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Analysis of genetic variation in *Pinus* species using SDS-page

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Abstract

Genetic diversity and phylogenetic characterization among five Pinus species (*wallichiana, roxburghii, brutia, strobus and elliottii*) were investigated in the present study. *Wallichiana, roxburghii* and *brutia* are native while *strobus* and *elliottii* are exotic in nature. Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) was used to explore phylogenetic characterization based on of Seed storage protein. The genetic distances were estimated among the 70 germplasms accessions, ranges from 0-100%. Using Popgen version 3.2, a total of 182 alleles were scored and Phylogenetic relationships were clustered in 2 main groups "A" and "B" comprising 36 and 34 accessions, respectively. At this end it is concluded that the sample size should be extended for better understanding of the phylogenetic relationship among these species.

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Introduction

Pinus belongs to *Pinaceae* family of the Gymnosperm. Species of this family comprise leaves in the form of needles or scales, and are evergreen in most cases (Vidakovic AND Mirko, 1991). Branches are usually in pseudo-whorls, shoots dimorphic, long shoots with scaly leaves and dwarf shoots with needle like leaves (ALI & NASIR 1987). Ovulate cones solitary and contain two seeds at the base of the cone scale, winged, in some the wing vestigial (KRAL *at al.* 1993). The interior structure of the *Pinus* leaf is important for identification (PEATTIE *et al.* 1950). Conifers have been widely established in forest plantations because of their innumerable uses and relative ease of plantation (ZHENG & RAVEN 1999). Proteins and lipids constitute the bulk of the storage reserves in seeds of most of the Pinaceae species have been examined to date (CHING *et al.* 1966; SIMOLA *et al.* 1974; KOVAC & KREGAR 1989; OWENS *et al*, 1993; GROOME *et al*, 1991). Proteolytic enzymes involved in protein reserve breakdown (SALMIA & MIKOLA 1976; SALMIA *et al.* 1981; GIFFORD *et al.* 1989). There also is some evidence that the urea pathway functions in seedlings of various Pine species (NAYLOR 1959).

In Pakistan conifer forests, principally *Pinus* species are the dominant forest cover over much of Hazara division, Swat and mountainous northern portion of NWFP. The wood of many species of *Pinus* is used for structural lumber, production of paper, fuel wood, posts, poles, essential oils, resin, fragrant, foliage, ornamental, decorative objects, edible seeds, flavorings and medicinal products. Despite enormous economic and social importance of *Pinus*, not much research has been done on Pines to investigate genetic variation in *Pinus* using SDS-PAGE in the selected area of Pakistan.

Aim of the current study is to investigate morphological characterization of the 5 *Pinus* germplasm, optimization of sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS-PAGE) Protocol for Pines, Profiling of various species of Pines based on morphological and biochemical characteristics, estimation of genetic diversity in the germplasm accessions, to establish phylogenetic relationship collected from Hazara division and Swat district.

Materials and method

Plant Material

Specimens, seeds and samples from five *Pinus* species viz. *Pinus wallichiana, Pinus roxburghii, Pinus strobus, Pinus brutia* and *Pinus elliottii* were collected from Northern areas of NWFP Pakistan, especially from Malakand, Swat and Hazara areas including Hazara University garden campus, Kaghan, Siran valley and Balakot (Table 1). Matured seeds were collected from each species, dried and were used to extract total seed storage protein.

Protein Extraction

For Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis, single seed from each species were taken and seed coat or testa from each seed was removed. Protein was extracted from *Pinus* seeds using various protocols previously described (PAYNE *et al.* 1987). Briefly, all seeds were placed in an oven over night at 37° c to remove the water content of the seed. The dry seed of each genotype were grounded in eppendorf tube with large needle. Then 500µl of protein extraction buffer (PEB) was added to 0.01g of seed flour and vortexes (using Gyromixer vortex) thoroughly to homogenize. The proteins were extracted at room temperature for 20 minutes. In order to purify, the homogenate samples were centrifuged (using Eppendorf centrifuge model No 0021586) at 12,000 rpm for 10 minutes at room temperature. The extracted crude proteins were recovered as clear supernatant and were transferred to a new 1.5 ml Eppendorf tubes and stored at 4°C until they were run on the polyacrylamide gel.

Electrophoresis and Documentation

In an attempt to optimize the Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) protocol suitable for Pine seed, different protocols were tested. For example the protocols used previously by Payne (1987) for protein analysis of

wheat and Lioi *et al.* (1999) for protein analysis of chickpea (*Cicer*) were used using modifications. Changes were made in these protocols to developed protocol suitable for protein analysis of *Pinus* species. The protocol that provided best results is summarized below.

Sodium Dodecyle Sulphate Polyaclrymide Gel Electrophoresis (SDS-PAGE) was run using procedure described by Laemmli (1970). The electrophoretic procedure was carried out using slab type SDS-PAGE Model: MGV-202, with 12.5% polyacrylamide gel. A 12.5% resolving gel (3.0M Tris-HCL (Sigma) pH 9, 0.4% SDS (Wako) and 4.5% stacking gel (0.4M Tris-HCL pH 7.0, 0.4% SDS) was prepared and polymerized chemically by addition of 17 µl of N, N', N', N' tetramethylene diamine (Wako) and 10% Ammonium persulphate (Circa reagent). The gels were run till the tracking dye "Bromophenol blue" (BPB) reaches the bottom of the gel. Gels were visualized on light plate and photographed using "UVITEC" gel documentation system.

Results

Seed storage protein profiles of seventy genotypes were constructed using SDS-PAGE. Based on principles outlined by Nei and Li (1979), only clearly scoreable major bands were included in the analyses. Minor bands which could not be scored reliably were excluded. Four various mobility bands (alleles) were scored in seventy germplasm entries used during present study. The individual protein bands were considered as allele/loci. Each allele/loci were scored as present (1) or absent (0), to generate bi-variate data matrix as shown in Table.1. In total 182 alleles were observed in 70 genotypes giving an average of 2.6 alleles per genotype. The result is summarized in Table.2. Genetic distances (GD) among the genotypes were calculated based on "Un Weighted Pair Group of arithmetic Means (UPGMA)" procedure (NEI & LI 1979). A high degree of genetic variability based on protein profile was observed in the material. Range of genetic distance observed among the germplasm accessions was 0–100%. 421 comparisons showed complete homozygosity (GD=0.0) for the protein loci detected during present study. While 184 comparisons showed maximum genetic distance (GD=100 %). Remaining 4295 comparisons showed various levels of genetic distances ranging from 25% to 75%.

Table 1. Bi-variate data matrix of 70 Pinus accessions used during present study.

Genotype	Alleles	Genotype	Alleles	Genotype	Alleles
1	0 0 0 1	25	0 0 0 1	49	1 1 1 0
2	1 1 1 1	26	1 1 0 1	50	0 0 1 1
3	0 1 1 1	27	1 0 0 1	51	0 0 0 1
4	0 1 0 1	28	0 0 1	52	0 0 0 1
5	1 1 1 1	29	0 1 1 1	53	1 1 0 1
6	0 1 1 1	30	0 0 0 1	54	0 0 1 0
7	0 1 1 1	31	0 1 1 1	55	1 1 1 0
8	1 1 0 1	32	0 1 0 1	56	0 0 1 1
9	0 0 0 1	33	0 0 0 1	57	1 1 1 1
10	1 1 1 1	34	1 1 1 1	58	0 1 1 1
11	0 0 0 1	35	0 0 1 0	59	0 1 1 1
12	0 1 1 1	36	0 0 1 1	60	0 1 1 1
13	0 1 1 1	37	0 1 0 1	61	1 1 1 1
14	0 1 1 1	38	1 1 1 1	62	0 1 1 1
15	1 1 1 1	39	1 1 1 1	63	0 1 1 0
16	1 1 1 1	40	1 1 1 1	64	1 1 1 1
17	1 1 1 1	41	1 1 1	65	0 1 1
18	1 1 1 1	42	1 1 1 1	66	0 1 1 1
19	1 0 0	43	1 1 1 1	67	0 0 0 1
20	1 1 0 1	44	0 1 1 1	68	0 1 1 0
21	0 1 0 1	45	0 0 0 1	69	0 1 1 0
22	0 1 1 1	46	0 1 1 1	70	1 0 0 0
23	0 1 0 1	47	1 1 1 0		
24	0 1 1 1	48	1 0 1 0		

1=Presence of allele, 0 = Absence of allele, 1=P. *wallichiana*, Batal 2=P. *wallichiana*, Swat 3=P. *roxburghii*, Mansehra 4=P. *roxburghii*, Garden campus 5=P. *wallichiana*, Swat 6= P. *wallichiana*, Batal, 7=P. *wallichiana*, Swat, 8=P. *roxburghii*, Garden campus, 9=P. *brutia*, Garden campus, 10=P. *brutia*, Garden campus, 11=P. *wallichiana*, Batal, 12=P. *wallichiana*, Batal, 13=P. *wallichiana*, Swat, 14=P. *roxburghii*, Shankiari, 15=P. *elliottii*, Garden campus, 16=P. *roxburghii*, Mansehra, 17=P. *roxburghii*, Batal, 18=P. *roxburghii*, Dadar, 19=P. *wallichiana*, Swat, 20=P. *roxburghii*, Mansehra, 21= P. *wallichiana*, Batal, 22=P. *wallichiana* Balakot, 23 = P. *brutia*, Garden campus, 24=P. *wallichiana*, Swat, 25=P. *wallichiana*, Swat, 26=P. *wallichiana*,, Batal (Hazara), 27=P. *wallichiana*, Batal (Hazara), 28=P. *wallichiana*, Swat, 29=P. *wallichiana*, Swat, 30=P. *wallichiana*, Swat, 31=P. *wallichiana*, Batal (Hazara), 32=P. *roxburghii*, Mansehra, 33=P. *wallichiana*, Batal (Hazara), 34=P. *wallichiana*, Batal (Hazara), 35=P. *roxburghii*, Garden campus, 36=P. *roxburghii*, Garden campus, 37=P. *roxburghii*, Batal (Hazara), 38=P. *roxburghii*, Mansehra, 39=P. *roxburghii*, Batal (Hazara), 40=P. *roxburghii*, Batal (Hazara), 41=P. *roxburghii*, Dadar valley, 42=P. *wallichiana*, Swat, 43=P. *wallichiana*, Batal (Hazara), 44=P. *roxburghii*, Mansehra, 45=P. *wallichiana*, Batal (Hazara), 46=P. *brutia*, Garden campus, 47=P. *roxburghii*, Mansehra, 48=P. *wallichiana*, Swat, 49=P. *wallichiana*, Swat, 50=P. *roxburghii*, Batal (Hazara), 51=P. *wallichiana*, Batal (Hazara), 52=P.*wallichiana*, Batal (Hazara), 53=P.brutia, Garden Campus, 54=P.strobus, Garden Campus, 55=P.*roxburghii*, Mansehra, 56=P. *wallichiana*, Batal (Hazara), 57=P. *brutia*, Garden Campus, 58=P. *roxburghii*, Garden campus, 59=P. *roxburghii*, Mansehra, 60=P. *wallichiana*, Batal (Hazara), 61=P. *wallichiana*, Batal (Hazara), 62=P. *roxburghii*, Dadar valley, 63=P. *roxburghii*, Batal (Hazara), 64=P. *wallichiana*, Batal (Hazara), 65=P. *wallichiana*, Swat, 66=P. *wallichiana*, Balakot, 67=P. *roxburghii*, Mansehra, 68=P. *brutia*, Garden Campus, 69=P. *roxburghii*, Garden campus, 70=P. *wallichiana*, Swat.

Discussion

Pinus is the large genus of *Pinaceae* contains tall, aromatic, evergreen, trees. Branches are dimorphic long shoots with scaly leaves and dwarf shoots with needle like leaves (ALI & NASIR 1987). In Pakistan five species reported by Riedl. 1963 and Nasir *et al*., 1969. Two newly reported species collected from Garden Campus of Hazara University, Mansehra were also included in the study viz *Pinus strobus* L., and *Pinus elliottii* Engelm. Total native and introduced including cultivated become seven species in Pakistan. Despite the enormous economic and social importance of family *Pinaceae* (especially Genus *Pinus)*, not too much work has been reported in Pakistan on biochemical /molecular analyses of the family. Present work is the first documented attempt to study proteomic analysis of some important species of the Genus *Pinus*. Research was initiated with aim to develop/optimize techniques suitable for isolation and separation of total seed storage protein using Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) as shown in figure 1. The objectives were met successfully; an easy, quick and cheap protocol has been developed for the biochemical analysis in Genus *Pinus*.

Table 2. Genetic distances among 70 *Pinus* accessions used during the present study (presented in percentage).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1																								
2	0.75																							
3	0.67	0.25																						
4	0.50	0.50	0.33																					
5	0.75	0.00	0.25	0.50																				
6	0.67	0.25	0.00	0.33	0.25																			
7	0.67	0.25	0.00	0.33	0.25	0.00																		
8	0.67	0.25	0.50	0.33	0.25	0.50	0.50																	
9	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67																
10	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75															
11	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75														
12	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67													
13	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00												
14	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00											

15	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25										
16	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00									
17	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00								
18	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00							
19	1.00	0.75	1.00	1.00	0.75	1.00	1.00	0.67	1.00	0.75	1.00	1.00	1.00	1.00	0.75	0.75	0.75	0.75						
20	0.67	0.25	0.50	0.33	0.25	0.50	0.50	0.00	0.67	0.25	0.67	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.67					
21	0.50	0.50	0.33	0.00	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.33				
22	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33			
23	0.50	0.50	0.33	0.00	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.33	0.00	0.33		
24	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	
25	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	0.50	0.67	0.50	0.67
26	0.67	0.25	0.50	0.33	0.25	0.50	0.50	0.00	0.67	0.25	0.67	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.67	0.00	0.33	0.50	0.33	0.50
27	0.50	0.50	0.75	0.67	0.50	0.75	0.75	0.33	0.50	0.50	0.50	0.75	0.75	0.75	0.50	0.50	0.50	0.50	0.33	0.67	0.75	0.67	0.75	
28	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	0.50	0.67	0.50	0.67
29	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
30	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	0.50	0.67	0.50	0.67
31	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
32	0.50	0.50	0.33	0.00	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.33	0.00	0.33	0.00	0.33
33	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	0.50	0.67	0.50	0.67
34	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.25	0.50	0.25	0.50	0.25
35	0.00	0.75	0.67	1.00	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	1.00	0.67	1.00	0.67
36	1.00	0.50	0.33	0.67	0.50	0.33	0.33	0.75	1.00	0.50	1.00	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.75	0.67	0.33	0.67	0.33
37	0.50	0.50	0.33	0.00	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.33	0.00	0.33	0.00	0.33
38	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.25	0.50	0.25	0.50	0.25
39	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.25	0.50	0.25	0.50	0.25
40	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.25	0.50	0.25	0.50	0.25
41	1.00	0.25	0.50	0.75	0.25	0.50	0.50	0.50	1.00	0.25	1.00	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.67	0.50	0.75	0.50	0.75	0.50
42	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.25	0.50	0.25	0.50	0.25
43	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.25	0.50	0.25	0.50	0.25
44	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
45	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	0.50	0.67	0.50	0.67
46	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
47	1.00	0.25	0.50	0.75	0.25	0.50	0.50	0.50	1.00	0.25	1.00	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.67	0.50	0.75	0.50	0.75	0.50
48	1.00	0.50	0.75	1.00	0.50	0.75	0.75	0.75	1.00	0.50	1.00	0.75	0.75	0.75	0.50	0.50	0.50	0.50	0.75	1.00	0.75	1.00	0.75	
49	1.00	0.25	0.50	0.75	0.25	0.50	0.50	0.50	1.00	0.25	1.00	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.67	0.50	0.75	0.50	0.75	0.50
50	0.50	0.50	0.33	0.67	0.50	0.33	0.33	0.75	0.50	0.50	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.75	0.67	0.33	0.67	0.33
51	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	0.50	0.67	0.50	0.67
52	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	0.50	0.67	0.50	0.67
53	0.67	0.25	0.50	0.33	0.25	0.50	0.50	0.00	0.67	0.25	0.67	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.67	0.00	0.33	0.50	0.33	0.50
54	1.00	0.75	0.67	1.00	0.75	0.67	0.67	1.00	1.00	0.75	1.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	1.00	1.00	0.67	1.00	0.67
55	1.00	0.25	0.50	0.75	0.25	0.50	0.50	0.50	1.00	0.25	1.00	0.50	0.50	0.50	0.25	0.25	0.25	0.25	0.67	0.50	0.75	0.50	0.75	0.50
56	0.50	0.50	0.33	0.67	0.50	0.33	0.33	0.75	0.50	0.50	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.75	0.67	0.33	0.67	0.33
57	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.25	0.50	0.25	0.50	0.25
58	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
59	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
60	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
61	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	0.75	0.25	0.50	0.25	0.50	0.25
62	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
63	1.00	0.50	0.33	0.67	0.50	0.33	0.33	0.75	1.00	0.50	1.00	0.33	0.33	0.33	0.50	0.50	0.50	0.50	0.50	0.75	0.67	0.33	0.67	0.33
64	0.75	0.00	0.25	0.50	0.00	0.25	0.25	0.25	0.75	0.00	0.75	0.25	0.25	0.25	0.00	0.00	0.00	0.00	1.00	0.25	0.50	0.25	0.50	0.25
65	0.50	0.50	0.33	0.67	0.50	0.33	0.33	0.75	0.50	0.50	0.50	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.75	0.67	0.33	0.67	0.33
66	0.67	0.25	0.00	0.33	0.25	0.00	0.00	0.50	0.67	0.25	0.67	0.00	0.00	0.00	0.25	0.25	0.25	0.25	1.00	0.50	0.33	0.00	0.33	0.00
67	0.00	0.75	0.67	0.50	0.75	0.67	0.67	0.67	0.00	0.75	0.00	0.67	0.67	0.67	0.75	0.75	0.75	0.75	1.00	0.67	0.50	0.67	0.50	0.67
68	1.00	0.50	0.33	0.67	0.50	0.33	0.33	0.75	1.00	0.50	1.00	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.75	0.67	0.33	0.67	0.33
69	1.00	0.50	0.33	0.67	0.50	0.33	0.33	0.75	1.00	0.50	1.00	0.33	0.33	0.33	0.50	0.50	0.50	0.50	1.00	0.75	0.67	0.33	0.67	0.33
70	1.00	0.75	1.00	1.00	0.75	1.00	1.00	0.67	1.00	0.75	1.00	1.00	1.00	1.00	0.75	0.75	0.75	0.75	0.00	0.67	1.00	1.00	1.00	1.00

Using currently developed protocols, seed storage protein profile of 70 specimens from five (5) *Pinus* species were constructed. A total of 182 alleles were scored in the 70 accessions. Genetic diversity among the accessions was estimated ranged from 0-100%. A total of 421 comparisons showed complete homozygosity (GD=0.0) for the protein loci detected during present study. While 184 comparisons showed maximum genetic distance (GD=100 %). Remaining comparisons showed various levels of genetic distances ranging from 25% to 75%. Phylogenetic elaboration among the *Pinus* accessions was studied using cluster analysis as shown in figure.2. The seventy accessions were clustered in 2 broad groups "A" and "B" comprising 36 and 34 accessions, respectively. Groups "A" was further subdivided into 3 sub groups "C", "D" and "E" comprising 13, 6 and 17 accessions, respectively. Main cluster "B" further

subdivided into 4 sub clusters viz; “F”. “G”.” H” and ”I” comprising 19, 4, 6 and 5 accessions, respectively.

Despite the enormous economic and social importance of Genus *Pinus*, not much work has been reported in Pakistan on biochemical /molecular analyses of the family. Present work is the first documented attempt to study proteomic analysis of some important species of the Genus *Pinus*. Research was initiated with aim to develop/optimize techniques suitable for isolation and separation of total seed storage protein using Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE). The objectives were met successfully; an easy, quick and cheap protocol has been developed for the biochemical analysis in Genus *Pinus*.

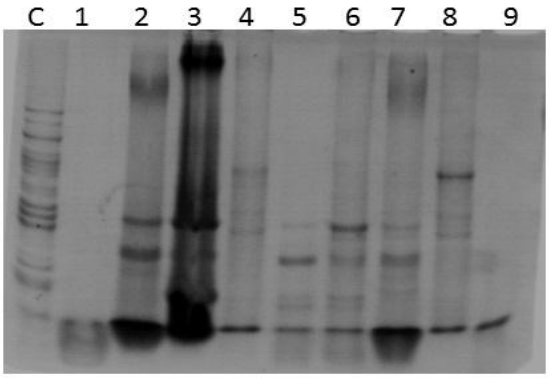

Fig. 1. Seed storage protein profile of nine accessions of Pines using SDS-PAGE. C= Check entry. 1=P. *wallichiana*, Batal 2=P. *wallichiana*, Swat 3=P.*roxburghii*, Mansehra 4=P. *roxburghii*, Garden campus 5=P. *wallichiana*, Swat 6= P. *wallichiana*, Batal, 7=P. *wallichiana*, Swat, 8=P. *roxburghii*, Garden campus, 9=P. *brutia*, Garden campus.

It is strongly recommended that more research work especially using recently developed DNA techniques (utilizing DNA based markers including RAPD, SSR, etc) should be conducted for better understanding of the genome structure in *Pinus*. It is also highly recommended that techniques developed during present work should be utilized for other *Pinus* accessions found in Pakistan which will help in construction of first molecular based data bank of this important family which is getting increasing importance these days mainly because of their socio economic values.

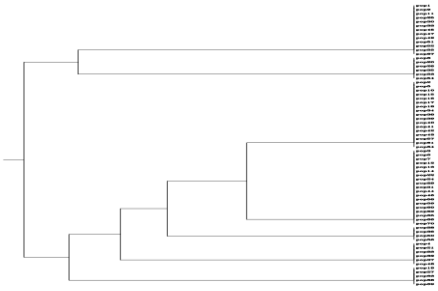

Fig. 2. Dendrogram constructed for 70 accessions of *Pinus* using 1-0 bivariate data matrix generated from SDS-PAGE analysis. 1= P. *wallichiana*, Batal 2= P. *wallichiana*, Swat 3= P. *roxburghii*, Mansehra 4= P. *roxburghii*, Garden campus 5= P. *wallichiana*, Swat 6= P. *wallichiana*, Batal, 7= P. *wallichiana*, Swat, 8= P. *roxburghii*, Garden campus, 9= P. *brutia*, Garden campus, 10= P. *brutia*, Garden campus, 11= P. *wallichiana*, Batal, 12= P. *wallichiana*, Batal, 13= P. *wallichiana*, Swat, 14= P. *roxburghii*, Shankiari, 15= P. *elliottii*, Garden campus, 16= P. *roxburghii*, Mansehra, 17= P. *roxburghii*, Batal, 18= P. *roxburghii*, Dadar, 19= P. *wallichiana*, Swat, 20= P. *roxburghii*, Mansehra, 21= P. *wallichiana*, Batal, 22= P. *wallichiana* Balakot, 23 = P. *brutia*, Garden campus, 24= P. *wallichiana*, Swat, 25= P. *wallichiana*, Swat, 26= P. *wallichiana*, Batal (Hazara), 27= P. *wallichiana*, Batal (Hazara), 28= P. *wallichiana*, Swat, 29= P. *wallichiana*, Swat, 30= P. *wallichiana*, Swat, 31= P. *wallichiana*, Batal (Hazara), 32= P. *roxburghii*, Mansehra, 33= P. *wallichiana*, Batal (Hazara), 34= P. *wallichiana*, Batal (Hazara), 35= P. *roxburghii*, Garden campus, 36= P. *roxburghii*, Garden campus, 37= P. *roxburghii*, Batal (Hazara), 38= P. *roxburghii*, Mansehra, 39= P. *roxburghii*, Batal (Hazara), 40= P. *roxburghii*, Batal (Hazara), 41= P. *roxburghii*, Dadar valley, 42= P. *wallichiana*, Swat, 43= P. *wallichiana*, Batal (Hazara), 44= P. *roxburghii*, Mansehra, 45= P. *wallichiana*, Batal (Hazara), 46= P. *brutia*, Garden campus, 47= P. *roxburghii*, Mansehra, 48= P. *wallichiana*, Swat, 49= P. *wallichiana*, Swat, 50= P. *roxburghii*, Batal (Hazara), 51= P. *wallichiana*, Batal (Hazara), 52= P. *wallichiana*, Batal (Hazara), 53= P. *brutia*, Garden Campus, 54= P. *strobus*, Garden Campus, 55= P. *roxburghii*, Mansehra, 56= P. *wallichiana*, Batal (Hazara), 57= P. *brutia*, Garden

Campus, 58= P. *roxburghii*, Garden campus, 59= P. *roxburghii*, Mansehra, 60= P. *wallichiana*, Batal (Hazara), 61= P. *wallichiana*, Batal (Hazara), 62= P. *roxburghii*, Dadar valley, 63= P. *roxburghii*, Batal (Hazara), 64= P. *wallichiana*, Batal (Hazara), 65= P. *wallichiana*, Swat, 66= P. *wallichiana*, Balakot, 67= P. *roxburghii*, Mansehra, 68= P. *brutia*, Garden Campus, 69= P. *roxburghii*, Garden campus, 70= P. *wallichiana*, Swat.

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Abbreviations

SDS-PAGE= Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis, GD=Genetic distances, UPGMA=Un Weighted Pair Group of arithmetic Means.