

MORPHO-MOLECULAR GENETIC DISTANCES REVEALED TAXONOMIC ANOMALIES IN *BERBERIS* SPECIES FROM PAKISTAN

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ABSTRACT

Berberis is an important genus both medicinally and economically. In traditional medicine it is used to treat various health conditions including different types of cancer, cardiovascular anomalies, hepatic disorders, diabetes mellitus and bone healing. Due to the plasticity of its morphology (leaf size, shape, and flower stalk – sometimes even on the same plant - and often subject to rampant hybridization) species identification and delineation has been and remains a challenge. Moreover, there is no efficient universal DNA extraction protocol to impact on the specific studies. The current study investigates apparent taxonomic differences using DNA based markers. Furthermore, it presents an optimized version of genomic DNA extraction. Twelve samples collected from different populations were treated with thirty RAPD primer combinations. A total of 44 polymorphic loci and 294 DNA fragments were amplified revealing a genetic distance ranging between 20 - 74%. While nineteen comparisons exhibited relatively higher genetic distance (46+ %). Cluster analysis indicated that different *Berberis* species have unique geographical and altitudinal adaptations. Morphological features considered for taxonomy showed little importance for classification. Research findings will improve taxonomic understanding, help effective DNA extraction, facilitate conservation of critically endangered *Berberis* species and could be supportive in optimizing breeding programs.

Keywords: Markers, Genetic Distance, Medicinal Plants, Himalaya, Primers, Breeding, Genomics

INTRODUCTION

Berberis is the largest genus of family Burseraceae. The Eurasia (the Himalayas and China) and Latin America (Ahrendt, 1961, Landrum, 1999, Adhikari *et al.* 2012) are two prime diversity centres. Landrum (1999) and Adhikari *et al.* (2012) proposed 407-447 species compared to 467 by Ahrendt (1961).

Berberis species are extensively used in a number of traditional herbal medicine systems including Eastern, Ayurvedic, Unani, Homeopathic and Modern systems (Chopra *et al.*, 1981; Chandra and Purohit, 1980; Jain, 1994). A number of British, Chinese, Indian, and Iranian herbal pharmacopoeias and the Homeopathic Materia Medica include it (Minaiyan *et al.* 2011; Chopra and Chopra, 1933; Xaio, 1983). *Berberis* species contain the alkaloid Berberine, an active and effective therapeutic agent (Sheng-Ji, 2011; Joshi *et al.* 2011; Tang *et al.*, 2009; Kong *et al.*, 2004). It is used to treat cancer, diabetes mellitus, jaundice, enlargement of spleen, osteoporosis, cardiovascular ailments, digestive complaints, leprosy and bone fractures treatments (Khan *et al.*, 2013).

Morphologically there are two primary subspecies of *Berberis pseudumbellata* are found in the area i.e. *Berberis pseudumbellata* subsp. *pseudumbellata* and *Berberis pseudumbellata* subsp. *gilgitica*. In the field they basically different in the berry colour and shape besides their leaf size and margin morphology. Subspecies *pseudumbellata* have more oval and dark blue to blackish berries as compared to subspecies *gilgitica* which has red/reddish oval to relatively elongated and larger berries. Both exhibit clear elevation differences; for example, subspecies *pseudumbellata* will not grow over 2100 m asl, while subspecies *gilgitica* will only grow above 2500 m asl (Khan *et al.*, 2014).

Sufficient genetic distance is the basic requirement of any species. It enables to adapt to changing environment and provides an insurance against unknown future threats (Levins, 1968). Previously, morphological, cytological, and biochemical markers were commonly used for GD estimation (Jarvis and Hodgkin, 1999). They are either limited in number or not free from influences. Therefore, they are not considered very suitable for in-depth and large scale GD studies (Clausen and Hiesey, 1958). DNA based molecular markers have facilitated GD estimation in plant / animal species of commercial importance (Varshney *et al.*, 2005). They are better to study genome structure due to their unlimited scope and being free from physico-environmental factors (Tripathi and Sandhya, 2013; Stuber *et al.*, 1992).

Some commonly used molecular markers are Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat (SSR), Amplified Fragment Length Polymorphism (AFLP), Randomly Amplified Polymorphic DNA (RAPD) (Paterson *et al.*, 1991, Lateef, 2015). Genetic variation estimations are increasingly based on DNA studies using different markers (Lynch 1988). Several scientists prefer RAPD technique (Welsh and McClelland 1990; Williams *et al.* 1990; Caetano-AnollCs *et al.*, 1991; Deragon 1992; Huff *et al.*, 1993) for having several advantages over RFLP and DNA fingerprints (Lynch and Milligan, 1994). Genetic distance is a reflection of genetic diversity between or among species and populations (Nei, 1987).

RAPDs are inexpensive, easy to perform and do not require any prior sequence information (Grattapaglia and Sederoff, 1994). They can discriminate closely related varieties (Fang and Roose, 1997; Yang and Quiros, 1993). Rajalakshmi *et al.* (2014) used RAPD primers to study phylogenetic relations among *Solanum* red berries (*Solanum dulcamara* L.) and *Solanum* blue (*Solanum nigrum*) berries. They found them genetically closely related. Recently scientists around the world used morphological characterization and DNA based markers for estimation of GD and to reach on better taxonomic conclusion of genus *Berberis* (Bottini *et al.*, 2007, Somayeh *et al.*, 2008; Iqbal *et al.*, 2013; Tripathi and Sandhya, 2013). Such reports are limited in number and reflect its neglected status and the extent of gap in the knowledge of the genus. Mostly previous studies were based on morphological characterization.

Bottini *et al.* (2007) analyzed sequence of the internal transcribed spacer (ITS) of the 18S (ITS1)-5.8S-26S (ITS2) rDNA region in 13 species of the genus *Berberis*. Along with morphological, biochemical, AFLP, and cytological data, they discovered existence of diploid and polyploid hybrid species in the genus. Similarly, Heydari, *et al.* (2009) used AFLP to evaluate genetic variation and Phylogenetic relationship among 30 samples of wild and cultured barberry in Iran. They concluded that the two genera *Berberis* and *Mahonia* belong to 2 completely distinct groups with significant genetic distance. Iqbal *et al.* (2013) studied red and black fruited *Berberis* accessions from Kunhar Valley, KPK, Pakistan using morpho-molecular (RAPD) markers. They reported 6-86% GD. Tripathi and Sandhya (2013) used RAPD primers to estimate GD in 50 accessions of *B. lycium*, related varieties and reported high level of GD (upto 85%).

The present work is the first documented attempt to utilize DNA technology for estimation of genetic diversity in genus *Berberis* in the Himalaya, Hindukush and Karakoram Ranges, Pakistan. It was aimed at resolving ongoing confusion about taxonomic position of *Berberis* species found in the area. Moreover, current investigation has optimized previous inefficient DNA extraction protocols for *Berberis* species.

MATERIAL AND METHODS

Study Area

Study was carried out in Central Karakoram National Park (CKNP) during 2012-2015. Due to globally important unique natural biodiversity and its fragile ecosystems, Government of Pakistan officially designated it a 'National Park' of IUCN category II in December 1993 (Nawaz *et al.*, 2009). It stretches across 36.0000° N, 75.0000° E and covers an area of 10,000 sq. km and six out of the nine administrative districts of GB i.e., Hunza, Nagar, Gilgit, Skardu, Ghanche and Shigar. The geographical scope of this study extends over three administrative districts i.e., Gilgit, Nagar and Hunza along with five separate valleys of Bagrot, Rahimabad-Nomal, Juglot-Goro, Naltar and Nagar (Fig. 1).

Species selection

A total of 32 researchers have reported 14 *Berberis* species from Gilgit-Baltistan and Chitral. Different reports are inconsistent and mostly contradict each other. For the present study we have chosen the most prevalent and frequent reported subspecies *Berberis pseudumbellata* subsp. *pseudumbellata* and *Berberis pseudumbellata* subsp. *gilgitica*.

Sampling

Twelve (n=12) *Berberis* populations (*Berberis pseudumbellata* subsp. *pseudumbellata*, n=8; *Berberis pseudumbellata* subsp. *gilgitica*, n=4) were identified from valleys of Bagrot, Nomal, Rahimabad, Naltar, Goro, Juglot, Hupaye, Thol and Ghulmet. Specimen (bark, berries, and leaves) were collected and transported to 'Water Quality Laboratory', Department of Biological Sciences, Karakoram International University, Pakistan. Samples were dried in the drier (Oven) at 48°C. Details of accessions are given in the Table 1.

DNA extraction

For DNA based analysis, 12 *Berberis* accessions (listed in Table 1) were processed at the Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan during 2014-15. For

DNA extraction CTAB procedure (Hoisington *et al.*, 1994; Doyle and Doyle, 1990) was used with minor modifications mentioned below.

Table 1. Geographic, subspecies and accession information of the *Berberis pseudumbellata* subspecies population used for DNA analysis. Under prevalent identification column, *B.p.pseudumbellata* refers to subspecies *Berberis pseudumbellata* subsp. *pseudumbellata* and *B.p.gilgitica* refers to *Berberis pseudumbellata* subsp. *gilgitica*.

Accession	Abbreviation	Origin/ location	Prevalent identification (subspecies)	Berry color	GPS Coordinates	Elevation (m a.s.l)
Pop 01	JugDwn	Juglot Down	<i>B.p.pseudumbellata</i>	Black	36°10'39.56"N 74°17'38.57"E	1879.6
Pop 02	Gor	Goro	<i>B.p.pseudumbellata</i>	Black	36°10'51.25"N 74°17'24.96"E	1708.2
Pop 03	No.Ed	Nomal End	<i>B.p.pseudumbellata</i>	Black	36°05'28.37"N 74°16'55.78"E	1639.3
Pop 04	Hup	Hupaye	<i>B.p.pseudumbellata</i>	Black	36°14'10.06"N 74°26'32.29"E	2042.1
Pop 05	Bag.Up	Bagrot Up	<i>B.p.gilgitica</i>	Red	36°02'23.34"N 74°35'16.66"E	2624.1
Pop 06	RBD	Rahimabad	<i>B.p.pseudumbellata</i>	Black	36°06'25.29"N 74°18'13.26"E	1733.8
Pop 07	JugUp	Juglot Up	<i>B.p.pseudumbellata</i>	Black	36°10'44.77"N 74°18'40.81"E	2033.5
Pop 08	N.B.Ed	Naltar Bala End	<i>B.p.gilgitica</i>	Red	36°10'23.71"N 74°09'58.29"E	2941.8
Pop 09	Thol	Thol	<i>B.p.pseudumbellata</i>	Black	36°14'12.24"N 74°26'04.24"E	1924.4
Pop 10	N.Mid	Naltar Midway	<i>B.p.gilgitica</i>	Red	36°09'22.67"N 74°11'57.19"E	2723.8
Pop 11	Ghl	Ghulmet	<i>B.p.pseudumbellata</i>	Black	36°14'22.96"N 74°29'03.89"E	1989.0
Pop 12	BagDwn	Bagrot Down	<i>B.p.gilgitica</i>	Red	36°02'00.67"N 74°34'00.51"E	2572.9

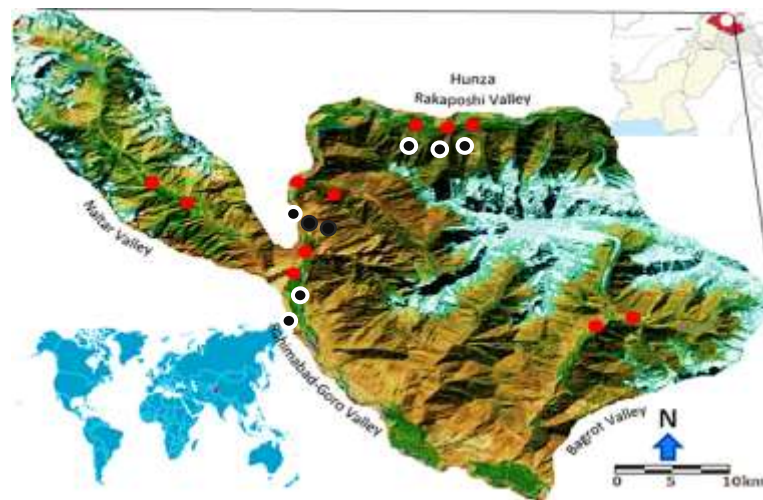


Fig. 1. Accessions sampling sites (marked as red and black dots). Dots and circles exhibit berry colour. Black dots in white circles represent *B. pseudumbellata* subsp. *pseudumbellata* and red spots represent *B. pseudumbellata* subsp. *gilgitica*. Inset: Gilgit-Baltistan location in Pakistan and the globe.

DNA quantification and quality check

The DNA concentration was quantified using a spectrophotometer (CECIL CE 2021 2000 Series). The samples from each parent were loaded on the spectrophotometer to measure their DNA concentration at 260 nm. The quality of DNA was checked by running 5 μ L DNA of each accession on 0.8 % agarose gel prepared in 0.5X TBE (Tris/Borate/EDTA) buffer. Bromophenol blue (3 μ L) was added in each sample of DNA.

DNA amplification

Working solution of DNA samples containing 15ng of DNA in 1.0 μ L of d_3H_2O was prepared. The concentration of genomic DNA, 10X PCR buffer with $(NH_4)_2SO_4$, $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), 10 mer random primer and Taq DNA polymerase were optimized for RAPD analysis (Williams *et al.*, 1990). Thirty (30), 10 base oligonucleotide RAPD primers were used during present study. These primers (listed in Tables 2) were obtained from Gene link company (USA). Taq polymerase together with buffer, $MgCl_2$, dNTPs and gelatin were purchased from Fermentas (Gene Link, Inc NY, USA).

Table 2. Sequence information of RAPD primer used for amplification of genomic DNA from *Berberis* accessions.

Sr. No	Primer (Oligo) Name	Sequence	% GC
1.	GL DecamerB-07	GGTGACGCAG	70
2.	GL DecamerB-10	CTGCTGGGAC	70
3.	GL DecamerB-11	GTAGACCCGT	60
4.	GL DecamerB-13	TTCCCCCGCT	70
5.	GL DecamerB-17	AGGGAACGAG	60
6.	GL DecamerD-06	ACCTGAACGG	60
7.	GL DecamerD-12	CACCGTATCC	60
8.	GL DecamerD-13	GGGGTGACGA	70
9.	GL DecamerD-19	CTGGGGACTT	60
10.	GL DecamerD-20	ACCCGGTCAC	70
11.	GL Decamer I-02	GGAGGAGAGG	70
12.	GL Decamer I-05	TGTTCCACGG	60
13.	GL Decamer I-06	AAGGCGGCAG	70
14.	GL Decamer I-07	CAGCGACAAG	60
15.	GL Decamer I-09	TGGAGAGCAG	60
16.	GL Decamer I-10	ACAACGCGAG	60
17.	GL Decamer I-11	ACATGCCGTG	60
18.	GL Decamer I-15	TCATCCGAGG	60
19.	GL Decamer I-17	GGTGGTGATG	60
20.	GL Decamer I-20	AAAGTGCGGG	60
21.	GL Decamer E-01	CCCAAGGTCC	70
22.	GL Decamer E-02	GGTGCGGGAA	70
23.	GL Decamer E-05	TCAGGGAGGT	60
24.	GL Decamer E-07	AGATGCAGCC	60
25.	GL Decamer E-16	GGTGACTGTG	60
26.	GL Decamer E-17	CTACTGCCGT	60
27.	GL Decamer E-20	AACGGTGACC	60
28.	GL Decamer F-01	ACGGATCCTG	60
29.	GL Decamer F-03	CCTGATCACC	60
30.	GL Decamer F-05	CCGAATTCCC	60

The master mix for the reactions against one primer was prepared through mixing of 6.5 μL of $\text{d}_3\text{H}_2\text{O}$, 2.5 μL of 10X PCR buffer, 4.0 μL dNTPs, 3.8 μL MgCl_2 , 3.5 μL of primer, 2.5 μL of gelatine and 0.2 μL of Taq polymerase. For each reaction, 2.5 μL of sample DNA was mixed with 22.5 μL of master mixture in one PCR tube. DNA amplification reactions were performed in a thermal cycler (Eppendorf AG No. 5333 00839). For PCR, Initial denaturation was carried out at 94°C for 5 minutes followed by 40 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 34°C and 2 minutes extension at 72°C.

Agarose gel electrophoresis

The 1.2% agarose gels were prepared dissolving 1.2 g agarose in 100 mL of TBE buffer and cooked in microwave oven for three minutes. After cooking, the liquid gels were cooled to 60 °C and add 3.0 μL of ethidium bromide (EtBr) solution (10mg of EtBr/100mL of TBE). The gel was run supplying 80 volts of current for 50 minutes. UV Transilluminator (Model - M-20E Upland-CA 91786 USA) was used for documentation.

Generation and analysis of marker data

PCR reactions were carried out in duplicate to reduce unreliability of the results if existed and only reliably score able bands were included in the analysis. Faint bands or bands not appearing in repeat gels were excluded from scoring. Data was scored from good quality photographs of each amplification reaction. Amplification profiles of all the genotypes were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). To rule out false negative and positives, replicates were made to ensure reproducible bandings. Dissimilarity matrix was generated using Nei's Measures of Genetic Identity and Genetic Distance (Nei, 1978). In addition population relationships were inferred using the Unweighted Pair Group of Arithmetic Means (UPGMA) clustering method using "Popgen" software (version 3.5).

RESULTS

DNA Analysis

The total genomic DNA was successfully extracted following the modified CTAB method (Hoisington *et al.*, 1994; Doyle and Doyle, 1990). The DNA quality was excellent as observed by running and visualizing the 0.8% agarose gel. A representative of the gels of *Berberis* DNA amplification using RAPD primers GLB B-13 is presented in figure 2. Forty-four polymorphic loci were recorded. A total of 294 DNA fragments were amplified using 30 RAPD primers. Which have mean value for each primer was 9.8. The genetic distances for RAPD data for *Berberis* accessions was constructed using procedure outlined by Nei and Li (1979) and the relationships between accessions were presented graphically in the form of a dendrogram in (Table 3 Figure 3). The values of genetic distances ranged from 0.2007 – 0.7397

Table 3. Average Genetic distance estimates among 12 *Berberis* accessions based on data obtained using 30 RAPD primers.

Pop ID	1	2	3	4	5	6	7	8	9	10	11
2	0.5261										
3	0.3502	0.5653									
4	0.3502	0.4884	0.3185								
5	0.2877	0.3502	0.2578	0.2007							
6	0.6487	0.5653	0.4520	0.3185	0.5261						
7	0.7397	0.5653	0.6931	0.5261	0.6061	0.3830					
8	0.4520	0.5261	0.4884	0.2877	0.3502	0.2288	0.3502				
9	0.5653	0.4884	0.4520	0.2578	0.4520	0.2578	0.3830	0.2288			
10	0.3830	0.3830	0.4884	0.2877	0.2877	0.4169	0.4884	0.3830	0.2877		
11	0.4520	0.5261	0.3502	0.3502	0.2877	0.4884	0.4169	0.2578	0.2288	0.3185	
12	0.4520	0.3185	0.5653	0.4169	0.3502	0.4169	0.4169	0.3830	0.4169	0.2578	0.4520

The lowest genetic distance of 20% was seen among Hupaye and Bagrot Up. While maximum genetic distance of 74% was observed among accessions Juglot Down and Juglot Up. Relatively higher genetic distances (46% or above) was observed among 19 comparisons.

The cluster analysis based on genetic distance values has classified all the accession in 3 major groups (A, B, and C) comprised of 4, 3 and 5 accessions respectively (Fig. 3). It is important to note that accessions collected from Naltar Midway, Bagrot Down, Naltar Bala End and Bagrot Up (all occupying central position of dendrogram)

all had red berries while rest of the accessions had black / dark bluish black berries.

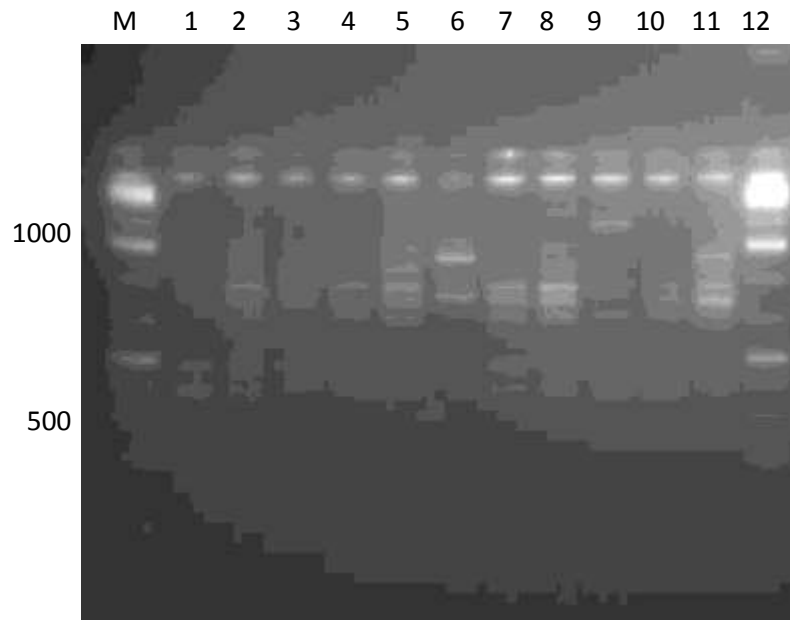


Fig. 2. PCR amplification of 12 accessions of *Berberis* using RAPD primer GL B-13. M= 100 bp Mol wt marker, 1= Juglot Down, 2= Goro, 3= Nomal End, 4= Hupaye, 5= Bagrot Up, 6= Rahimabad, 7= Juglot Up, 8= Naltar Bala End, 9= Thol, 10= Naltar Midway, 11= Ghulmet and 12= Bagrot Down.

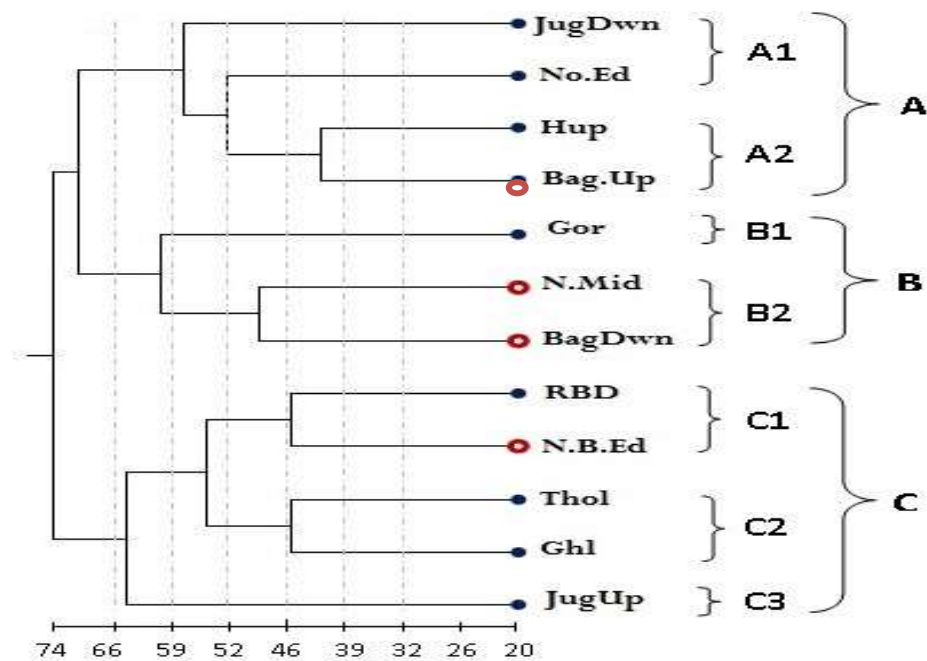


Fig. 3. Cluster analysis of 12 *Berberis* accessions based on data generated using 30 RAPD primers. Dots and circles exhibit berry colour. Black dots represent *B. pseudumbellata* subsp. *pseudumbellata* and red circles represent *B. pseudumbellata* subsp. *gilgitica*.

DISCUSSION

The findings of the current study establish the efficient use of RAPD markers in estimating genetic distance within and among medicinal plant species, supporting evidence from earlier research on *Berberis* and other genera (Tripathi and Sandhya, 2013; Iqbal *et al.*, 2013). Genetic analysis via RAPDs presents a very appealing method for non-model species such as *Berberis* since it is not based on any advanced knowledge of the DNA sequence and hence makes it possible to assess genetic diversity rapidly (Wolfe and Liston, 1998). The method allows simultaneous analysis of many loci throughout the genome within one PCR reaction, taking the form of a genomic snapshot (Michelmore *et al.*, 1991). While some researchers have criticized RAPD markers for possible reproducibility problems (Atienzar and Jha, 2006), the rigorous protocols used in our study - duplicate PCR reactions and the rejection of weak or non-reproducible bands - were intended specifically to avoid such problems and guarantee the integrity of our data.

In this study, all 30 primers were able to amplify a high percentage of the fragments from the 12 *Berberis* accessions, most of which were polymorphic. The high level of polymorphism points to the high genetic diversity that exists in these populations. Multivariate analysis yielded a wide range of genetic distances (20% to 74%), with the greatest divergence between the morphologically identical accessions Juglot Up and Juglot Down (both originally classified as *B. pseudumbellata* subsp. *pseudumbellata*). This observation is significant, as it indicates that classical morphological classification is not an accurate indicator of the underlying genetics. Such a divergence raises the possibility of a taxonomic anomaly and suggests that these two populations may have experienced remarkable genetic differentiation, due to local adaptation, genetic drift, or other evolutionary pressures in spite of their phenotypic convergence.

Cluster analysis also contradicts the use of classical phenotypic markers to classify. The dendrogram divided the 12 accessions into three large clusters (A, B, and C), but these did not directly align with the prevailing subspecies delimitation based on berry colour and elevation. Interestingly, Cluster B held all four accessions of *B. p.* subsp. *gilgitica* (red berries and higher elevations), but Cluster B also embraced one accession of *B. p.* subsp. *pseudumbellata* (Pop 06: Rahimabad). On the other hand, Clusters A and C were almost entirely made up of the black-berried *B. p.* subsp. *pseudumbellata*. Rahimabad (black berry) falling under the mostly red-berries group (B) and the high degree of genetic similarity between subgroups A2 and C1—which have various morphological characteristics—strongly suggest that traits like berry colour, number of thorns, and structure of leaves are not good indicators of genetic closeness. Excess dependency on such plastic morphological features can thus be deceptive for taxonomic identification and demarcation, a finding agreed with by parallel results for other plant lineages (Bottini *et al.*, 2007).

These findings require a plea for further refined and thorough taxonomic studies, possibly combining stronger molecular methods (e.g., ISSR, AFLP, or ITS sequencing) with precise morphological and ecological information to establish a stable and phylogenetically stable classification of the *Berberis* species of this area (Kim *et al.*, 2004; Heydari *et al.*, 2009).

Apart from taxonomy, the genetic divergence patterns revealed here have immediate conservation and breeding implications. The high genetic diversity found, even within subspecies, is a precious resource. Conservation efforts for germplasm can be maximized through prioritizing accessions with unique genetic lines, like the highly divergent Juglot Up population, to best preserve genetic variation. This diversity gives a reservoir of alleles that can facilitate adaptation to new environmental conditions and future danger (Levins, 1968). Furthermore, this genetic information is invaluable for devising informed breeding programs. Selecting genetically distinct and advantageous accessions can contribute significantly to meeting the rising demand from the pharmaceutical industry for uniform and high-quality raw material, both in Pakistan and internationally.

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Conflict of interest

We, the authors unanimously declare that there is no conflict of interest.

Researchers' individual contribution

Mr. Tika Khan and Dr. Abdul Rehman have collected data and samples from field and its initial processing at Water Quality Lab, Karakoram International University. Moreover, first author has executed DNA work at Centre for Biochemistry and Biotechnology, Agriculture University Faisalabad under supervision and facilitation of Dr. Nisar Ahmed. Prof. Dr. Imtiaz Ahmed Khan has helped with overall research planning, data interpretation and data analysis along with Dr. Nisar Ahmed. Ms. Nasreen has helped in data processing and compilation.

REFERENCES

- Adhikari, R., H. N. Kumar and S. D. Shruthi (2012). A review on medicinal importance of *Basella alba* L. *International Journal of Pharmaceutical Sciences and Drug Research*, 4(2): 110-114.
- Ahrendt, L. W. A. (1961). *Berberis* and *Mahonia*: A taxonomic revision. *Journal of the Linnean Society of London, Botany*, 57(369): 1-410. <https://doi.org/10.1111/j.1095-8339.1961.tb00889.x>
- Atienzar, F. A. and A. N. Jha (2006). The random amplified polymorphic DNA (RAPD) assay and related techniques applied to genotoxicity and carcinogenesis studies: a critical review. *Mutation Research*, 613(2-3): 76-102.
- Bottini, M.C.J., A. De Bustos, A. M. Sanso, N. Jouve and L. Poggio (2007). Relationships in Patagonian species of *Berberis* (Berberidaceae) based on the characterization of rDNA internal transcribed spacer sequences. *Botanical Journal of the Linnean Society*, 153(3): 321-328. DOI: 10.1111/j.1095-8339.2007.00586.x
- Caetano-Anollés, G., B. J. Bassam and P. M. Gresshoff (1991). DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Bio/Technology*, 9(6), 553-557. <https://doi.org/10.1038/nbt0691-553>
- Chandra, P. and A. N. Purohit (1980). Berberine contents and alkaloid profile of *Berberis* species from different altitudes. *Biochemical Systematics and Ecology*, 8(4): 379-380.
- Chopra, M., A. Chatterji and S. C. Pakrashi (1981). *The treatise on Indian medicinal plants*. CSIR, New Delhi, pp. 33-35.
- Chopra, R.N. and I. C. Chopra (1933). *Indigenous drugs of India*. Academic publishers.
- Clausen, J. and W. M. Hiesey (1958). *Experimental studies on the nature of species. IV. Genetic structure of ecological races*. Carnegie Institution of Washington Pub. 615. 312 pp.
- Deragon, J. M. and B. S. Landry (1992). RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. *PCR Methods and Applications*, 1(3): 175-180. <https://doi.org/10.1101/gr.1.3.175>
- Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker and N. Vidal (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97(4): 654-660.
- Doyle, J. J. and J. L. Doyle (1990). Isolation of plant DNA from fresh tissue. *Focus*, 12(1): 13-15.
- Duke, J. A., M. B. Godwin, J. Decellier and P. A. Duke (2002). *CRC- Hand Book on Medicinal Plants*. Taylor and Francis. Inc pub.SBN-13, Edition 2, pp 821
- Fang, D.Q., and M. L. Roose (1997). Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theoretical and Applied Genetics*, 95(3): 408-417.
- Grattapaglia, D. and R. Sederoff (1994). Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. *Genetics*, 137(4): 1121-1137.
- Heydari, S., H. Marashi, M. Farsi and K. A. Mirshamsi (2009). Assessment of genetic structure and variation of native *Berberis* populations of Khorasan provinces (Iran) using AFLP markers versus morphological markers. *Iranian Journal of Biotechnology*, 7(2): 101-107.
- Hoisington, D., M. Khairallah and D. González-de-León (1994). *Laboratory protocols: CIMMYT Applied Molecular Genetics Laboratory*. CIMMYT.
- Huff, D. R., T. Peakall and P. E. Smouse (1993). RAPD variation within and among natural populations of outcrossing buffalograss [*Buchloë dactyloides* (Nutt.) Engelm.]. *Theoretical and Applied Genetics*, 86(8): 927-934. <https://doi.org/10.1007/BF00211043>
- Iqbal, A., I. Ahmad, H. Ahmad, S. Ghafoor, A. Razzaq and I. Ahmed (2013). Genetic characterization of *Berberis* species collected from Kunhar River catchment using morphological and molecular markers. *International Journal of Biosciences*, 3(5): 35-42.
- Jain, S. K. (1994). Ethnobotany and research in medicinal plants in India. *Ethnobot. Search New Drugs*, 185: 153-168.
- Jarvis, D. I. and T. Hodgkin (1999). Wild relatives and crop cultivars: detecting natural introgression and farmer selection of new genetic combinations in agroecosystems. *Molecular ecology*, 8(s1): S159-S173.

- Joshi, P. V., A. A. Shirkhedkar, K. Prakash and V. L. Maheshwari (2011). Antidiarrheal activity, chemical and toxicity profile of *Berberis aristata*. *Pharm Biol.*, 49(1): 94–100.
- Khan, T., I. A. Khan, A. Rehman, J. Alam and S. Ali (2013). Exploration of near-extinct folk wisdom on medicinally important plants from Shinaki Valley Hunza, Pakistan. *International Journal of Biosciences* 3(10): 180-186.
- Khan, T., I. A. Khan, K. Ahmed and A. Rehman (2014). Differential levels of susceptibility of *Berberis* species to insect attack at various altitudes in Karakoram Ranges. *International Journal of Biosciences* 4(5): 92-101. DOI: <http://dx.doi.org/10.12692/ijb/4.5.92-101>
- Kim, Y. D., S. H. Kim and L. R. Landrum (2004). Taxonomic and phylogeographic implications from ITS phylogeny in *Berberis* (Berberidaceae). *Journal of plant research*, 117(3): 175-182.
- Kong, W., J. Wei, P. Abidi, M. Lin, S. Inaba, C. Li, and S. Wang (2004). Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins. *Nature medicine*, 10(12): 1344-1351.
- Kunwar, R.M., B. K. Nepal, H. B. Kshhetri, S. K. Rai and R. W. Bussmann (2006). Ethnomedicine in Himalaya: a case study from Dolpa, Humla, Jumla and Mustang districts of Nepal. *Journal of Ethnobiology and Ethnomedicine*, 2(1): 1.
- Landrum, L. R. (1999). Revision of *Berberis* (Berberidaceae) in Chile and adjacent southern Argentina. *Annals of the Missouri Botanical Garden*, 86(4): 793–834. <https://doi.org/10.2307/2666172>
- Lateef, D.D. (2015). DNA Marker Technologies in Plants and Applications for Crop Improvements. *Journal of Biosciences and Medicines*, 3(05): 7.
- Levins, R. (1968). *Evolution in changing environments: some theoretical explorations* (No. 2). Princeton University Press.
- Loconte, H. (1993). Berberidaceae. The Families and Genera of Vascular Plants. II. In: *Flowering Plants-Dicotyledons* (K. Kubitzki, J.G. Rohwer and V. Bittrich, Eds.). Springer-Verlag: Berlín.
- Lynch, M. and B. G. Milligan (1994). Analysis of population genetic structure with RAPD markers. *Molecular ecology*, 3(2): 91-99.
- Michelmore, R. W., I. Paran and R. V. Kesseli (1991). Identification of markers linked to disease resistance gene by bulk segregation analysis: A rapid method to detect markers in specific genome region using segregation population. *Proc. Natl. Acad. Sci.*, 88: 9828-9832.
- Minaiyan, M., A. Ghannadi, P. Mahzouni and E. Jaffari-Shirazi (2011). Comparative study of *Berberis vulgaris* fruit extract and berberine chloride effects on acetic acid-induced colitis in rats. *Iranian journal of pharmaceutical research*, 10(1): 97.
- Nawaz, M. A., P. Shadie and V. Zakaria (2009). Central Karakoram Conservation Complex: Draft Management Plan. The International Union for Conservation of Nature. Retrieved from:
- Nei, M. (1987). *Molecular Evolutionary Genetics*. (Chapter 9). New York: Columbia University Press.
- Paterson, A. H., S. D. Tanksley and M. E. Sorrells (1991). DNA markers in plant improvement. *Adv. Agron.*, 44: 39–90. [10.1016/S0065-2113\(08\)60578-7](https://doi.org/10.1016/S0065-2113(08)60578-7)
- Perveen, A. and M. Qaiser (2010). Pollen flora of Pakistan—LXV. Berberidaceae. *Pakistan Journal of Botany*, 42(1): 1-6.
- Rajalakshmi, A., N. Krithiga and A. Jayachitra (2014). Molecular Marker Studies of Selected Medicinal Plants for Assessment of Genetic Diversity. *Mintage Journal of Pharmaceutical and Medical Sciences*, 3(3): 1-3.
- Sheng-Ji, P. (2011). Ethnobotanical approaches of traditional medicine studies: some experiences from Asia. *Pharmaceutical biology*, 39(s1): 74-79.
- Singh, R., S. S. Tiwari, S. Shrivastava and A. K. S. Rawat (2012). Botanical and phytochemical studies on roots of *Berberis umbellata* Wall. ex G. Don. *Indian Journal of Natural Products and Resources*, 3(1): 55-60.
- Stuber, C. W., S. E. Lincoln, D. W. Wolff, T. Helentjaris and E. S. Lander (1992). Identification of genetic factors contributing to heterosis in a hybrid from two elite maize inbred lines using molecular markers. *Genetics*, 132(3): 823-839.
- Tang, J., Y. Feng, S. Tsao, N. Wang, R. Curtain and Y. Wang (2009). Berberine and Coptidis rhizoma as novel antineoplastic agents: a review of traditional use and biomedical investigations. *Journal of ethnopharmacology*, 126(1): 5-17.
- Tripathi, V. and G. Sandhya (2013). Assessment of genetic diversity in *Berberis lycium* Royle complex using RAPD markers. *J. Cell Biol.*, 3(1): 1-13.
- Varshney, R. K., A. Graner and M. E. Sorrells (2005). Genic microsatellite markers in plants: features and applications. *TRENDS in Biotechnology*, 23(1): 48-55.
- Welsh, J. and M. McClelland (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*, 18(24): 7213–7218. <https://doi.org/10.1093/nar/18.24.7213>

- Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski and S. V. Tingey (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, 18(22): 6531–6535. <https://doi.org/10.1093/nar/18.22.6531>
- Wolfe, A. D. and A. Liston (1998). Contribution of PCR-based methods to plant systematics and evolutionary biology. In: *Molecular systematics of plants II: DNA sequencing*. pp. 43-86.
- Xaio, P. (1983). Recent developments on medicinal plants in China. *Journal of ethnopharmacology*, 7(1): 95-109.
- Yang, X. and C. Quiros (1993). Identification and classification of celery cultivars with RAPD markers. *Theoretical and Applied Genetics*, 86(2-3): 205-212.
- Yeh, F.C., R. Yang, T. J. Boyle, Z. Ye and J. M. Xiyang (2000). *Pop Gene 32, Microsoft Windows Based Freeware for Population Genetic Analysis*. Molecular Biology and Biotechnology Centre, University of Alberta, Edmonton, Canada.