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Full Length Research Paper

Characterization of indigenous *Pyrus* germplasm of Azad Jammu and Kashmir revealed by SDS-PAGE analysis

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Soluble proteins of sixty pear genotypes/varieties were extracted from their leaves, separated by polyacrylamide gel electrophoresis and distinguished by protein banding pattern. Seven types of protein with 12 to 20 bands were observed. SD45 (Kashmiri nakh) showed the maximum (20) and KT60 (Keiffer) the minimum (12) number of bands. Remaining accessions exhibited less variability having 15 to 19 bands. The accessions were classified into 12 groups and individual accessions varied from 0.71 to 0.97 similarity level. Moreover, the highest similarity was expressed among groups 1 to 7. UPGMA cluster analysis distributed the accessions into three clusters, seven sub-clusters along with 11 identical groups, one independent group and two independent accessions. There were 37 accessions in cluster I, 16 in II, 3 in III, 2 in independent group 12 and 2 accessions existed independently. The most variable accession, KT60 (Keiffer) fell independently, had the highest genetic diversity. The findings show that the pear accessions have different protein profile irrespective of their geographic locations and climatic conditions.

Key words: Accessions, pear, *Pyrus* germplasm, SDS-PAGE, soluble protein, variability.

INTRODUCTION

Pear ranks 2nd after apple in the world and has great economic and nutritional importance (Blattny, 2003). The genus *Pyrus* originated in Central Asia, the mountainous regions of western and southern China and further diversified both in southern and eastern directions from its primary centre of origin (Watkins, 1976). The genus belongs to family *Rosaceae* and subfamily *Pomoideae* with 17 basic chromosomes and somatic number 34, rarely 51 or 68 (Westwood, 1978). Botanically, it is characterised into 22 primary species and some non-primary species or botanical varieties/inter-specific hybrids (Bell et al., 1996). Rich genetic diversity in *Pyrus* germplasm found in mountainous region of Azad Jammu and Kashmir, consist of traditional varieties, wild relatives and species due to seed based propagation and severe incompatibility which lead to high levels of heterozygosity and diversity within genus communities. The potential of this genus remained largely unexplored in mountainous region of Azad Jammu and Kashmir and genetic variation existing within this genus needs to be characterised to meet the demand for more food, provide reservoir of genetic variation and to find particular characters such as

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Abbreviations: AFLP, Amplified fragment length polymorphism; **APS,** ammonium per sulphate; **RAPD,** random amplified polymorphic DNA; **RFLP,** random fragment length polymorphism; **SDS-PAGE,** sodium dodecyl sulphate-polyacrylamide gel electrophoresis; **TEMED, N,N,N,N**-tetramethylethylenediamine; **UPGMA,** unweighed pair group method with arithmetic means.

resistance genes for diseases and insect-pests. High yielding potential accessions/genotypes need to be collected and characterised (Engelmann, 1991). Characterization based on morphological and horticultural traits have some disadvantages as being influenced by many factors. Genetic diversity in pear has not been identified due to low morphological variation and lack of differenttiating characters among species and varieties (Yuan and Du, 1980). Moreover, variability in phenotypic characters among the accessions growing in different areas with different environments and production practices demonstrate problems with their heritable approach (Kresovich and McFerson, 1992; Hokanson et al., 1998). These morphological characters have been used traditionally for identification and phylogenetic analysis of pear species and cultivars (Yuan and Du, 1980) which is difficult, tedious and unreliable. Phenotypical characterization proved useful for limited number of species under certain conditions only (Shen, 1980; Westwood, 1982). Therefore, germplasm characterization based on morphological traits need to be complimented with molecular and protein profiling.

Availability of genetic variation is important for genetic improvement of the crop; local and primitive germplasm can be used as a source of genetic variation. Different types of marker systems have been used for biodiversity analysis. Protein markers can act effectively to study the genetic variation of germplasm for its utilization in breeding programs. Many workers used protein electrophoresis to characterize and investigate genetic variation of many cultivated and wild plant species (Isemura et al., 2001; Fan et al., 2001; Ahmed et al., 2003; Rehan et al., 2004; Asif et al., 2004; Gulen et al., 2005). These studies showed that diversity exists for protein profiles and have the potential for aiding species classification and for serving as markers for interspecific hybridization studies.

The use of storage proteins provides a more appropriate option to characterize and classify plant germplasm. Total protein is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop plants. It has been widely suggested that such banding patterns could be an important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivars or when cultivars are to be patented (Harborne and Turner, 1984). Electrophoretic banding pattern revealed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE) have provided a valid source of taxonomic evidences and were used to address taxonomic relationships at the generic and specific levels of various crop species. Variation of seed storage proteins has also been analyzed to estimate the center of genetic diversity and possible dissemination pathway in common bean (Gepts and Bliss, 1988). Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice (Thanh and Hirata, 2002). The proteins can represent primary gene products and seeds are considered physiological stable materials and easy to handle (Ladizinsky and Hymowitz, 1979). Analysis of SDS-PAGE is fairly simple and inexpensive, which are added advantages for use in practical plant breeding (Rahman and Hirata, 2004). However, the information on the SDS-PAGE on different species of *Pyrus* for genetic diversity is still limited.

Electrophoresis patterns of the protein fractions directly represent the genetic background of the proteins to certify the genetic make-up (Rehana et al., 2004). The study of banding pattern of SDS extract of proteins in PAGE is one of the most commonly used methods (Laemmli, 1970) to characterize plant germplasm because protein profiles are specific, highly stable and storage proteins are not affected by environment (Singh et al., 2004). Band patterns of protein profiles are advocated for cultivar identification and identifying phylogenetic relations among species (Gepts, 1988, 1990; Dinelli and Lucchese, 1999; Duran et al., 2005).

Total leave protein profiles and isozymes were used to characterize coconut palms (Parthasarathy et al., 2004). Protein profiles studies by using SDS-PAGE were useful to predict graft incompatibility in *Prunus* (Huang et al., 1984; Schmid and Feucht, 1985). Further, this technique has also been effectively used to investigate compatibility/incompatibility between pear scion and quince rootstocks peach/plum, pear scion grafted on quince and pear rootstocks (Moreno et al., 1994; Gulen et al., 2005).

In general, genetic improvement of crops can be accelerated when broad genetic diversity and information about the genetic resources are available. Research on *Pyrus* germplasm could enhance the genetic information regarding pear germplasm to get the nutritional benefits for local and national requirements. The collection and assessment of genetic diversity could be beneficial to develop better methods for evaluation and preservation of pear germplasm resources. Moreover, this genus has received little attention with respect to estimated genetic diversity with SDS-PAGE. The present study attempted, therefore, to compare the local *Pyrus* germplasm and its relatives growing in different agro-ecological zones of Azad Jammu and Kashmir by using protein markers.

MATERIALS AND METHODS

Mature leaves of sixty accessions of pear including two primitive varieties were collected from different ecological zones of Azad Jammu and Kashmir (Pakistan) and brought to the Laboratory of Plant Genetic Resources, National Agricultural Research Centre (NARC), Islamabad, Pakistan for extraction of proteins and their analysis. Sodium dodecyl sulphate – poly acrylamide gel electrophoresis (SDS-PAGE) was used to characterize the protein profiles of the accessions by using 12.25% (w/v) separating gel and 4.5% (w/v) stacking gel (as developed by Laemmli (1970) with some modifications. Leaves were ground in liquid nitrogen in a mortar with pestle. About 0.15 g of crushed leaf powder was suspended in 1 ml cold extraction buffer. Homogenized mixture was incubated at 40°C overnight and the mixture was vortexed. Later, samples were centrifuged at 15,000 rpm for 15 min at 4°C, 1 ml of acetone was

added in each sample and the resultant suspension was left overnight at -20°C. Extraction was done with 0.05 M Tris-HCl at pH 8.0, 0.2% SDS, 5.0 M urea and 1% ß-mercaptoethanol. A few drops of bromophenol blue (BPB) was added to the prepared buffer solution and stored in a refrigerator. Clean extracts after centrifugation were used for electrophoresis. Solutions A, B and C were prepared for electrophoresis, A with 3.0 M Tris-HCl at pH 7.0, 0.4% SDS, B with 0.49 M Tris-HCl at pH 7.0, 0.4% SDS and C with 30% acrylamide, acrylamide/bis = 30:0.8. Electrode buffer solution with 0.025 M Tris, 0.129 M glycine, 0.125% SDS were prepared and stored at room temperature.

Protein samples were pelleted at 10,000 rpm for 10 min at 4°C in an Eppendorff centrifuge. The supernatant (acetone) was discarded and pellet was air-dried. Pellet of each sample was resuspended in 400 µl protein extraction buffer. It was properly mixed by vortexing for 2 - 3 min. The solubilized samples were centrifuged at 10,000 rpm for 10 min and the supernatant was saved and stored at -20°C for further use. Next day, the samples were recentrifuged for 5 min at 10,000 rpm at -4°C and were loaded to vertical gel with 10 µl of solubilized sample in each well.

Electrophoretic gels (12.25%) were prepared with solution A (5 ml), C (7.5 ml) and 10% APS 200 µl and TEMED (N, N, N, N-tetramethylethylenediamine) 15 µl and stacking gel (4.5%) was prepared with solution B (2.5 ml), solution C (1.5 ml), 10% APS 70 µl, TEMED 17 µl with total volume of 10 ml. Each well was rinsed and loaded with 10 µl of each sample one by one. Upper and lower chambers were assembled in buffer. After electrophoresis, staining of the gels was done in 0.2% Coomassie Brilliant Blue R-250 solution, containing 10% acetic acid and 40% methanol, for about one hour. Gels were then destained by washing with a solution containing 5% acetic acid and 20% methanol until the colour of background disappeared and electrophoresis bands were clearly visible. The protein bands were visualized by transilluminator and photographs were taken for comparison of results. Depending upon the presence (1) or absence (0) of polypeptide bands, similarity index was calculated for all possible pairs of protein types. Presence and absence of bands were entered in a binary data matrix. Based on result of electrophoresis band spectra, Nei and Li's (1979) similarity matrix was calculated for all possible pairs of protein type's electrophoregrams by the formula of Sneath and Sokal (1973) as given below:

$$S = W/(A + B - W)$$

Where 'W' is the number of bands of common mobility, 'A' is the number of bands in protein type A and B is the number of bands in protein type B. The similarity matrix thus generated was converted to a dissimilarity matrix (Dissimilarity = 1 - similarity) and used to construct dendrogram by the unweighed pair group method with arithmetic means (UPGMA). All computations were carried out using the NTSYS-pc, software package, version 2.2 (Rohlf, 2004).

RESULTS AND DISCUSSION

Germplasm characterization based on morphological trait is unreliable and needs to be confirmed on molecular or at least protein level. Genetic diversity of *Pyrus* germplasm elucidated through SDS-PAGE of proteins separated from leaves, revealed distinct electrophoretic banding pattern. Twenty polypeptide bands (major and minor bands) ranging from 14.4 to 116 kDa were recognised among 60 accessions/ primitive varieties and screened (Table 1, Figure 1). Gogorcena and Ortiz (1993) separated soluble proteins from leaves of *Citrus aurantium* L. and its relatives by polyacrylamide gel electrophoresis and distinguished different species and cultivars by protein banding pattern. These electrophorograms of the *Pyrus* genotypes/accessions can be used as passport data for their genetic identity and could be good tool for testing diverse germplasm abundantly found in mountainous region of Northern Pakistan. Electrophoretic banding pattern of protein profiles is considered to be a source of taxonomic evidences and relationships at genetic level in field crops as well as fruit plants like citrus and coconut (Ladizinsky and Hymowitz, 1979; Gogorcena and Ortiz, 1993; Geethalakshmi et al., 2005). This technique is equally important for the identification of rootstocks as well as scion stock compatibility in fruit trees (Huang et al., 1984; Schmid and Feucht, 1985). SDS-PAGE is considered as a reliable method of genetic characterization because electrophoretic patterns of the protein fractions are directly related to the genetic background of the proteins and can be used to certify the genetic make-up (Rehana et al., 2004).

In order to estimate the variability at genetic level, SDS-PAGE banding pattern of the gel using total leave protein was investigated. Relationship of the 60 accessions was based on estimated number of total bands present in protein profiles. The accession SD45 (Kashmiri nakh) yielded the highest number (20) of bands while minimum bands (12) were found in KT60 (Keiffer). However, the remaining accessions showed less diversity and close relationship in banding pattern. Likewise, fifteen bands in protein type 2 were counted in sample of 3 accessions, sixteen bands in protein type 3 were present in 8 accessions, seventeen bands in protein type 4 were found in the samples of eighteen accessions, eighteen bands in protein type 5 were found in twenty two accessions and nineteen bands in protein type 6 were recorded in seven accessions (Table 2). Protein profiles of the accessions further showed variability on the basis of presence or absence of protein bands (Table 3). Some protein bands among the accessions e.g. bands 2, 8,12,13,14, 17 and 20 were same and present in all accessions showing genetic relationship and had monomorphic banding pattern. Overall out of 20 bands, 14 were found polymorphic and other 6 as monomorphic (Table 3). Moreover, visualised electrophoertic banding pattern exhibited a considerable range of variability with regard to their mobilities and intensities. The variability in SDS-PAGE profile indicated genetic diversity in pear genotypes collected from diverse geographical and environmental conditions of Northern Pakistan (Azad Jammu and Kashmir) would be due to diverse environmental conditions. Results corresponded with Choong et al. (1996) who reported that diversity in coconut leaf protein bands was likely to be distributed among the populations due to natural selection and environmental conditions.

Cluster analysis

Cluster analysis for germplasm based on similarity matrix

Table 1. Total numbers of bands present/absent in each pear germplasm sample.

Accession #	Local name	No. of present bands	No. of absent bands
RT1	Khurolli	17	3
RT2	Bagugosha	16	4
RT3	Glass	17	3
RT4	Pathar nakh	18	2
RT5	Kotharnul	19	1
RT6	Khurolli	18	2
RT7	Desi nash	18	2
RT8	Btung	18	2
RT9	Btangi	16	4
RT10	Kashmiri nakh	16	4
RT11	Glass	17	3
RT12	Bagugosha	17	3
RT13	Frashishi	18	2
BG14	Desi nashpati	17	3
BG15	Btung	17	3
BG16	Khurolli	17	3
BG17	Btangi	17	3
BG18	Kashmiri nakh	15	5
BG19	Kotharnul	15	5
BG20	Desi nakh	18	2
BG21	Kotharnul	18	2
BG22	Khurolli	19	1
BG23	Kashmiri nakh	18	2
BG24	Bagugosha	18	2
BG25	Frashishi	17	3
MZ26	Kotharnul	18	2
MZ27	Desi nashpati	16	4
MZ28	Glass	19	1
MZ29	Kashmiri nakh	17	3
MZ30	Raj btung	18	2
MZ31	Raj btung	17	3
MZ32	Frashishi	17	3
MZ33	Btangi	18	2
MZ34	Pathar nakh	18	2
MZ35	Desi nakh	19	1
MZ36	Pathar nakh	18	2
MZ37	Raj btung	18	2
SD38	Btangi	16	4
SD39	Desi nakh	18	2
SD40	Frashishi	18	2
SD41	Desi nakh	17	3
SD42	Khurolli	16	4
SD43	Pathar nakh	17	3
SD44	Bagugosha	18	2
SD45	Kashmiri nakh	20	0
SD46	Glass	16	4
SD47	Desi nakh	16	4
SD48	Nashpati	19	1
SD49	Frashishi	17	3
KT50	Kotharnul	18	2
KT51	Kashmiri nakh	15	5

Table 1.contd.

KT52	Desi nakh	17	3
KT53	Btung	18	2
KT54	Btangi	17	3
KT55	Raj btung	18	2
KT56	Bagugosha	19	1
KT57	Pathar nakh	18	2
KT58	Khar nakh	17	3
KT59	LeConte	19	1
KT60	Keiffer	12	8

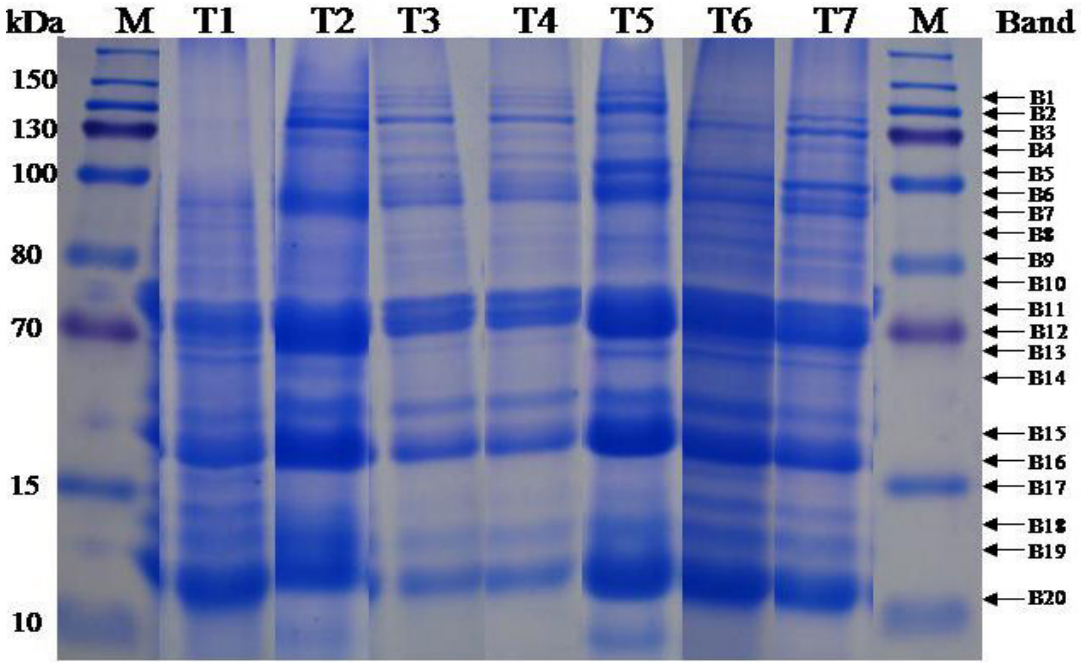

Figure 1. Electrophoretic banding pattern of pear accessions generated by SDS-PAGE analysis.

Table 2. Protein type composition based on SDS-PAGE.

Protein types	No. of bands	No. of accessions	Name of accessions
1	12	1	KT60
2	15	3	BG18-19, KT51
3	16	8	RT2, RT9-10, MZ27, SD38, SD42, SD46-47
4	17	18	RT1, RT 3,RT11-12, BG14-17, BG25, MZ29, MZ31-32, SD41, SD43, SD49, KT52, KT54, KT58
5	18	22	RT4, RT6-8, RT13, BG20-21, BG23-24, MZ26, MZ30, MZ33-34, MZ36-37, SD39-40, SD44, KT50, KT53, KT55, KT57
6	19	7	RT5, BG22, MZ28, MZ35, SD48, KT56, KT59
7	20	1	KT45

of SDS-PAGE of protein was sorted and classified the accessions into 12 groups and 19 individuals ranging from 0.71 to 0.97 similarity of coefficient level (Figure 2). The results further revealed that the highest similarity ranged from 0.91 to 0.97 that existed among groups 1 to 7. On the other hand, KT60 (Keiffer) exhibited the lowest

Table 3. Presence and absence of protein peptides in SDS-PAGE analysis of pear germplasm.

Protein band	Present	Absent	Polymorphic/Monomorphic
1	18	42	Polymorphic
2	60	0	Monomorphic
3	58	2	Polymorphic
4	56	4	Polymorphic
5	53	7	Polymorphic
6	58	2	Polymorphic
7	59	1	Polymorphic
8	21	39	Polymorphic
9	58	2	Polymorphic
10	58	2	Polymorphic
11	55	5	Polymorphic
12	60	0	Monomorphic
13	60	0	Monomorphic
14	60	0	Monomorphic
15	59	1	Polymorphic
16	42	18	Polymorphic
17	60	0	Monomorphic
18	26	34	Polymorphic
19	59	1	Polymorphic
20	60	0	Monomorphic

similarity and was the most distant from all other accessions. A dendrogram was constructed by using method of UPGMA, which classified the accessions into three major clusters along with seven subclusters, 12 identical groups and two independent accessions (starting top to bottom) (Figure 2). This division is consistent with the molecular studies of Kawata et al. (1995), Iketani et al. (1998) and Monte-Corvo et al. (2000), who divided *Pyrus* into occidental and oriental groups using RFLP, AFLP and RAPD markers. The major clusters were separated from each other at the level of 0.91 with coefficient of similarity of 0.92, 0.92 and 0.93. Number of accessions present in cluster I were 37 (61.7%), 16 in cluster II (26.7%), 3 in cluster III (5%), 2 in independent group 12 and 2 accessions existed independently (with same percentage, that is, 1.7%) (Table 5). The accessions of cluster I differenttiated into 3 subclusters, separated from major cluster varying from 0.94, 0.95 and 0.92 coefficient of similarity, showed less diversity. However, 7 identical groups had greater homogeneity among them as compared to those accessions branched with these subclusters and groups. (Table 4).

The second major cluster comprised three subclusters with four groups, that is, group 7, 8, 9 and 10, along with 7 independent accessions positioned at 0.92 to 0.97 coefficient of similarity levels. Furthermore, a subcluster having two identical accessions, that is, RT6 (Khurolli) and SD49 (Frashishi) branched with cluster III had close relationship at 0.97 showing the highest genetic relatedness. However, one more accession SD47 (Desi nakh) separated from the subcluster at 0.93 affinity level indicating variability and distantly related to other two accessions of this cluster. The individual group (12) of dendrogram consisted of two identical accessions, RT9 (Btangi) and RT10 (Kashmiri nakh) positioned distantly ranging from 0.87 to 0.97 and exhibited the highest genetic variability from all the accessions except KT60 (Figure 2). This group showed independent status in cluster analysis at 0.87 affinity level. The genetically diverse accessions, SD46 (Glass) had a lower affinity ranging from 0.81 to 0.94 separated from clusters at 0.87 coefficients of similarity (Figure 2). The most variable accession, KT60 (Keiffer) fell absolutely independently showing the highest genetic diversity and the lowest affinity range (0.71 to 0.97) from all the accessions. Moreover, this accession also inferred variability in number of protein subunits in banding pattern reflecting its genetic uniqueness. Although some morphological traits are similar to many accessions, yet Keiffer (KT60) being primitive variety and introduced for long in the mountainous region of Kashmir genetically differed with other accessions.

In the present study, total leaf proteins provide a clear idea about the accessions that segregate from main group. The results indicated that cluster I composed of seven groups which had same genetic relatedness and seven branched accessions showing variation to each other clearly separated from cluster and subclusters. Genetic similarities of the accessions found in groups were partially matched with morphological evidences

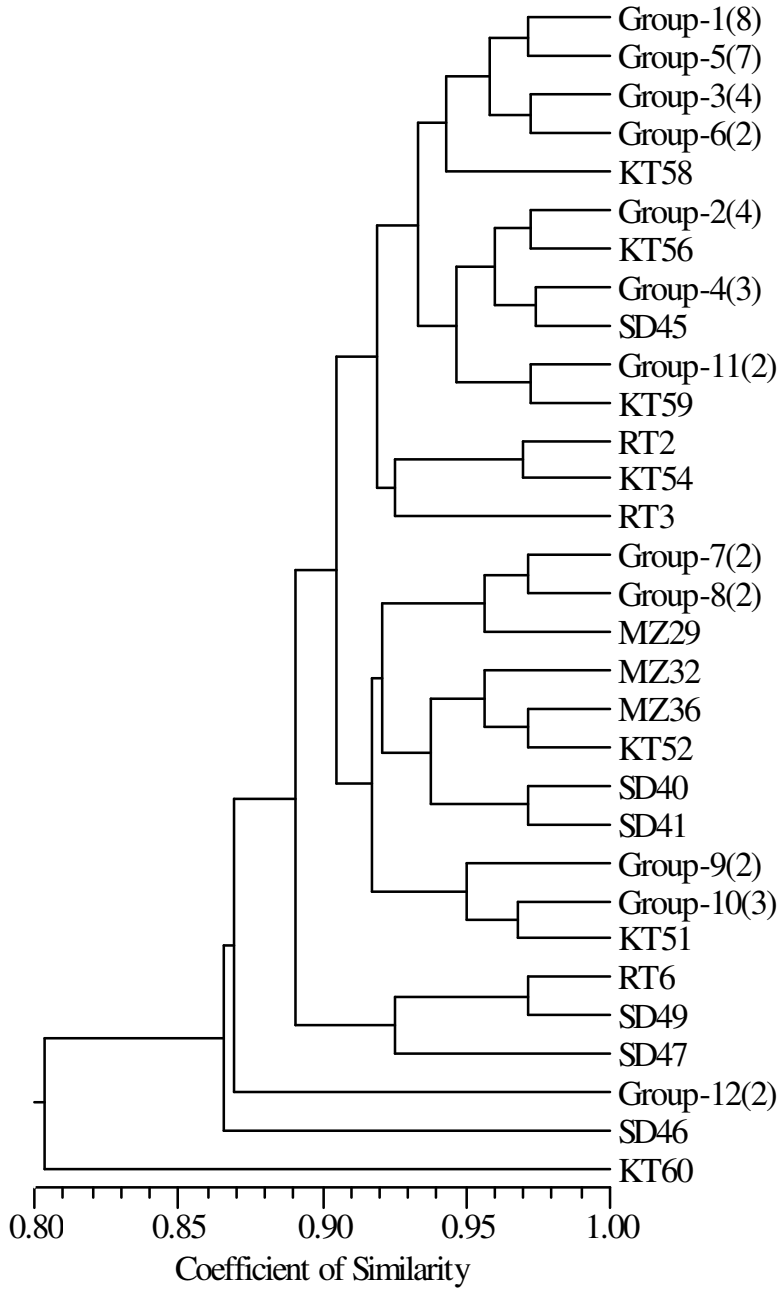

Figure 2. UPGMA dendrogram showing the relationships among 60 pear accessions revealed by SDS-PAGE cluster analysis.

which indicated that the accessions RT1 (Khurolli), RT11 (Glass) and RT12 (Bogugosha) were supported by morphological traits, whereas, some accessions like BG25 (Frashishi) and SD43 (Pathar nakh) showed 100% similarity to each other on the basis of protein bands analysis, whereas phenotypical and morphological evidences did not show any close relationship. Phenotypical characters are also used as sources of diversity identification markers (Challice et al., 1973; Kajiura et al., 1979). Such type of variation in morphological traits might be due to seedling origin or their interaction with environment. Genetic variation among species and populations might occur due to natural selection, genetic diversification and environmental impacts (Choong et al., 1996). It was further depicted that the accessions RT7 (Desi nash), BG23 (Kashmiri nakh) and SD39 (Desi nakh) showed close relationship in clustering, indicated genetically same gene source but phenotypically had slight resemblance with each other. RT4 (Pathar nakh), RT8 (Btung), MZ33 (Btangi) and MZ34 (Pathar nakh) were detected as similar genetic pattern but only two (RT4 and MZ34) were supported by morphological evidence. How-

Table 4. Detail of accessions present in groups by SDS-PAGE analysis of pear germplasm.

Group	No. of accessions	Name of accessions
1	8	RT1, RT11, RT12, BG14, BG15, BG17, BG25 and SD43
2	4	BG20, BG21, KT50, and KT55
3	4	RT4, RT8, MZ33 and MZ34
4	3	RT5, MZ28 and SD48
5	7	RT7, RT13, BG23, BG24, BG26, SD39 and SD44
6	2	BG16 and MZ31
7	2	MZ30 and MZ37
8	2	BG18 and BG19
9	2	KT53 and KT57
10	3	MZ27, SD38 and SD42
11	2	BG22 and MZ35
12	2	RT9 and RT10

Table 5. Cluster analysis of pear germplasm through SDS-PAGE.

Clusters/ individuals	No. of accessions	%age	No. of sub-clusters	No. of groups	# of branched /independent accessions	Name of accessions
I	37	61.7	3	7	7	RT1-RT5, RT7-RT8, RT11-RT13, BG14-BG17, BG20-BG26, MZ28, MZ31, MZ33-MZ35, SD39, SD43-SD45, SD48, KT50, KT54-KT56, KT58-KT59
II	16	26.7	3	4	7	BG18-BG19, MZ27, MZ29-MZ30, MZ32, MZ36-MZ37, SD38, SD40-SD42, KT51-KT53, KT57
III	3	5.0	1	-	3	RT6, SD47, SD49
VI	2	3.3	-	1	2	RT9-RT10
-	1	1.7	-	-	1	SD46
-	1	1.7	-	-	1	KT60

ever, Phenotypic similarities between BG21 (Kotharnul) and KT50 (Kotharnul), RT8 (Btung) and MZ33 (Btangi) and RT5 (Kotharnul) and MZ28 (Glass) represent true genetic relationship although grown at different geographical localities with different environmental conditions. Thus their genetic relationship seemed not to be influenced by climatic variations and showed parent-offspring relationship in this study. Similar results have been reported by (Yu, 1979) that Chinese white and sand pears resemble in leaf morphology and fruit characters with same or different protein profiles banding pattern. The maximum extent of similarity was shown by these accessions in their protein banding pattern and resemblance in their morphological characters suggesting similar genetic background. In addition to identical groups of cluster I, seven accessions (Figure 1) were branched with these groups individually, exhibited close relation with higher affinity level. Whereas, five of them showed variability in their morphological and phenological traits, while two accessions (KT56 and RT2) had close relationship both in phenotypic as well as cluster analysis. Environmental factors might be responsible for morphological variation among these accessions which did not show in protein based cluster analysis.

Cluster analysis further revealed genetic relationship of accessions corresponded with 4 genetically identical groups (7, 8, 9 and 10) along with some more accessions branched with these groups placed in cluster II with less variability with each other. However, morphological evidences did not support genetic relation, while two accessions of group 7, that is, MZ30 (Raj btung) and MZ37 (Raj btung) showed resemblance in fruit characters. Furthermore, branched accessions showed variation to some extent on protein based analysis but entirely differed phenotypically and morphologically with each other as in KT51 (Kashmiri nakh) branched with group 10. Thus, it would be concluded that branched accessions were the progenies of their corresponding groups of clusters. This hypothesis is partially supported by the findings of (Lin and Shen, 1983) who used isozymic patterns and predicted that wild *P. pyrifolia* is a common progenitor species of Chinese sand and white pears.

The accessions RT 6 (Khurolli) and SD49 (Frashishi) showed relationship between them and are placed at less

distance with each other in cluster III but their phenotypic relation with regard to fruit colour did not support to protein based relationship. However, the accession SD47 (Desi nakh) distantly branched with these two, showed variability both in their fruit character as well as banding pattern based analysis. In view of above cluster analysis, protein based analysis of some accessions corresponded with morphological traits and on the other hand, different genetic pattern were also observed in some cases in the present study. The high range of affinities might be due to parent offspring relationship as a result of cross pollination (Kimura et al., 2002). The variability in phenoltypical and morphological traits might be due to interaction between environment and genotypes (Pu and Wang, 1963; Yu, 1979; Teng et al., 2002).

Accessions RT9 (Btangi) and RT10 (Kashmiri nakh) of group 12 were genetically identical to each other, exhibitted the highest diversity with other clusters and fell independently in dendrogram. Moreover, RT9 (Btangi) grows abundantly in wild form with seedling origin and widely used as hardy rootstock for pear. Its fruit is small, round and matures late. As State of Jammu and Kashmir is situated in Himalayan region which is near to centre of origin of *Pyrus* germplasm, it seems quite logical that Btangi and its other kind of accessions namely BG17, MZ33, SD38 and KT54 might have parental relations with Chinese origin germplasm. Pu and Wang (1963) and Yu (1979) reported that pea pear belongs to *Pyrus betulaefolia* having small sized fruit, distributed from east to west in North China and is extensively used as rootstock in East Asia.

SD46 (Glass) had high degree of genetic diversity which did not exhibit genetic relationship on protein based analysis. Phenotypical and morphological evidences showed close relationship of this accession with RT1 (Khurolli), RT3 (Glass) and BG19 (Kotharnul) with its fruit shape and colour. Such distant affinity of this accession with other accessions of pear indicates that it might be a hybrid clone. Variability and some relationship among all these accessions showed inheritance relation with each other as a result of hybridization as suggested by Westwood and Bjornstad (1971) and Bell and Hough (1986). The most diverse accession/variety KT60 (Keiffer), had different genetic make-up and the highest polymorphism. This accession did not show any relation with all other accessions in the present study and existed independently with unique genetic background. The high degree of variability in this accession (KT60) for protein profile analysis might be because it was a diverse clone introduced a long ago as a cultivar. The highest diversity in other wild as well as primitive varieties of genus *Pyrus* still need to be explored on molecular level that might be helpful to understand the wide range of native accessions for genetic variability or similarities (Volk et al., 2006). Moreover, the variation of the existing germplasm could be of enormous value to breeders for developing new cultivars and to design their hybridization program with greater success. In light of these results, the highly diverse individuals of genotypes SD46 (Glass) and KT60 (Keiffer) containing polymorphisms are the most threatened and should be explored and secured from human activities and natural disasters. Collection and maintenance of diverse populations are more secured instead of collecting a few samples from each popula-tions (Kaundun and Park, 2002). Also those individuals had more polymorphism and should be secured (Leyla et al., 2009).

A very limited report on SDS-PAGE for leaf protein is available for characterization of diverse fruit germplasm (cultivars and species). So it seemed that more divergence in fruit species and their wild relatives are necessary to find out polymorphism in leaf protein. SDS-PAGE technique has proven to be a useful tool in supporting classical taxonomy studies. Protein types and their variation differed among different species, which information will help us for early identification of the species at seed level as well as to get the information on purity of genetic resources. Protein banding patterns as revealed by SDS-PAGE produces reproducible band pattern (profile) when proteins are prepared in a standard method and hence have valid value in taxonomic purpose. Consequently, proteins with identical electrophoretic mobility are deemed to represent the same unit character. Therefore, characters derived from seed proteins have been utilized in plant taxonomy at different levels to construct phenetic classifications (Boulter, 1981). Hence they can be considered as traits to study genetic variation among the plant taxa. Storage proteins are direct, stable products of genes that can reflect DNA diversity of plants (Jin et al., 2006). Electrophoretic markers appear to be due to neutral genes, which are not linked to any loci that affect the cultivar and the value. They are also independent of cultivar morphology and physiology and offer significant advantages over morphological methods of variety and/or species identification in that they are rapid, relatively cheap, eliminate the need to grow plants to maturity and largely unaffected by the growth environment. Recently, the number of available markers in plants has increased dramatically with the use of molecular biology techniques. With these techniques it is now possible to identify variation at the DNA level which may not be expressed to differentiate protein phenotype. However these techniques need enough capital outlay which is not available for most of the scientists in the developing countries (Thanh et al., 2006).

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