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INTRA AND INTER SPECIFIC PROFILING OF PAKISTANI *QUERCUS* SPECIES GROWING IN THE HILLY AREAS OF DISTRICT DIR KHYBER PAKHTUNKHWA

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Abstract

The intra and inter genetic diversity among 20 genotypes of Pakistani *Quercus* species viz., ten genotypes of *Q. incana*, eight *Q. baloot* and two *Q. dilatata* were analyzed using morphological characterization and proteomic profiling. A total of 14 morphological traits were scored for estimation of genetic diversity through descriptive statistics, traits similarity index and cluster plotting. Similarly, seven loci (bands) were detected in the collected germplasm of *Quercus* sp. Intra species locus contribution to genetic diversity (*LCGD*) was 42.9% in *Q. baloot* and 14.2% in *Q. incana*. Similarly, inter species *LCGD* was 71.43% in the collected germplasm. Out of seven loci, locus-1, 5 and 6 showed polymorphic in *Q. baloot* and locus 6 in *Q. incana*. Importantly, locus 3 and 4 was monomorphic in all collected lines and marked as generic specific locus for *Quercus* (sp.). SDS-PAGE profiling based on one-way cluster plotting successfully resolved the three species into separate clusters. The present data reflect that though the *Quercus* sp. showing intra and Inter species genetic diversity, but maintained species specific identity in the area regardless of environmental fluctuation.

Key words: *Quercus* species, Morphological characterization, Proteomic profiling.

Introduction

Quercus (Banj) is a genus of family Fagaceae, that consists of 8 genera and 900 species, widely distributed in temperate regions of the world, including Pakistan, where this family is represented by two genera (*Castana* and *Quercus*) as reported by Nasir (1976). However, later 6 species were reported in the Northern temperate mountains of Pakistan, whereas is *Quercus rouber* an introduced species (Shah *et al*., 2005). Its wood is durable, tough, attractively grained and especially valued in shipbuilding, flooring, furniture, railroad ties, barrels, tool handles, veneers and also for timber purposes. The dried bark of *Quercus* is used for medicinal purposes that are a rich source of tannin, used for tanning leather. The Acorns (fruit) are used for making flour or roasted for acorn coffee. The primary wood is used to make barrels for ageing wine. It is hard and watertight, but perhaps more importantly, it imparts phenolics such as tannins and important flavor compounds to wine (Nasir, 1976).

The woody *Quercus* species are widespread, long-lived, out crossing by wind. Therefore, it can spread into wide geographic regions that show high genetic variations as compared to other woody plant species (Neophytou *et al*., 2010). It is known that hybridization among species in the same genus *Quercus* (Jensen *et al*., 2009) due to weak reproductive barriers among oak species, consequently hybrid species spring up vigorously. Therefore the genus *Quercus* is taxonomically one of the most problematic groups (Bacilieri *et al*., 1996). For classification, morphological and molecular characterizations are widely used to delimit genera and species more clearly, also to investigate levels of genetic diversity (Kelleher *et al*., 2005; Yilmaz *et al*., 2011).

Morphological characterization is the prerequisite to investigate the genetic composition of species; but adversely affected by environmental changes. Contrarily, molecular characterizations (protein and DNA based Makers) are reliable and free of environmental effects. Kelleher *et al*. (2005) found that morphological traits clearly distinguished Irish Oak population in comparison with DNA markers (AFLP) and the results are not in good agreement. Similarly, storage seed proteins (SDS-PAGE) have proved to be a useful tool to evaluate the genetic variability in many plant species and useful indicator for estimation of taxonomic relationships. SDS-PAGE is as an important genetic marker in some species, mainly in cereals (Duran *et al*., 2005; Amar *et al*., 2014). The main advantages of proteins as markers are the high polymorphism level, simple genetic control, environmental independence, and the economy, easiness and expeditiousness of their analysis. The role of proteins in forest species has been scarcely studied, and a few works have been reported in the family Fagaceae (Galvan *et al*., 2011) that explored its biochemical compositions (Alvarez *et al*., 2003). Duran *et al*. (2005) reported that SDS-PAGE differentiate plant species and as a useful indicator of taxonomic relationships, whereas Rogle *et al*. (1996) observed that SDS-PAGE an important tool for differentiation of *Quercus* species. Furthermore, Galvan *et al*. (2012) concluded that Physiological parameter and proteomics makers separate Oak genera into different species regardless of environmental changes.

Keeping in view the applicability of morphological and proteomic analyses for distinguishing (estimation of genetic distances) of Quercus species, the present study was designed to investigate the level of genetic diversity based on morphological characterizations and SDS-PAGE markers. The main focus was to investigate intra and inter specific variation in Pakistani *Quercus* species growing in the mountainous areas of Khyber Pkhtunkhwa, Pakistan.

Materials and Methods

Exploration and collection: Exploratory trips were arranged to 20 different localities (Zyam, Barcharai-Talash, Rogbanai, Barcharai-Top, Jaleel-Baba, Shen-Kamar, Degan, Ghaljo, Khadango, Shaheed, Jawaro, Nawagai, Oleganda, Khawas, Shorshing, Kumbanar, Hamza-Banda, Bambolai-Bala, Danda and Babakrai) of District Dir (Lower), Khyber Pakhtunkhwa during two consecutive years, i.e., 2013-2014. Map of the area is represented in Fig. 1. Twenty genotypes of three species were identified and investigated for morphological characterization and protein profiling. *Quercus incana* was abundant followed by *Quercus baloot*, whereas *Quercus dilatata* was least in occurrence.

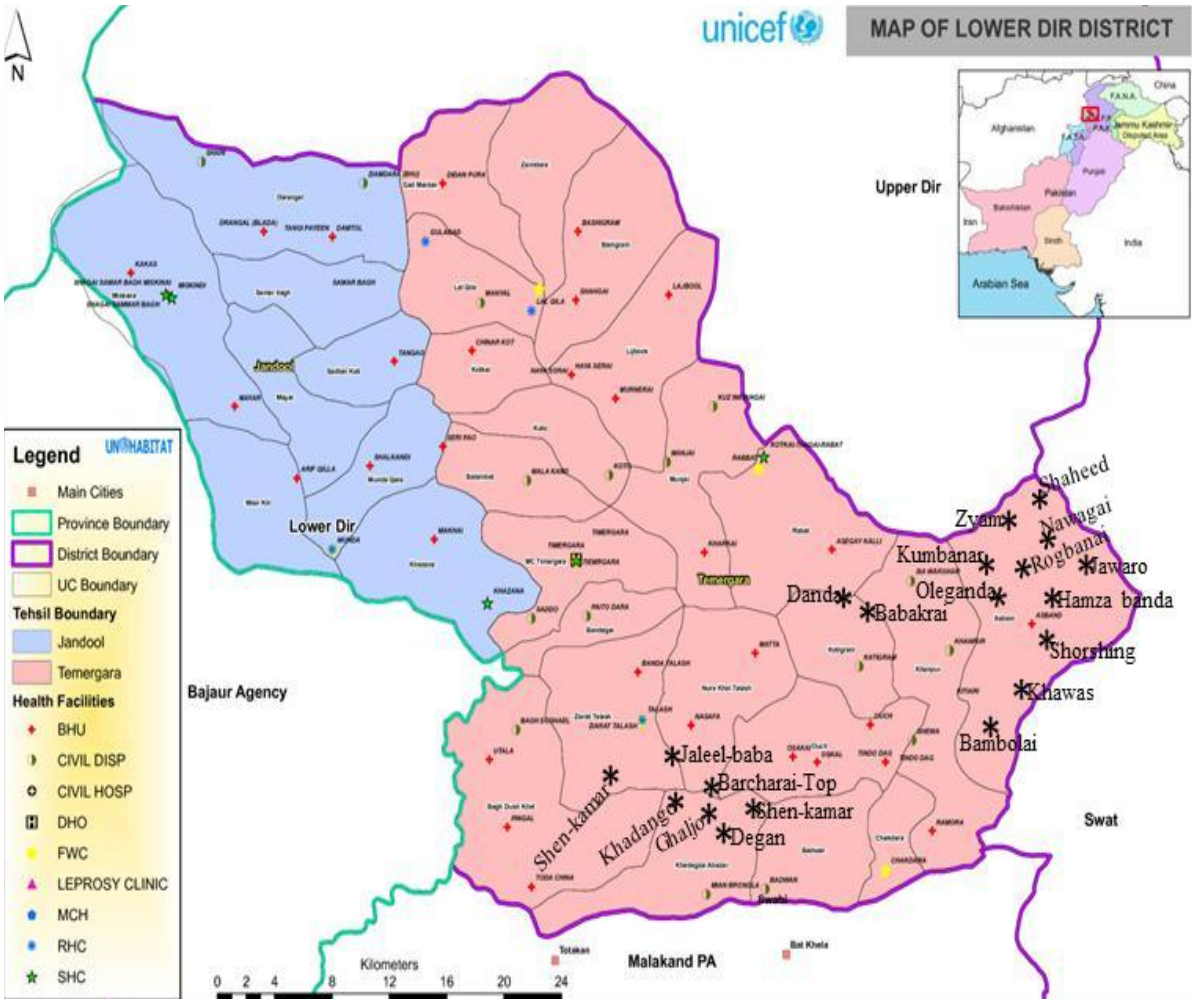

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Fig. 1. Map of research area. * representing loclities of Quercus species.

Morphological characterization: Morphological characterization was carried out for estimation of inter and intra species genetic diversity, a total of 14 morphological characters was scored. Out of 14 characters 5 were qualitative and 9 quantitative. Qualitative traits include leaf type (LTY), leaf margin (LM), leaf upper surface color (LUSC), leaf lower surface color (LLSC) and seed shape (SS). While, quantitative traits scored were petiole length (PL), leaf length (LL), leaf width (LW), leaf thickness (LT), seed length (SL), seed width (SW), cupule length (CL), cupule width (CW) and seed weight (SWt). The data of 14 morphological characters were recorded and cluster analysis was carried out using software PC-ORD.

SDS-PAGE characterization: For SDS-PAGE analysis single seed of each genotype was ground into a fine powder with the help of mortar and pestle for the extraction of proteins. About 400µl of protein extrication buffer (0.5 M Tris-HCL pH 8.0, 0.2%SDS, 5 M Urea, 1%B-mercaptoethanol) was added to 0.01g of seed flour taken in 1.5 ml Effendorf tube. The E-tube was vortexed thoroughly to homogenize the mixture. Bromo-Phenol Blue (BPB) was added to the protein extraction buffer as tracking dye to monitor the movement of protein in the gel. The homogenated samples were centrifuged at 13,000rpm for 10 minutes at 10^{o}C.The electrophoretic procedure was carried out using 15% polyacrylamide gel, separation gel (3.0M Tris-HCl pH9.0, 0.4% SDS) and 4.5% stacking gel (0.4M Tris-HCl pH 7.0, 0.4% SDS). Electrode buffer (0.025 M Tris, 129 M Glycine, 0.125% SDS) was poured into the top pool of the apparatus. A total volume of 20 µl of the protein extract solution was loaded in each well of the gel with the help of micropipette. The electrophoresis were run at 100V until the blue line passed through the bottom of gel plates. The gels were then stained in staining solution containing 0.2% BPB dissolved in 10% glacial acetic acid, 40% methanol and water in the ratio of 10:40:50. Gels were de-stained in a solution containing 5% acetic acid and 20% methanol for 15 minutes. The data were recorded from the destined gel on the basis of presence and absences of protein bands, i.e., '1' for the presence and '0' for the absence of bands and cluster analysis was carried out using software PC-ORD.

Results

Morphological trait analysis: Among 14 quantitative traits, petiole length (mm), leaf thickness and seed weight were significantly variable (Table 1). Using the Pearson correlation coefficient the results for association among various traits for two species (*Q. incana* and *Q. baloot*), whereas due to less number of genotypes for *Q. dilatata,* the correlations were not performed and the variance was measured statistically (Tables 2 and 3). Interestingly, in correlation study, petiole length (mm) was significantly ($p>0.01$) positively correlated with leaf length in *Q. incana*; while negatively with leaf length in *Q. baloot*. The leaf length in *Q. baloot* was significantly ($p>0.01$) positively correlated with leaf and seed width, whereas negative correlation in the same traits was observed in *Q. incana*. Leaf thickness was negatively correlated with seed weight in *Q. incana* but positive correlation was observed in *baloot*. Cupule length showed negative correlation with seeds length in the germplasm of *Q. incana* and *Q. baloot* (Tables 2 & 3). Genetic similarity indexes were calculated for all the genotypes of all the three genera. It was observed that *Q. baloot* and *Q. dilatata* were 57.15% similar morphologically while *Q. incana* and *baloot* exposed revealed 7.14% genetic similarity. Likewise *Q. incana* and *Q. dilatata* shared 21.43% homology based on present investigation (Table 4).

Phylogenetic relationships indicate the relatedness of various species or genera and all the three species of Oaks distributed in Khyber Pakhtunkhwa were investigated for the similarities and the dendrogram was constructed (Fig. 2). At half similarity the species were grouped into two major groups. The group-1 comprised all the genotypes of *Q. incana* species and both the other species (*Q. baloot* and *Q. dilatata*) were genetically dissimilar from the species *Q. incana*. Further at 70% genetic distance two major groups were separated *Q. baloot* and *Q. dilatata* forming two distinct clusters (Fig. 1). Similar same pattern of grouping was observed through a scattered plot that was constructed on the basis of principal components (Fig. 3), that indicated the coordination of both these numerical taxonomic techniques for investigating the phylogenetic relationship among three species of Oak.

Table 1. Coefficient of variation (%age) in three genera of *Quercus* sp. based on quantitative traits.

Traits	*Q. incana*	*Q. baloot*	*Q. dilatata*
Petiole length (mm)	**29.80**	**32.30**	**15.25**
Leaf length (mm)	13.49	17.06	0.58
Leaf width (mm)	8.38	14.96	12.33
Leaf thickness (mm)	**10.65**	**28.47**	**27.29**
Seed length (mm)	17.96	6.05	9.88
Seed width (mm)	11.79	5.09	1.64
Cupule length (mm)	9.61	7.85	7.03
Cupule width (mm)**	11.39	5.46	2.24
10 Seed weight (g)	**23.03**	**12.80**	**9.67**

Table 2. Correlation coefficient among nine quantitative traits of *Quercus incana*.

Quercus incana	PL	LL	LW	LT	SL	SW	CL	CW	10SWt
Petiole length (mm)	1.00								
Leaf length (mm)	**0.83**	1.00							
Leaf width (mm)	0.02	**-0.01**	1.00						
Leaf thickness (mm)	0.17	0.24	**0.45**	1.00					
Seed length (mm)	-0.22	-0.25	**0.44**	0.45	1.00				
Seed width (mm)	-0.32	**-0.55**	-0.28	-0.60	-0.07	1.00			
Cupule length (mm)	-0.10	0.24	-0.06	-0.18	-0.37	0.20	1.00		
Cupule width (mm)**	-0.13	-0.23	-0.35	-0.60	-0.37	**0.90**	**0.50**	1.00	
10 Seed weight (g)	-0.32	0.02	-0.57	**-0.23**	**-0.52**	-0.14	**0.48**	0.05	1.00

Table 3. Correlation coefficient among nine quantitative traits of *Quercus baloot*.

Quercus baloot	PL	LL	LW	LT	SL	SW	CL	CW	10SWt
Petiole length (mm)	1								
Leaf length (mm)	**-0.24**	1.00							
Leaf width (mm)	0.19	**0.76**	1.00						
Leaf thickness (mm)	0.06	-0.09	**0.40**	1.00					
Seed length (mm)	-0.06	-0.33	**-0.10**	0.36	1.00				
Seed width (mm)	-0.26	**0.43**	-0.02	-0.40	-0.75	1.00			
Cupule length (mm)	0.20	0.29	0.31	-0.19	-0.55	0.34	1.00		
Cupule width (mm)**	-0.29	0.28	-0.13	-0.62	-0.60	**0.83**	0.29	1.00	
10 Seed weight (g)	-0.60	0.12	0.07	**0.63**	**0.00**	0.06	**-0.29**	-0.24	1.00

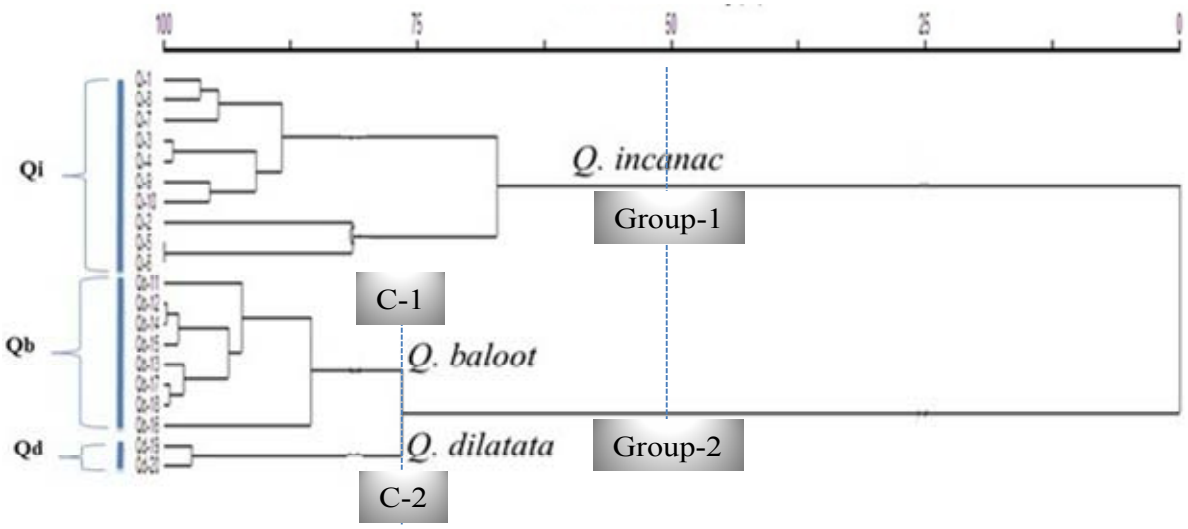

Fig. 2. Inter and intra-species phylogenetic relationship detected through morphological traits analysis in 20 different genotypes of *Quercus* species collected from Dir, Khyber Pakhtunkhwa, Pakistan. *Q. indicate* genotypes of *Quercus incana*, Qb indicates genotypes of *Quercus baloot* and Qd indicate genotypes of *Quercus dilatata*. Qi-1, Qi-2, Qi-3, Qi-4, Qi-5, Qi-6, Qi-7, Qi-8, Qi-9 and Qi-10 collected from zyam, barcharaitalash, rogbanai, barcharai top, Jaleel baba, shenkamar, degan, ghaljo, khadango and shaheed respectively. Qb-11, Qb-12, Qb-13, Qb-14, Qb-15, Qb-16, Qb-17 and Qb-18 collected from jawaro, nawagai, oleganda, khawas, shorshing, kumbanar, hamzabanda and bambolaibala respectively. Qd-19 and Qd-20 collected from danda and babakrai respectively.

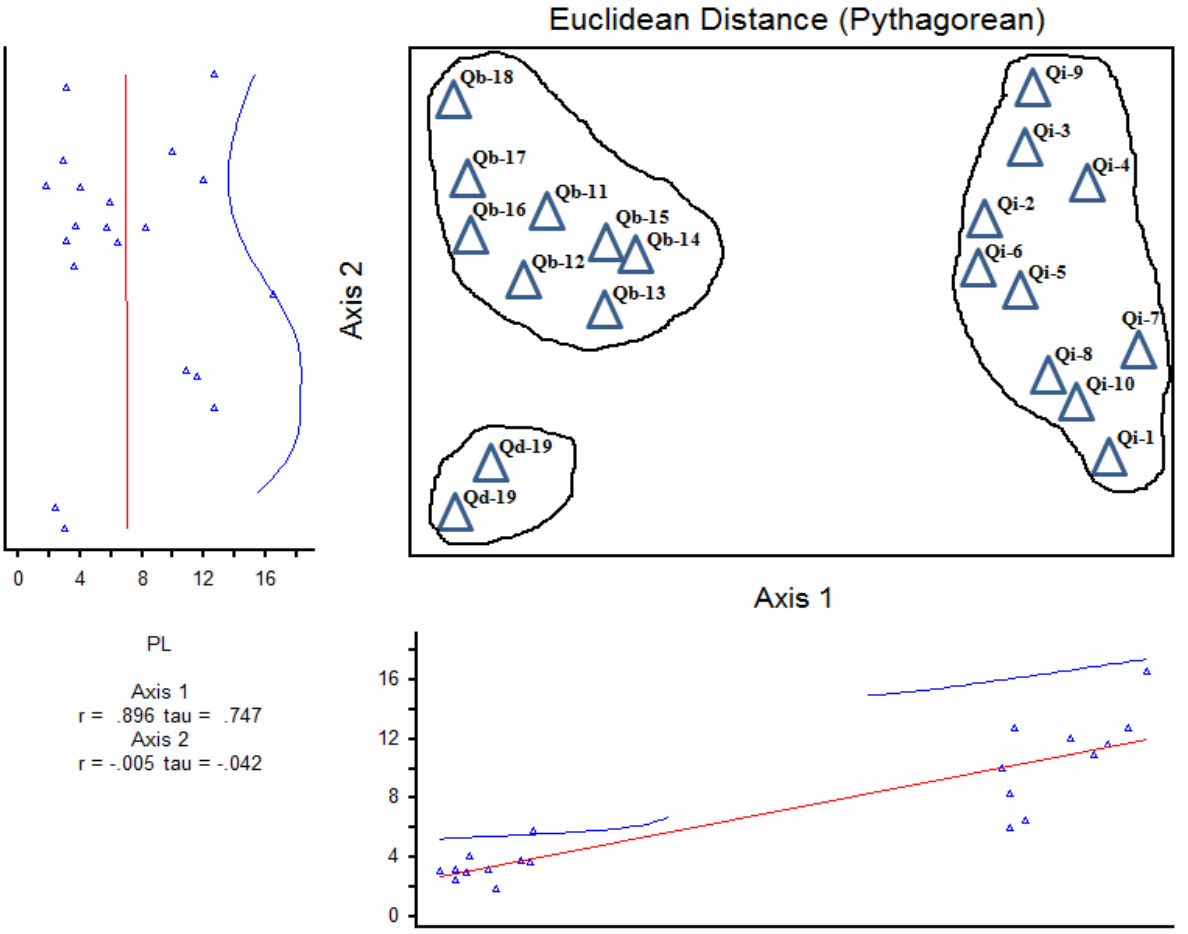

Fig. 3. Confirmation of phylogenetic relationship by scattered plot detected through cluster analysis in 20 different genotypes of Quercus species collected from Dir, Khyber Pakhtunkhwa, Pakistan.

Table 4. Intra and interspecific genetic diversity in 14 morphological characters studied in *Q. incana*, *Q. baloot* and *Q. dilatata*.

Morphological traits		*Q. incana*	*Q. dilatata*	*Q. baloot*	Traits similarity indexes		
					Qi &Qb	*Qi & Qd*	*Qb & Qd*
Nine quantitative traits	Petiole length (mm)	11.255	2.69	2.29	NA	NA	2.490*
	Leaf length (mm)	82.36	38.41	25.41	NA	NA	NA
	Leaf width (mm)	29.995	28.72	18.52	NA	29.358*	NA
	Leaf thickness (mm)	0.565	0.285	0.335	NA	NA	0.310*
	Seed length (mm)	13.515	24.8	30.525	NA	NA	NA
	Seed width (mm)	8.865	8.895	11.025	NA	8.880*	9.960*
	Cupule length (mm)	9.64	12.64	12.065	NA	NA	12.353*
	Cupule width (mm)**	**11.67****	**10.82****	**12.485****	**12.078****	**11.245****	**21.210****
	Seed weight (g)	6.45	20.47	21.95	NA	NA	21.210*
Five qualitative traits	Leaf type	Lanceolate	Elliptic ovate	Oblang ovate	NA	NA	NA
	Leaf margin	Serrate	Spiny	Entire	NA	NA	NA
	Leaf upper surface color	Dark green	Green	Green	NA	NA	Green*
	Leaf lower surface color	White tomentose	Green	Pale green	NA	NA	NA
	Seed shape	Oval	Elongate	Elongate	NA	NA	Elongate*
Total TSI = ((homologous trait/total traits)*100)					**7.14%**	**21.43%**	**57.14%**

*- Traits similarity within two species

**- Traits similarity within three species

Table 5. Inter specific locus variation among *Quercus incana*, *Quercus baloot* and *Quercus dilatata* reported from Dir (L), Pakistan.

Locus (L)	Present (%)	Absent (%)	Variation (%)	Status	Genetic disagreement (band present)
L-1 (Band-1)	12 (60%)	8 (40%)	40%	Poly	0.60
L-2 (Band-2)	18(90%)	2(10%)	10%	Poly	0.90
L-3 (Band-3)** (generic specific locus)	20(100%)	0.00	Nil	Mono	1.00
L-4 (Band-4)** (generic specific locus)	20 (100%)	0.00	Nil	Mono	1.00
L-5 (Band-5)	5 (25%)	15 (75%)	75%	Poly	0.25
L-6 (Band-6)	11 (55%)	9 (45%)	45%	Poly	0.55
L-7 (Band-7)	2(10%)	18(90%)	90%	Poly	0.10
Locus contribution toward genetic diversity GD = ((Poly loci/total loci)*100)			**71.43 %**		

SDS-PAGE analysis: Seven reproducible bands were observed for SDS-PAGE among all the three species that is presented on the Fig. 4. The phylogenetic relationship among all the three species through dendrogram has been presented in the Fig. 5. One forth linkage distance divided all the genotypes of three species into two lineages, whereas these were further divided into six clusters.

The cluster 1 is represented by three genotypes of *Quercus incana*, collected from Zya-Dir, Rogbanae-Dir and Shaheed-Dir. Importantly; the cluster 2 have two genotypes of *Quercus baloot* collected from Kumbanar-Dir and from Hamzabanda-Dir. The cluster-3 consists of four genotypes of *Quercus baloot* collected from Jawaro-Dir, from Nawagae-Dir, from Khawas-Dir and from Bambolae Bala-Dir. The cluster 4 consisted genotypes of *Quercus baloot* collected from Oleganda-Dir and from Shorshing-Dir. Cluster 5 comprised of two genotypes of *Quercus dilatata* collected from Danda-Dir and from Babakrai-Dir. Similarly, the cluster 6 contained seven genotypes of *Quercus incana* collected from Barcharai-Talash-Dir, Barcharai-Top-Dir, Jaleel-Baba-Dir, Shen-Kamar-Dir, Degan-Dir, Ghaljo-Dir and Khadango-Dir that that revealed low levels of genetic diversity for protein banding profile among various genotypes clustering together. Conclusively, the cluster 1 and cluster 6 represented ten genotypes of *Quercus incana,* cluster 2, cluster 3 and cluster 4 grouped eight genotypes of *Quercus baloot* and the cluster 5 represented two genotypes of *Quercus dilatata* that revealed a clear-cut evidence for species identification on the basis of protein profiling. The scattered conformed the phylogenetic tree according to the species orientation (Fig. 6). The cluster 1 and cluster 6 of dendogram encircled the species *Quercus incana,* the cluster 2, 3 and 4 separated the species *Quercus baloot* and the cluster 5 encircled *Quercus dilatata*.

Locus variation: Interestingly, table 5 represents inter-specific locus variation among 20 genotypes of *Quercus* species. Among all the genotypes, seven loci (L-1 to L-7) were detected; out of which L-3 and L-4 were monomorphic. L-3 and L-4 were tagged as generic specific locus/band which could be used to distinguish Pakistan Oak genera. Furthermore, the loci L-1; L-2; L-5; L-6 and L-7 spotted as polymorphic with 40, 10, 75, 45 and 90 percent genetic diversity, respectively.

The inter species comparative locus contribution toward genetic disagreement *(CLCTGD)* was 71.43% in the three species of 20 Oak genotypes (Table 6). Intra-specific locus variation among 10 genotypes of *Quercus incana* is represented in Table 3 and among seven loci, the loci L-5 and L-7 were missing throughout the *incana* species, hence this marker/locus can be used to identify this species. Importantly, the loci 1, 2, 3 and 4 were monomorphic in *incana*. Locus-6 explained 70% genetic diversity *incana* species which were collected from Zyam-Dir, Rogbanai-Dir and Shaheed-Dir. The locus contribution toward genetic disagreement *(LCTGD)* of *Quercus incana* was 14.2%.

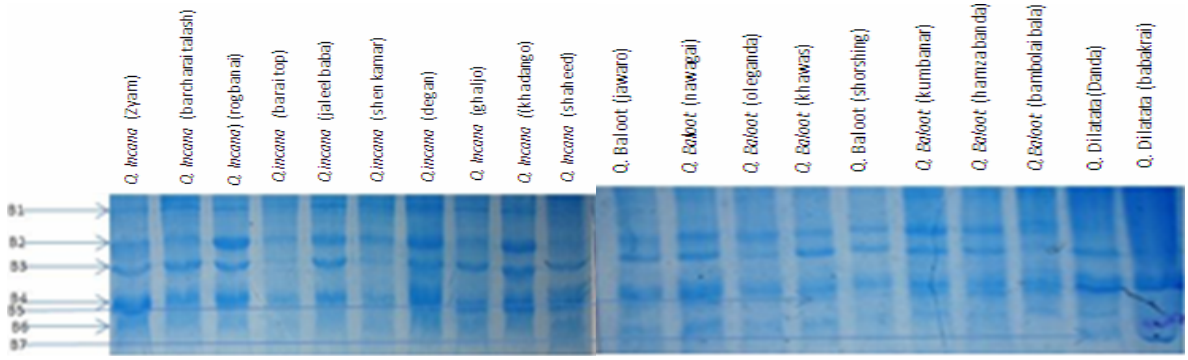

Fig. 4. Electrophoregram showing inter and intra-species variation in 20 different genotypes of Quercus species collected from Dir, Khyber Pakhtunkhwa, Pakistan.

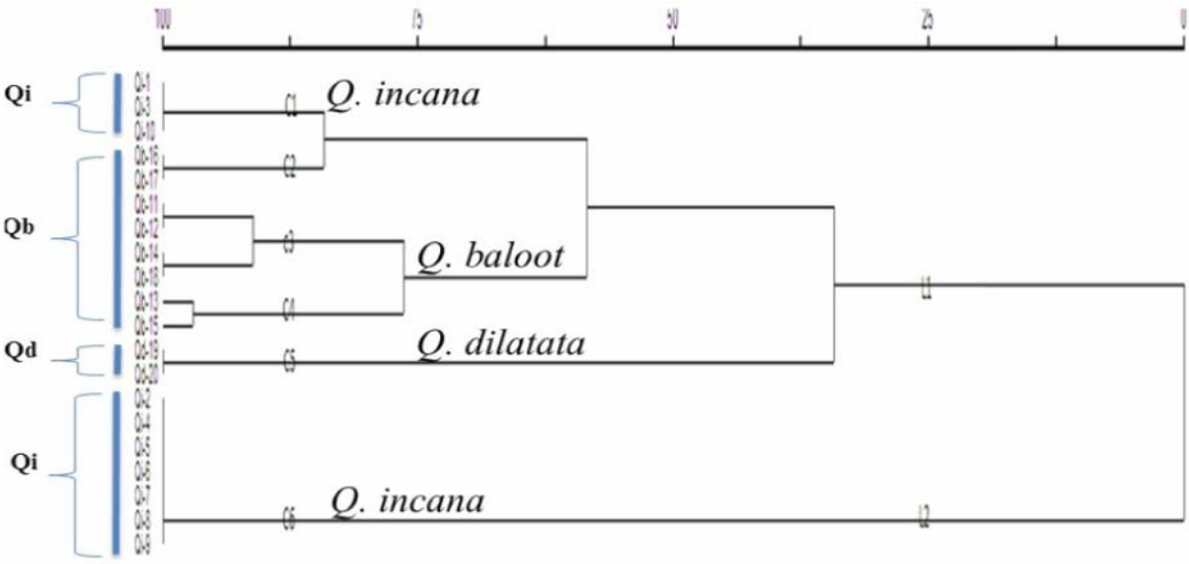

Fig. 5. Inter and intra-species phylogenetic relationship detected through SDS-PAGE in 20 different genotypes of *Quercus* species collected from Dir, Khyber Pakhtunkhwa, Pakistan. *Q. indicate* genotypes of *Quercus incana*, Qb indicates Genotypes of *Quercus baloot* and Qd indicate genotypes of *Quercus dilatata.* Qi-1, Qi-2, Qi-3, Qi-4, Qi-5, Qi-6, Qi-7, Qi-8, Qi-9 and Qi-10 collected from zyam, barcharaitalash, rogbanai, barcharai top, Jaleel baba, shenkamar, degan, ghaljo, khadango and shaheed respectively. Qb-11, Qb-12, Qb-13, Qb-14, Qb-15, Qb-16, Qb-17 and Qb-18 collected from jawaro, nawagai, oleganda, khawas, shorshing, kumbanar, hamzabanda and bambolaibala respectively. Qd-19 and Qd-20 collected from danda and babakrai respectively.

The Table 7 represents intra-specific locus variation among 8 genotypes of *Q. baloot*. As compared to *Q. incana*, the *Q. baloot* exhibited high intra-specific locus variation. Among seven loci, out of which L-2, L-3 and in L-4 were monomorphic, while L-1, L-5 and L-6 were polymorphic. The L-7 were missing in 8 *baloot* genotypes. In L-1 displayed 75% intra-locus variation and was present in the genotypes collected from Kumbana and Hamzabanda. L-5 were present in five genotypes (*Q. baloot* (khawas), *Q. baloot* (shorshing), *Q. baloot* (kumbanar), *Q. baloot* (hamzabanda) and *Q. Baloot* (bambolaibala) and showed 37.5% genetic diversity. Similarly L-6 were observed in the genotypes collected from Jawaro, Nawagai), Khawas), Kumbanar, Hamzabanda) and Bambolaibala. The remaining two accessions in this locus have no band and thus show 25% variation. The genetic disagreement of L-1, L-5 and L-6 was 0.25, 0.63 and 0.75%, respectively with LCTGD of 42.9%.

In two genotypes of *Quercus dilatata*, the L-1, L-2 and L-5 had no band while in the remaining L-3, L-4, L-6 and in L-7, 100% bands were present and have no intra-specific locus variation (Table 8).

Discussion

Twenty Pakistani *Quercus* accessions (ten accessions of *Quercus incana*, eight of *Quercus baloot* and two of *Quercus dilatata*r were analyzed for plant descriptons and proteomic profiling indicated varying degrees of diversity. Intra-specific variation was for nine quantitative traits while inter-specific variation was observed among three species for all the traits. We observed variation for leaf morphological traits that is a precise tool for taxonomic identification various species of *Quercus* and that has been suggested by Mehrnia *et al.* (2012). However, establishment of phylogenetic relationships by using a single parameter of morphological traits has been

challenged and is not supported by recent results, and more analyses for molecular markers are required (Neophytou *et al*., 2007). On the other hand protein profiling and DNA markers can provide a large number of neutral and independent markers that are extremely useful in genetic analysis and species relationships (Kumar & Kumar, 2014). Hence electrophoresis of protein have been published as a powerful tool, especially for identification of various species of the same genus or different genera (Ghafoor, 2013). As the SDS-PAGE has been used as a practical and reliable method for species identification, therefore, the present study was conducted that exhibited encouraging results with low inra-specific and high inter-specific divergence that could enable us to distinguish all the three species through SDS-PAGE. Low heterogeneity in protein bands was attributed to the conservative nature of seed proteins within one species that was ultimately DNA signaling made possible, but to identify specific primer encoding for the conserved gene require huge database and sophisticated lab facilities that could be accomplished through reverse genetics. Due to High inter-species locus contribution toward genetic disagreement (*ISLCTGD*) SDS-PAGE could be a reliable technique for identification of these three species, while intra-specie locus contribution toward genetic disagreement was high in genotypes of *Quercus baloot* (42.9%) as compare to *Quercus incana* (14.2%) and *Quercus dilatata* (0.00%), respectively.

Rogle *et al.* (1996) used to modify SDS-PAGE from the protocol proposed by Giulian and Graham, (1992 & 1993) for investigating protein profile of *Q. rouber*, *Q. rubra*, *Q. petraea* and *Q. pubescens*. They reported 7 bands in electrophoregram and differentiate species on the basis of specie specific protein bands. Similarly our present study indicated that variation was present in *Quercus* species at both morphological and molecular levels and the seed protein profile is species-specific and SDS-PAGE method presented here could be an important tool for differentiation of *Quercus* species that is robust and cheap as compared to DNA markers.

Table 6. Intra specific locus variation 10 genotypes of *Quercus incana* reported from Dir (L), Pakistan.

Locus (L)	Present (%)	Absent (%)	Variation (%)	Status	Genetic disagreement (band present)
L-1 (Band-1)	100	0.00	Nil	Mono	1.00
L-2 (Band-2)	100	0.00	Nil	Mono	1.00
L-3 (Band-3)** (generic specific locus)	100	0.00	Nil	Mono	1.00
L-4 (Band-4)	100	0.00	Nil	Mono	1.00
L-5 (Band-5)	0.00	100	Nil	Mono	0.00
L-6 (Band-6)	30.0	70.0	70*	Poly	0.30
L-7 (Band-7)	0.00	100	Nil	Mono	0.00
Locus contribution toward genetic diversity GD = ((Poly loci/total loci)*100)			**14.2%**		

Table 7. Intra specific locus variation 8 genotypes of *Quercus baloot* reported from Dir (L), Pakistan.

Locus (L)	Present (%)	Absent (%)	Variation (%)	Status	Genetic disagreement (band present)
L-1 (Band-1)	25.0	75.0	75*	Poly	0.25
L-2 (Band-2)	100	0.00	Nil	Mono	1.00
L-3 (Band-3)** (generic specific locus)	100	0.00	Nil	Mono	1.00
L-4 (Band-4)** (generic specific locus)	100	0.00	Nil	Mono	1.00
L-5 (Band-5)	*62.5*	*37.5*	37.5*	Poly	0.63
L-6 (Band-6)	*75.0*	*25.0*	25*	Poly	0.75
L-7 (Band-7)	0.00	100	Nil	Mono	0.00
Locus contribution toward genetic diversity GD = ((Poly loci/total loci)*100)			**42.9 %**		

Table 8. Intra specific locus variation 2 genotypes of *Quercus dilatata* reported from Dir (L), Pakistan.

Locus (L)	Present (%)	Absent (%)	Variation (%)	Status	Genetic disagreement (band present)
L-1 (Band-1)	0.0	100	Nil	Mono	0.00
L-2 (Band-2)	0.0	100	Nil	Mono	0.00
L-3 (Band-3)** (generic specific locus)	2(100%)	0.0	Nil	Mono	1.00
L-4 (Band-4)** (generic specific locus)	2(100%)	0.0	Nil	Mono	1.00
L-5 (Band-5)	0.0	100	Nil	Mono	0.00
L-6 (Band-6)	2(100%)	0.0	Nil	Mono	1.00
L-7 (Band-7)	2(100%)	0.0	Nil	Mono	1.00
Locus contribution toward genetic diversity GD = ((Poly loci/total loci)*100)			**0.00 %**		

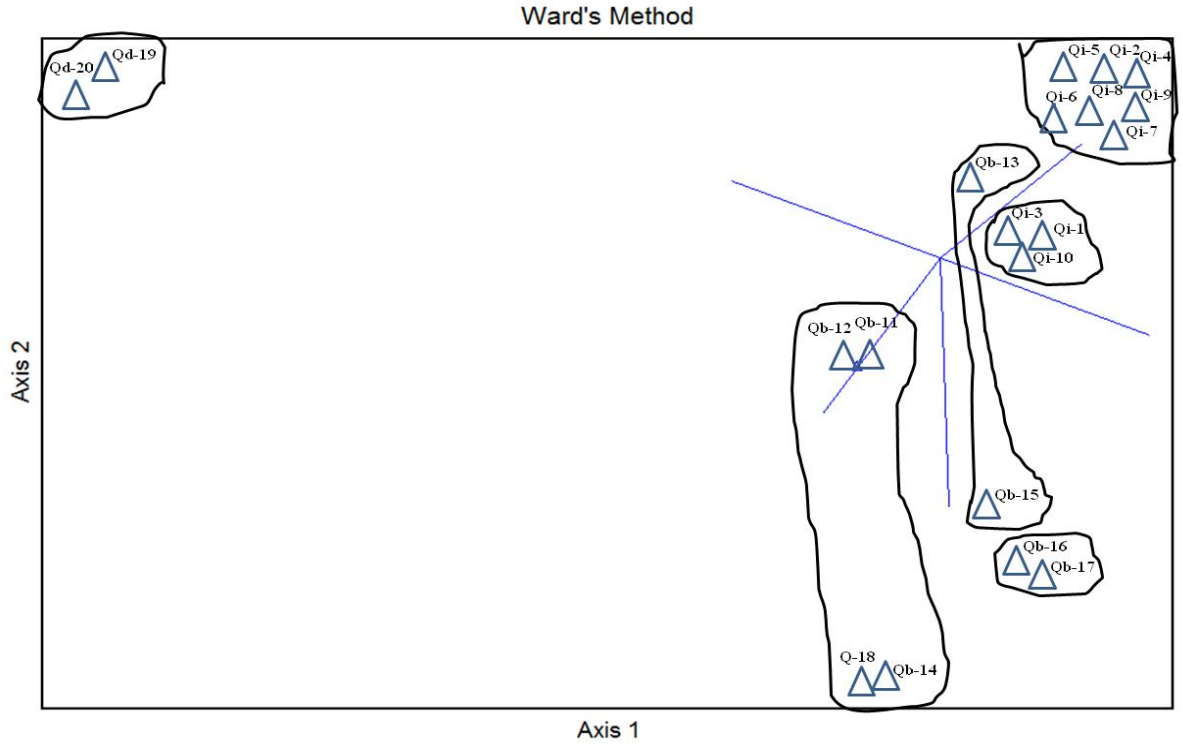

Fig. 6. Confirmation of phylogenetic relationship by scattered plot detected through SDS PAGE in 20 different genotypes of *Quercus* species through cluster analysis collected from Dir, Khyber Pakhtunkhwa, Pakistan.

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