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PHYLOGENETIC ANALYSIS OF FORTY *SOLANUM MELONGENA* L. ACCESSIONS BY SDS-PAGE

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Abstract

Work was carried out to resolve the existing intraspecific taxonomic relation and protein richest accessions of *Solanum melongena* by using SDS-PAGE with the reference of their genetic diversity. Phylogenetic relatedness within samples was studied by cluster analysis using an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to construct a dendrogram. Electrophoretogram of accessions No. (1-19) 018477, 018482 (Faisalabad), 18484 (Sahiwal), whereas accessions from 20 - 40 from D. I Khan (18504, 18500, 18505, 14466(3), Sahiwal (20344) and Batgram (20509) was unique in protein banding position. Largest dendrogram of cluster 1 divided into 6 (6a,b), 7 (7a,b), 8 (8a,b) and 9 (9a,b) sub clusters including accessions 20425 - 4745(3). The results demonstrated that accessions have low level of genetic diversity and almost similar protein contents. No relationship was found between genetic divergence and genetic status of the samples.

Introduction

Solanum melongena L. (Eggplant/Brinjal), belonging to Solanaceae is a common and popular vegetable grown in the tropics, subtropics, and temperate regions (Lai 1993). It is a warm weather crop grown extensively in India, Bangladesh, Pakistan, China, Japan, and Philippines (Agnieszka *et al.* 2007). This vegetable is very sensitive and insect infestation is very common.

Scientists are forced to introduce such species, which have high protein contents and genetically resistant in characteristics (Neilyn *et al.* 1995). Genetic diversity among the species of *S. melongena* is the best source for the plant breeders and hybridized specie of *S. melongena* (Lantican 1993). The breeder can produce very unique species by the combination of hybridized and genetically diverse specie of it (Welsh 1981). Morphological diversity ranges from wild and weedy to semi or fully-cultivated forms of inter- and intraspecific landraces (Lester and Hassan 1991, Karihaloo *et al.* 2002).

Molecular markers have been used to explore genetic diversity (Clain *et al.* 2004). SDS-PAGE which is a simple, cheap and rapid technique (Leisner *et al.* 2001) provides a valid source of taxonomic evidence for addressing taxonomic relationships at the generic and specific level (Harborne and Turner 1984). Seed protein profiling by SDS-PAGE (Quicke 1993, Abou-El-Enain 1995) is helpful for identification of inter- and intraspecific protein richest species. The work was aimed at resolving the existing intraspecific taxonomic problems and to determine the protein rich accessions of *S. melongena* by using SDS-PAGE method.

Materials and Methods

The experiment was designed to evaluate taxonomic confusion among the genetically diverse 40 accessions of *Solanum melongena* by protein markers through SDS-PAGE. Seed flour of 5 to 12 cotyledons was prepared by pestle and mortar. Soluble proteins were extracted by soaking 0.01 g of seed protein flour in 0.5 ml H_2O in a centrifuge vial. The suspension was centrifuged and 100 µl of protein solution (supernatant) was incubated with 100 µl of tris-HCl buffer (pH 6.8)

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consisting of 20% glycerol and 5 mM mercaptoethanol. Bromophenol blue (0.1 ml w/v) was added into the samples as a tracking dye. Seed flour was thoroughly mixed with buffer by vortexing. The extracts were then centrifuged at 15,000 rpm for 5 min. The crude proteins were recovered as supernatant, transferred into a new 1.5 ml Eppendorf tube and stored at −20°C until further analysis (Kamel 1996). Proteins in the supernatants were quantified by protein markers.

One-dimensional SDS-PAGE was performed according to the modified Laemmli method (1970) to carry out protein analysis by vertical slab gel (12.5 or 15%) in the discontinuous buffer system, tris-HCl at pH of 6.8 and 8.8, respectively were used. The stacking gel (2.5%) was loaded upon resolving gel for 40 min until polymerization started. The 100 µl protein buffer was loaded. Ammonium persulphate (10%) initiated polymerization and TEMED was used as a catalyst. The 20 µl sample solution of each accession was loaded into each well. The electrophoresis at 5°C with a constant current of 30 mA for 5 hrs was set. Gels were stained by 44% methanol, 6% acetic acid, 500 ml dH_2O and 2.25 g of Coomassie brilliant blue (Sigma Aldrich Chemie, Germany) for 45 min. The destaining solution contained 20% methanol, 5% acetic acid and 750 ml of dH_2O until the background color disappeared and protein bands were clearly visible (Yousaf *et al.* 2006). The destained gel was dried in drying processor at 60 - 70°C for 1 hr (Yousaf *et al.* 2006). Wet/dried gel photographs were scanned on the flat bed HP Scanjet 4°C with a resolution of 100 or 300 dots/inch to resolve the band 1 mm apart (Figs 4 - 7). Dissociated polypeptide weight can be determined by plotting a standard curve of log 10 molecular weight of standard polypeptide against the calculated relative mobility (R_f) value of each protein (Fig. 1).

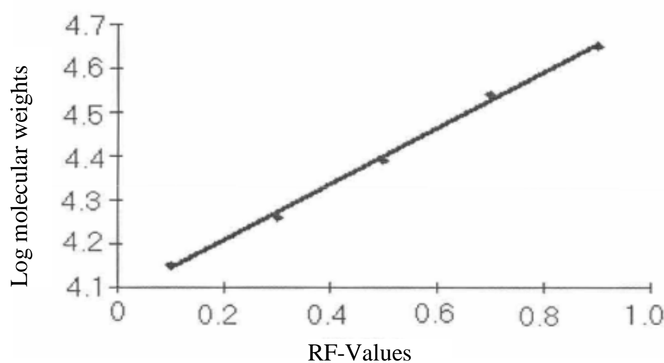

Fig. 1. Standard graph for analysis of protein gel bands.

Table 1. Protein standards with molecular weight, log size and their Rf value.

Standard proteins	Molecular weight (Kda)	Log size	Rf value
Albumin, bovine plasma	66	4.82	0.23
Albumin, egg ovalbumin	45	4.65	0.35
Pepsin porcine stomach mucosa	34.7	4.54	0.48
Trypsinogen, bovine pancreas	24	4.38	0.52
B-lactoglobulin, bovine milk	18.4	4.26	0.62
Marker (dye at the end)			0.85

A standard curve was used to determine the log molecular weight. Unknown protein value was determined by antilog of this number. The protein ladder consisted of albumin (66 kDa), egg oval albumin (45 kDa), pepsin from porcine, the stomach mucosa (34.7 kDa), trypsinogen from

bovine pancreas (24kDa) and β-lactoglubulins (18.4 kDa) (Sigma Chemical Company, USA) (Table 1).

The genetic diversity among the accessions was evaluated by using cluster analysis. Protein bands were scored depending on their presence (1) or absence (0). The principal components of data were used as input variables for cluster analysis using the UPGMA. Using the 'Graphics' option, the computed UPGAM data were used to construct a dendrogram.

Results and Discussion

the degree of relationship and phylogenetic variations of *Solanum melongena* accessions have been explored by 12.5% SDS-PAGE. The electrophoretic banding patterns of seed proteins of eggplant (*S. melongena* L.) accessions were representing morphological groups and geographical origins (Figs 2 - 7). The protein bands ran as separate groups with distinct MW ranging from 15 - 50 kDa (Figs 4 - 7). For convenient description, all bands were classified into three groups designated as α (MW < 20 ×103), β (20 × 103 < MW < 50×103) and χ (50 ×103 < MW > 80 × 103) according to MW protein markers. Eighteen resolving bands of 40 accessions with molecular weight ranged between 80 - 21 KDa in electrophoretogram (Figs 2 and 3) were used to evaluate the genetic variability. The banding patterns revealed variations in the numbers, positions, width, staining intensities, molecular weight and presence or absence of bands in different accessions. Each accession or group exhibited a unique banding pattern. The intensity of bands is represented by three different colors categorized into major (dark color) and minor bands (light color) (Figs 4 - 7).

Electrophoretic analysis of soluble seed proteins used for identification of cultivar, breeder, plant varieties and accessions to check the pedigree (Thanh *et al.* 2006) and systematics of sub-specie level (Quake 1994). Seed protein profiles are known for stability, uniformity and additive nature (Rao *et al.* 1990, Stegemann *et al.* 1992, Youpsanis *et al.* 1992). It is common for the agricultural cultivar seed protein banding pattern to be very stable during commercial seed production, but the pattern may alter slightly when an accession is multiplied, particularly if it is regenerated from a very small sample size (Gardiner and Forde 1988). Kamel (1996) had studied some relationship between certain taxa, species and tribes of certain families. Abou El-Enain (1995) had determined the taxonomic and phylogenetic relationship of *S. melongena* seed protein by SDS-PAGE.

Diffusion of crop from its primary center of diversity to secondary regions (Behera *et al.* 2006), where primitive cultivars and weed (Daunay 2008) caused the diversification and evolution (Prohens *et al.* 2005) of crop due to micro-evolutive factors like mutation, selection (natural and artificial), genetic drift, gene flow and recombination.

Protein profile of 1 - 20 accessions indicated that 18 (21 kDa) and 7 (49 kDa) band showed exclusive similarity in banding position (Figs 2, 4 and 5). In electrophoretogram of all 40 accessions (Figs 2 and 3) bands 1 (80 kDa), 5 (52 kDa), 8 (44 kDa), 15 (27 kDa), and 16 (26 kDa) was highly constant, uniform and might be the species-specific and remained same generation after generation and not affected by environmental stresses. This result was similar with the earlier findings that diverse accessions of cultivated eggplant still possess essentially the same major seed protein profiles (Pearce and Lester 1979). Karihaloo *et al.* (2002) concluded that most of the accessions had identical band patterns, supporting the interbreeding complex with limited genetic differentiation. So these bands may be an important marker for the identification of *S. melongena*. Minor bands contributed to determining the variation in this experiment. It might be possible to divide the genotypes into different groups and cultivars.

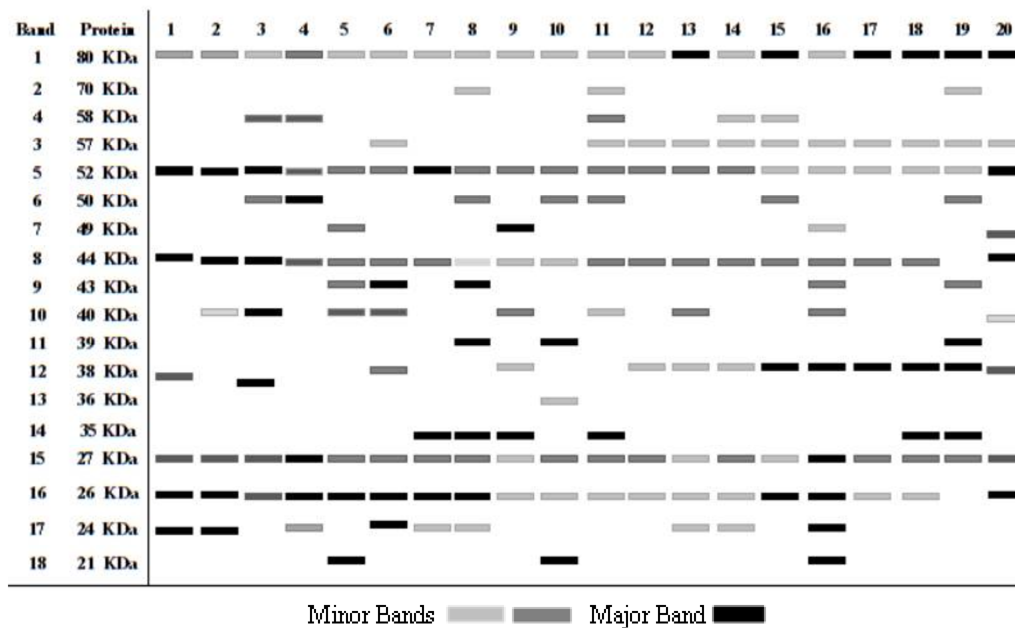

Fig. 2. Electrophoretogram of 1 - 20 accessions of *Solanum melongena* based on 12% acrylamide gel. (1: 018473, 2: 018474, 3: 018475, 4: 018476, 5: 018477, 6: 018478, 7: 018479, 8: 018481, 9: 018482, 10: 018484, 11: 018485, 12: 018489, 13: 018491, 14: 018494, 15: 018495, 16: 018496, 17: 018497, 18: 018498, 19: 018499, 20: 018500).

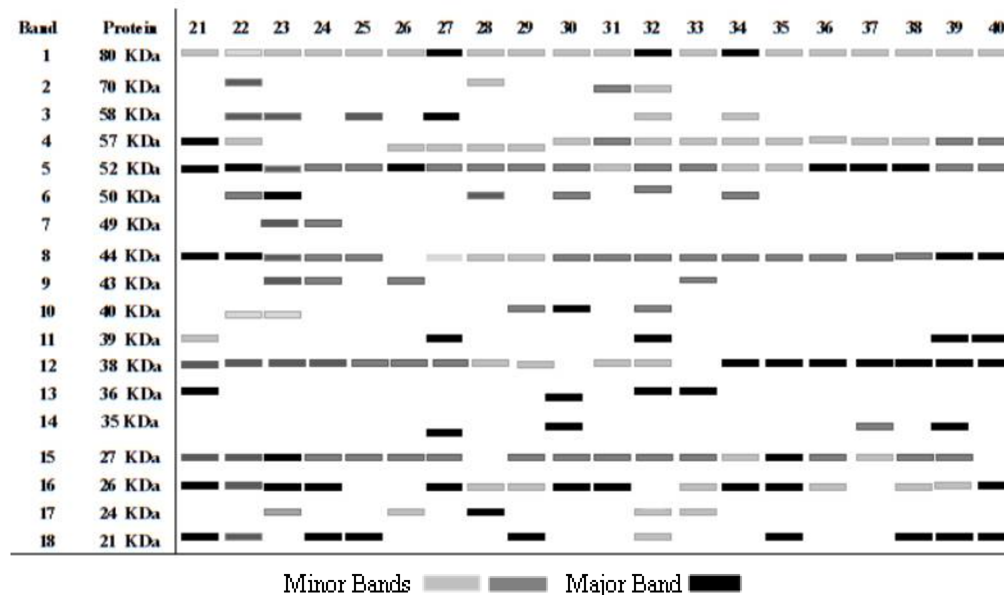

Fig. 3. Electrophoretogram of 21 - 40 accessions of *Solanum melongena* based on 12% acrylamide gel. (21: 018502, 22: 018504, 23: 018505, 24: 019868, 25: 020257, 26: 020281, 27: 020295, 28: 020344, 29: 020452, 30: 020480, 31: 020507, 32: 020509, 33: 020537, 34: 4466(3), 35: 4745(3), 36: 4792(3), 37: A-58, 38: MK-95, 39: White-egg, 40: XISANGZUE-6).

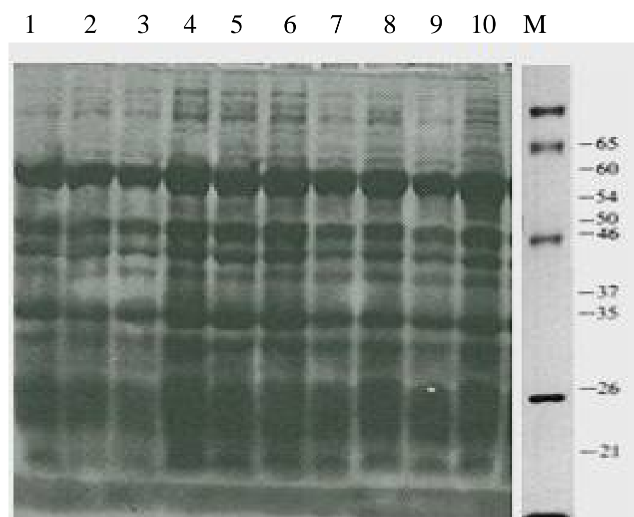

Fig. 4. The electrophoresis profile of seed storage proteins of 1-10 accessions. (1: 018473, 2: 018474, 3: 018475, 4: 018476, 5: 018477, 6: 018478, 7: 018479, 8: 018481, 9: 018482, 10: 018484). M: molecular mass markers are bovine (66Kda), ovalbumin (45Kda), pepsin (34.7 Kda), trypsinogen (24 Kda), B-lactoglobulin (18.4 Kda).

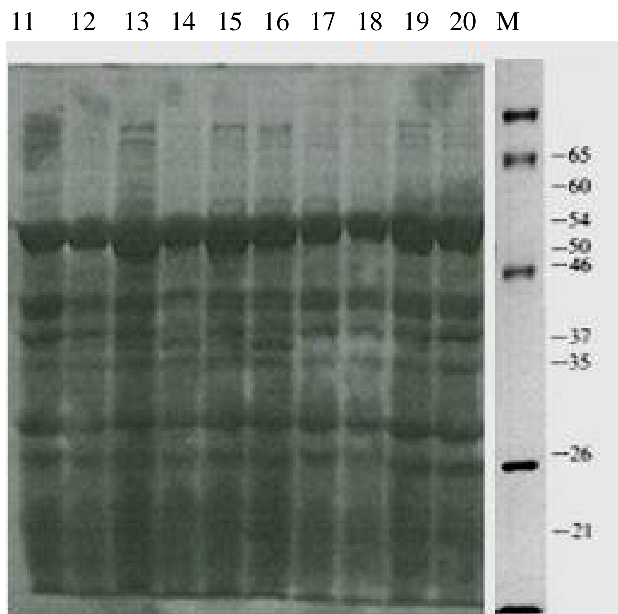

Fig. 5. The electrophoresis profile of seed storage proteins of 11-20 accessions (11: 018485, 12: 018489, 13: 018491, 14: 018494, 15: 018495, 16: 018496, 17: 018497, 18: 018498, 19: 018499, 20: 018500). M: molecular mass markers are bovine (66Kda), ovalbumin (45 Kda), pepsin (34.7 Kda), trypsinogen (24 Kda), B-lactoglobulin (18.4 Kda).

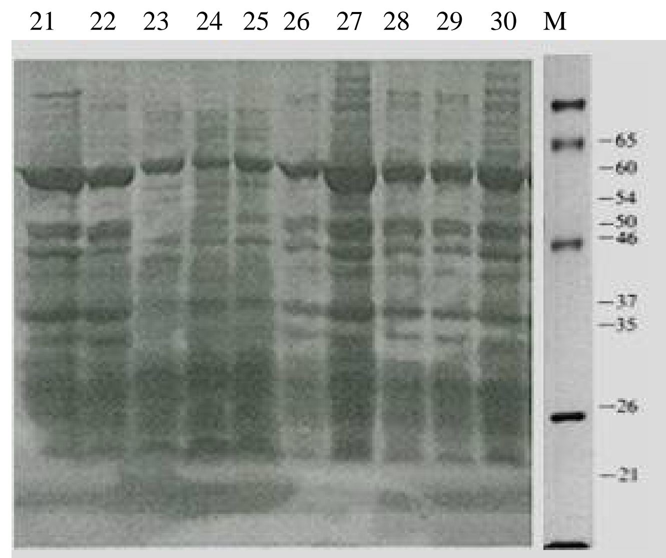

Fig. 6. The electrophoresis profile of seed storage proteins of 21-30 accessions (21: 018502, 22: 018504, 23: 018505, 24: 019868, 25: 020257, 26: 020281, 27: 020295, 28: 020344, 29: 020452, 30: 020480). M: molecular mass markers are bovine (66Kda), ovalbumin (45 Kda), pepsin (34.7 Kda), trypsinogen (24 Kda), B-lactoglobulin (18.4 Kda).

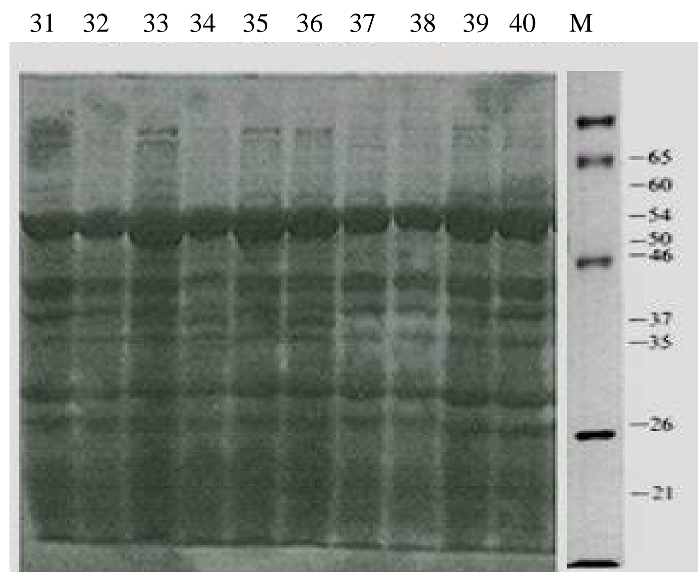

Fig. 7. The electrophoresis profile of seed storage proteins of 31-40 accessions (31: 020507, 32: 020509, 33: 020537, 34: 4466(3), 35: 4745(3), 36: 4792(3), 37: A-58, 38: MK-95, 39: White-Egg, 40: XISANGZUE-6). M: molecular mass markers are bovine (66 Kda), ovalbumin (45Kda), pepsin (34.7 Kda), trypsinogen (24 Kda), B-lactoglobulin (18.4 Kda).

The approximate number of deeply stained major bands was 36 in 40 accessions belong to Faisalabad, Sahiwal, Punch, D.I Khan, Bahawalpur, T.T Singh, Swat and Vehari. But only two accessions were lightly stained i.e. 18489 (Acc.) and 18494 (Acc.) from Punch and D.I Khan, respectively.

To divide the accessions into distinct groups, variation in the staining intensities of minor bands was not enough. Syed and Isa (1998) worked on the accessions of different regions of world to evaluate the similarity and differences among accessions. Results of electrophoretic banding pattern showed a great deal of genetic variation within and between groups in terms of number, size, position, staining intensities and presence or absence of bands in the profile of round and elongated fruits (Karihalo and Rai 1995).

Protein profile 2 and 7 (1 - 40 accessions) showed specific protein banding pattern of *S. melongena,* which indicated the soil and environmental impact on the accessions. The band 2 (70 kDda) of Mandi Bahiddin (18481), Sahiwal (18485), D.I Khan (18499, 18504), Swat (20344) and Batgram (20509) and band 7 from Faisalabad (18477, 18482), D.I Khan (18496, 18499, 18500, 18505) and Bahawalpur (4466-3) indicated that their soil has similar characteristics. Anu and Peter (2003) analyzed seed protein of 29 accessions of *Capsicum annum* L. by polyacrylamide gel electrophoresis. They observed distinct genotypic bands but certain bands were shared by several genotypes.

Table 2. List of 40 accessions of *Solanum melongena* used in this study.

Cluster	Accession No.	Geographical position
1	20509	Batgram
2	18499, 18481	D. I. Khan, Mandibahauddin
3	18477	Faisalabad
4	18502, 18484	Sahiwal
5a	18476, 18475	Faisalabad
5b	18495, 18494	D. I. Khan
6	18473, 18479, 18474	Faisalabad
7a	20452, 20344	Vehari, Sawat
7b	18504, 18485	D. I. Khan, Sahiwal
8a	4466(3), 18505, 18496	Bahawalpur, D. I. Khan
8b	18500, 18482	D. I. Khan, Faisalabad
9a	18497, 18478, 20537, 20480, 18491, 18498, 18489	Faisalabad, D. I. Khan, Kohat, Punch
9b	20295, 18868, 4745(3), 20507, 20281, A-58, 4792(3), XIANGZUE-6, white-egg, MK-95	T. T. Singh, Bahawalpur, Rahim Yar Khan, Mansehra

The electrophoretogram of (1 - 20 accessions) (Fig. 2) band 13(36 kDa) was considered accession (018484) specific band (minor band) due to weak staining because the seeds were poorly filled as a result of the hybrid origin from the original source, whereas accessions 20 - 40 (Figs 2 and 3) categorized as major bands. But this band did not provide enough data to identify and divide the species into groups. The results were verified by UPGMA. Variability of the protein banding pattern made it possible to divide the genotypes into different cultivar and groups. Nunome *et al.* (2001) proved that *Solanum melongena* accessions have minor variation and low frequency of polymorphism.

Diversity and relationship of cultivated species facilitate the establishment of conservation strategies, genetic resources in breeding programs and crop evolution (Singh *et al.* 2006, Maria *et al.* 2012).

Cluster analysis helps to characterize the accessions into several distinct and significant groups (sub-species, botanical or variety group, cultivar and population). So accessions collected from different areas are intermixed showed no genetic barrier (Table 2).

Cluster 9 was the largest one comprising most of the accessions. It was divided into two main sub-clusters 9a and 9b. Sub-cluster 9a contained seven accessions 18497, 18478, 20537, 20480, 18491, 18498, 18489 while 9b comprised of 11 accessions 20295, 19868, 4745(3), 20507, 20281, A-58, 4792(3), XIANGZUE-6, 20257, white-egg, MK-95. In sub-group 9b accessions comprised genetic variability up to 2 - 26%, collected from T.T. Singh, Bahawalpur, Rahim Yar Khan and Mansehra (Fig. 8).

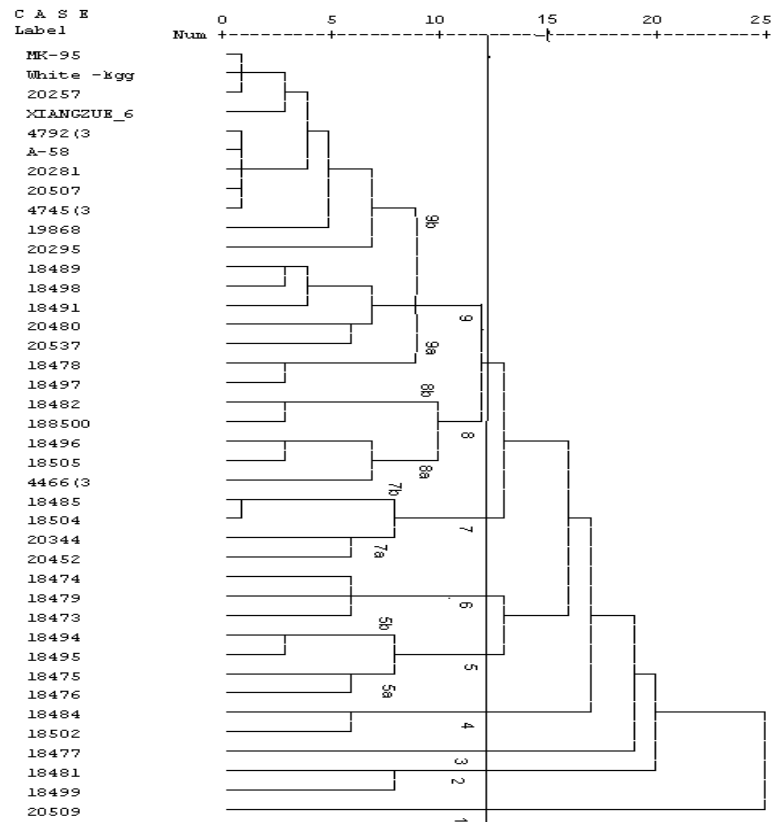

Fig. 8. Cluster analysis dendrogram of 40 accessions of *Solanum melongena* based on seed protein gel electrophoresis (0.30 - 0.95%).

Cluster 8 has also been divided into two sub-clusters 8a and 8b. Sub-groups 8a included three accessions 4466(3), 18505, 18496 collected from (Bahawalpur, D.I. Khan) and in 8b two accessions (188500, 18482) from D.I. Khan, Faisalabad. (Fig. 3) This showed genetic variability up to 27%. Cluster 7 was divided into two sub-clusters 7a and 7b which showed genomic variation 2-32 % (Fig. 8). Sub cluster 7a containing 20452, 20344 collected from Vehari, Swat. Sub clusters 7b containing 18504, 18485 from D.I. Khan, Sahiwal showed more similarity than 7a accessions.

Cluster 5 was divided into two sub-groups 5a (18476, 18475) and 5b (18495, 18494), whereas cluster 6 containing three sub-clusters (18473, 18479, 18474) showed that geographical areas were not contributing to genetic variation in accessions. Cluster 4 contained 18502, 18484 and cluster 3 contained only one accession 18477. Cluster 2 comprised of 18499 and 18481 accessions, Cluster1 contained one accession 20509 (Fig. 8). Tümbilen *et al.* (2011) studied the genetic variability of 67 Turkish eggplant accessions with 30 morphological traits. Dendrogram of high genetic similarity in related *Solanum* species ranged from 0.30 to 0.95 whereas 0.68 to 0.95 indicating low genetic diversity. Behera *et al.* (2006) worked on closely related species of *Solanum* clustered along *S. melongena* accessions, being crossable with cultivated species, constitute important sources of genes that can be introgressed by backcross breeding. Molecular markers can be employed to identify the hybrids and also to monitor introgression of useful genes.

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