixe vortex) carefully. The homogenates were centrifuged (Termo Fisher Scintific) at 13,000 rpm for ten minutes at room temperature in order separate the undissolved solids. The isolated crude proteins were isolated as clear supernatant, and specified amount (1.5 µL) was shifted to a new Eppendorf tube and placed at 4°C temperature until they were run on the gel.

Different protocols were used to optimize the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis for *Acacia modesta*. Protocols devised by Payne (1987), for protein investigation of wheat and that of Lioi et al. (1999), for storage protein analysis of chickpea were tested with minor changes.

The electrophoresis process was carried out using slab type Model: (MGV-202), SDS-PAGE with 12.5% polyacrylamide gels. A 12.5% resolving gel containing 3.0 M HCL Tris (Sigma) having pH 9, 0.4% SDS (Wako) and 4.5% stacking gel containing Tris-HCL 0.4 M of pH 7.0 and 0.4% SDS was prepared which were then polymerized chemically by adding 10% ammonium persulphate and 17 mL of N, N, N, N- tetramethylenediamine. The Electrode buffer solution was taken into the base pool of the apparatus. The gel plates were placed in the apparatus with care so as to avoid bubbles formation at the base of gel plates. After that electrode buffer (0.025 M Tris-HCL, 0.125% SDS, 1.29 M Glycine) was added to the top pool of the apparatus. 15 µl of the isolated protein were loaded into the wells of the gels with the help micropipette. Then the apparatus was linked with constant electric supply (110V). The gels were run until bromophenol blue (the tracking dye) reached to the base of the gel.

The gels after developing the electrophoretogram was treated with staining solution containing methanol (44%, V/V) and Comassie Brilliant Blue R 250 (0.2%, W/V) dissolved in acetic acid (6%, V/V) for about ten to twenty minutes at room temperature. Then the gels were treated with destaining solution {methanol (0%, V/V) and acetic acid (5%, (V/V))}. During staining and destaining processes gels were shaken gently using Shaker Mixer (Rabilloud et al., 2001).

3. Results

In this study proteomics assay was applied to estimate the extent of genetic diversity in *Acacia modesta*. Out of the various protocol used for the development of electrophoretogram, best resolution was achieved with 12.5% acryl amide gel (Figure 1).

Dendrogram was constructed for proteomic of *A. modesta* using software STATISTICA 6.0 in Windows XP2005 (www.statsoft.com). Dendrogram (Figure 2 and 3) divided 40 combinations into two Linkages at 20% linkage distance linkage 1 and linkage 2. The binary data matrix of protein bands of *A. modesta* have been shown in Table 1 while the intra-specific locus variation among them have been shown in Table 2. Linkage 1 was further divided into seven clusters: C1, C2, C3, C4, C5, C6, and C7, at 75% linkage distance. Cluster C1 consist A30, A29, cluster C2 consist A19, A17, A16, A15, A13, cluster C3 consist A37, A32, A38, A31, cluster C4 A24, A23, A10, cluster C5 A12, A20, A14, A11, cluster C6 consist A36, A35, A34, A33, A28, A25, A6 and cluster C7 consist A18, A40, A39, A2. Linkage 2 was also further divided into two clusters C8 and C9 (at 75% linkage distance). Cluster C8 was consist of A26, A27, A22, A21 and cluster C9 consist of A9, A8, A3, A5, A4, A7 and A1 varieties. The codes of different areas from the germplasms were collected are given in Table 3.

4. Discussions

In the present study intra-specific locus variation in 40 genotypes of *A. modesta* were detected through SDS-PAGE. A total of 12 loci were observed, out of which 2 loci i.e. locus 1 and locus 2 were monomorphic which were evident from the fact that all protein polypeptide bands were present in all the studied samples. The remaining loci (3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) showed variation and were marked as polymorphic loci.

Out of polymorphic loci, locus 12 showed 75% variation because this band was missing in 30 genotypes of *Acacia modesta*. The genetic disagreement of this locus was 0.1%. Similarly locus 5 showed 32.5% variation due to the absence

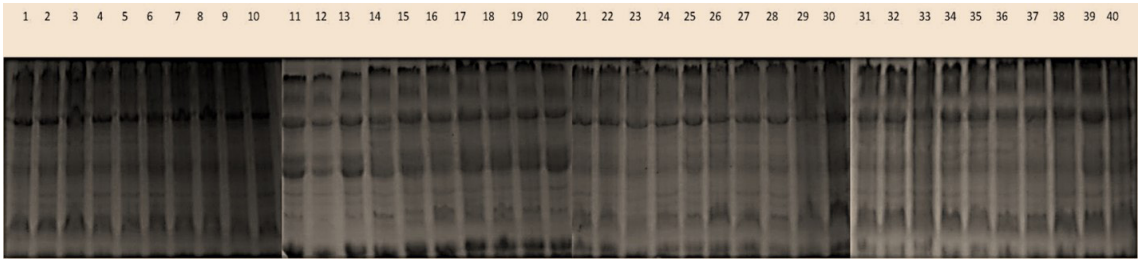

Figure 1. Proteomic profiling of 40 genotype of *A. modesta* based on SDS-PAGE.

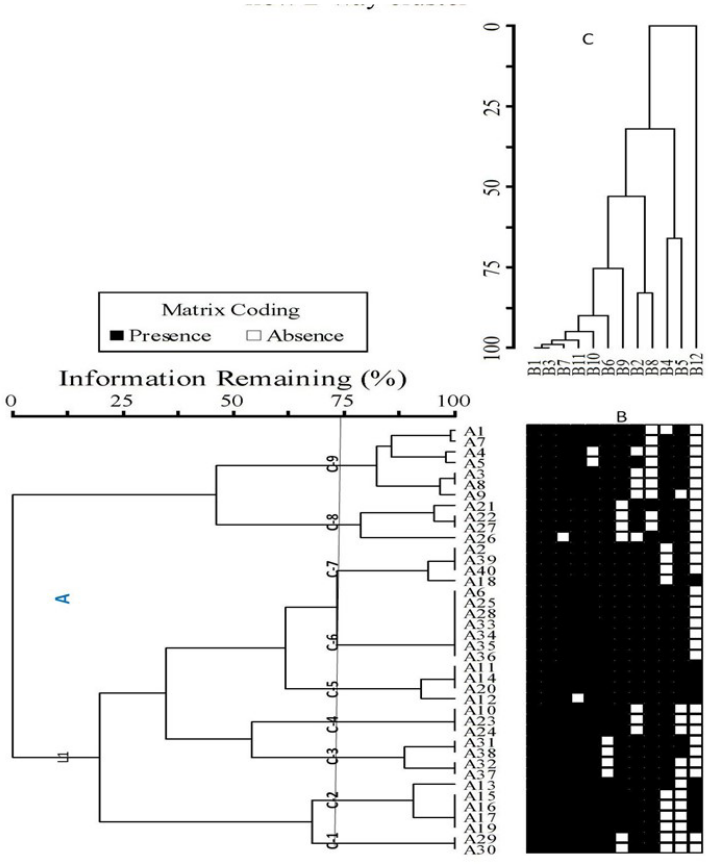

Figure 2. Two way cluster analysis of molecular traits matrix coding indication the presence and absence of protein band using PCA; **A**; cluster analysis of 40 among 40 cultivars of *Acacia modesta*. **B**; genetic polymorphism based on protein polypeptide distributed in 40 cultvars of *Acacia modesta*. **C**; zygomorph of 12 bands reported in *Acacia modesta* seed.

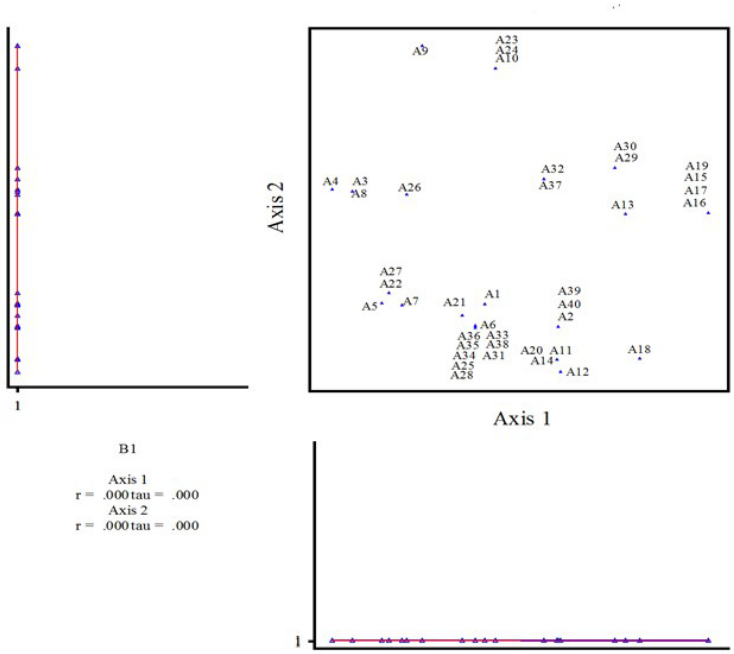

Figure 3. The groups are shown as plots by principal component analysis.

Table 1. Binary Data Matrix of Protein Bands of *A. modesta.*

	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
A1	1	1	1	0	1	1	1	0	1	1	1	0
A2	1	1	1	0	1	1	1	1	1	1	1	0
A3	1	0	1	1	1	1	1	0	1	1	1	0
A4	1	0	1	1	1	1	1	0	1	0	1	0
A5	1	1	1	1	1	1	1	0	1	0	1	0
A6	1	1	1	1	1	1	1	1	1	1	1	0
A7	1	1	1	1	1	1	1	0	1	1	1	0
A8	1	0	1	1	1	1	1	0	1	1	1	0
A9	1	0	1	1	0	1	1	0	1	1	1	0
A10	1	0	1	1	0	1	1	1	1	1	1	0
A11	1	1	1	1	1	1	1	1	1	1	1	1
A12	1	1	1	1	1	1	1	1	1	1	0	1
A13	1	1	1	1	0	1	1	1	1	1	1	1
A14	1	1	1	1	1	1	1	1	1	1	1	1
A15	1	1	1	0	0	1	1	1	1	1	1	1
A16	1	1	1	0	0	1	1	1	1	1	1	1
A17	1	1	1	0	0	1	1	1	1	1	1	1
A18	1	1	1	0	1	1	1	1	1	1	1	1
A19	1	1	1	0	0	1	1	1	1	1	1	1
A20	1	1	1	1	1	1	1	1	1	1	1	1
A21	1	1	1	1	1	1	1	1	0	1	1	0
A22	1	1	1	1	1	1	1	0	0	1	1	0
A23	1	0	1	1	0	1	1	1	1	1	1	0
A24	1	0	1	1	0	1	1	1	1	1	1	0
A25	1	1	1	1	1	1	1	1	1	1	1	0
A26	1	0	1	1	1	1	0	1	0	1	1	0
A27	1	1	1	1	1	1	1	0	0	1	1	0
A28	1	1	1	1	1	1	1	1	1	1	1	0
A29	1	1	1	0	0	1	1	1	0	1	1	0
A30	1	1	1	0	0	1	1	1	0	1	1	0
A31	1	1	1	1	1	0	1	1	1	1	1	0
A32	1	1	1	1	0	0	1	1	1	1	1	0
A33	1	1	1	1	1	1	1	1	1	1	1	0
A34	1	1	1	1	1	1	1	1	1	1	1	0
A35	1	1	1	1	1	1	1	1	1	1	1	0
A36	1	1	1	1	1	1	1	1	1	1	1	0
A37	1	1	1	1	0	0	1	1	1	1	1	0
A38	1	1	1	1	1	0	1	1	1	1	1	0
A39	1	1	1	0	1	1	1	1	1	1	1	0
A40	1	1	1	0	1	1	1	1	1	1	1	0

1= Present, 0= Absent.

of 13 polypeptide bands. The genetic disagreement of this locus was 0.675. The locus contribution toward genetic disagreement was 83.33.

In the current research high degree of germplasm protein differences recorded were in range of 0 to 75%. A total of 16.66% average similarity and 83.33% genetic

Table 2. Intra-specific locus variation in *A. modesta* detected through SDS-PAGE for samples collected from different districts of Khyber Pakhtunkhwa, Pakistan.

S. No.	Band present	Band absent	Variation	Status	Genetic disagreement
Locus-1	40 (100%)	0 (0.00%)	0.00%	Mono	1.0
Locus-2	32 (80%)	8 (20%)	20%	Poly	0.8
Locus-3	40 (100%)	0 (0.00%)	0.00%	Mono	1.0
Locus-4	29 (72.5%)	11(22.5%)	22.50%	Poly	0.72
Locus-5	27 (67.5%)	13(32.5%)	32.50%	Poly	0.675
Locus-6	36 (90%)	4 (10%)	10%	Poly	0.9
Locus-7	39 (97.5%)	1 (2.5%)	2.50%	Poly	0.97
Locus-8	31 (77.5%)	9 (22.5%)	22.50%	Poly	0.77
Locus-9	34 (85.5%)	6 (15%)	15%	Poly	0.85
Locus-10	38 (95%)	2 (5%)	5%	Poly	0.95
Locus-11	39 (97.5%)	1 (2.5%)	2.50%	Poly	0.97
Locus-12	10 (25%)	30 (75%)	75%	Poly	0.1
Locus contribution toward genetic disagreement	**GD= (Poly loci/Total loci)100 = 83.33**				

Table 3. Information about codes used for samples collected from different localities of Khyber Pakhtunkhwa Pakistan.

S. No.	Code	Locality (collected from)
1.	A1	Gorgoria Hill
2.	A2	Barhawalo Hill
3.	A3	Baghragia Hill
4.	A4	Zeenin Hill
5.	A5	Meshta
6.	A6	Banudir
7.	A7	Bararosar 1
8.	A8	Darorha upper Dir
9.	A9	KandoBajawar
10.	A10	Pingal
11.	A11	Naritangi
12.	A12	Qulangi
13.	A13	Ormalo
14.	A14	Boshaq
15.	A15	Manogi
16.	A16	Ghowan kali
17.	A17	Bango
18.	A18	Dandoki
19.	A19	Sharbatia
20.	A20	Bararosar 2
21.	A21	Band
22.	A22	Shenki
23.	A23	Band Agra

Table 3. Continued...

S. No.	Code	Locality (collected from)
24.	A24	Soghyar
25.	A25	Dir Upper
26.	A26	Manki
27.	A27	Enzargi
28.	A28	Dherijulagram
29.	A29	Nawagi Banda
30.	A30	Matkani
31.	A31	Kamala
32.	A32	Kozsapari
33.	A33	Temargara
34.	A34	Menagha
35.	A35	Bajawarkhar
36.	A36	Towda Chena
37.	A37	Tor Baba
38.	A38	Doda Pirkhel
39.	A39	Hazarnow
40.	A40	Lasho

diversity were recorded (band profile). The results obtained showed that estimation of genetic diversity in terms of euclidean distance, topography, genetic distance and linkage distances through SDS-PAGE is superior one as compared to other techniques.

5. Conclusions

In the present study high degree of variations in the germplasm's protein were recoded ranging from 0 to 75% which is very important for a plant to grow them in varied environment along with selection of better varieties. From the obtained results a possibilty seemed to improve the plant and develop new varieties of *A. modesta* as it is believed that more diverse plant can survive in environment more easily, because diverse plant are well adopted in the natural environment. Due to high degree of diversity it is suggested that *A. modesta* plant can be improved according to the changed environmental coditions and the plant can be propagated for the welfare of human beings.

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