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Full Length Research paper

Evaluation of hereditary variety in *Berberis lycium* Royle complex using RAPD markers

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Genomic diversity among fifty accessions of *Berberis lycium* Royle complex was studied, using random amplified polymorphic DNA (RAPD) markers. Out of 80 RAPD primers, 28 were polymorphic and showed reproducible results. Total of 11,683 amplicon generated 50 accessions of *B. lycium* complex with 28 primers. 332 amplification products scored, 284 (85%) were polymorphic and 48 monomorphic. Maximum numbers of 21 amplification products were obtained with primers OPAP-3 and 20 products with OPB-4. Average number of 11.5 bands obtained per primer and amplificon size ranged from 100 to 4, 500 bp and after study, no primer gave single band among all accessions. Polymorphic Information Contents (PIC) ranged from 0.013 to 0.52 with an average of 0.12. Dendrogram grouped all the accessions into five major groups. Principle Component Analysis (PCA) was also supporting result obtained by dendogram. Present study is not supporting previous taxonomic classification of *B.lycium* Royle complex (based on morphological characters) but showed large diversity among them.

Key words: *Berberis lycium* Royle complex, inter-varietal-relationship, RAPD (random amplified polymorphic DNA), principle component analysis.

INTRODUCTION

Berberis lycium, economically and medicinally important genera (Bhakuni et al., 1968), belongs to the family Berberidaceae. *B. lycium* (2n = 28) Royle complex is one of the important species of this genera and interesting for molecular study due to its misclassification (Rao et al., 1998). *B. lycium* is being abundantly distributed in Western Ghat Himalaya, West Pakistan and Nepal. In India, it is frequently distributed in Himachal Pradesh and Uttrakhand. It is extensively used for the treatment of several human diseases (Watt, 1893; Kirtikar et al., 1933; Anonymous, 1988; Khan, 2001; Chand et al., 2007; Lahiri et al., 1967). It is used as a single plant remedy or in polyhedral formulation in organized medicine such as Ayurveda, Siddha and Unani (Khare, 2004). The plant contains major alkaloid known as berberine (Khosla, 1992; Rastogi et al., 1993), an isoquinoline alkaloid, known for its activity against cholera (Rabbani, 1996), severe diarrhea (Yamamoto et al., 1993), amoebiasis, and latent malaria (Ghosh et al., 1985). In the British pharmacopoeia patented, a drug made from Berberine is Orisol.

Population studies and genetic diversity studies in the family is almost non-existent. Inspite of three major revisions (Rao et al., 1998), the taxonomy of *B. lycium* Royle complex still remains, and utter confusion, perhaps due to difficulty in their correct identification. Due to the great variation among them, the taxonomic identification is difficult. In the present study, an attempt has been done to clarify the existing confusion and efforts have also been made clear to rearrange their taxonomic position.

Ahrendt (1941) surveyed the *Berberis* spp. and published a detailed revision of *Berberis*. Recently, Rao and Hajra (1993) while treating the family for the flora of India, included 54 species of *Berberis* in Indian region. The conclusion of most of the mentioned workers are primarily based on previous herbarium collections and often on solitary collections scattered in different herbaria. Study of the live plants in the natural habitat is rarely attempted.

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Molecular genetic diversity studies of this family were not attempted before, which raveled the neglect status and the extent of gap in the knowledge of family. Rao et al. (1998) solved the identification and taxonomy of *B. lycium* complex based on morphological basis. These identifications were based on extensive field studies as well as herbarium specimens. This helped extremely in solving the taxonomic problem of several species-complexes. It is important to use DNA based markers to study genetic diversity in the species as they are expected to reveal results that are less affected by environmental factors.

The objective of the current research is to deploy RAPD-DNA marker to study the genetic diversity of fifty accessions of *B. lycium* complex.

MATERIALS AND METHODS

Plant materials

In the present study fifty accessions of *Berberis lycium* complex were collected from different parts of Uttrakand, and Himachal Pradesh, India (Table 1). Different accessions of *B. lycium* complex were identified on morphological basis. The gross morphological attributes were taken into consideration for the identification of taxa which include terete or sulcate nature of stem, colour of bark, colour of leaf surface, nature of inflorescence, etc. The morphologically-closely related plants have been identified and categorized in to four major groups; *B. lycium* var. *lycium*, *B. lycium* var. *simlensis*, *B. lycium* var. *subfascicularis* and *B. lycium* var. *subvirescens*. Some species were collected from mixed wild populations and some were maintained by local people in India.

Total genomic DNA extraction

Total genomic DNA was extracted from young leaves of each variety using CTAB method. The leaves were first ground into a fine powder in liquid nitrogen, using mortar and pestle, and then, following the steps of the protocol of Doyle and Doyle (1987) with some minor modification, DNA was extracted. A fluorometer (Hoefer DyNA Quant^{200} pharmacia Biotech, USA) was used to determine the quantity and quality of the DNA. The stock DNA samples were diluted with sterile TE buffer to make a working solution of 5 ng µl^{-1} for use in PCR analysis.

Polymerase chain reaction (PCR) analysis

A total of eighty decamer random primers from kits AP, B, C and U from Operon Technologies (Alameda, Calif.) were used for amplification of template DNA. A standard 20 µl reaction contained 50 ng template DNA, 1.5 U Taq DNA polymerase (Bangalore genei, India) , 2 × PCR reaction buffer containing 1.5 mM $MgCl_2$, 10 picomoles primer and 100 µmoles of each dNTPs. DNA amplification was performed in Perkin Elmer DNA thermal cycler 9700 according to Williams et al. (1990). The following thermal cycling protocol was used: (1) One cycle for 2 min at 94°C; (2) 44 cycles of 94°C for 1 min, 36°C for 1.30 min and 72°C for 1.30 min; (3) one cycle for 5 min at 72°C, followed by a soaking at 4°C. The RAPD products were separated by electrophoresis according to their molecular weight on 1.4% (w/w) agarose gels submerged in 0.5 × TBE buffer and then stained with ethidium bromide (100 µg ml^{-1}) solution for 15 min. The DNAs were visualized on a UV-trans-illuminator and documented using the gel documentation system of AlphaImager (System and Control, India). The λ DNA digested by EcoRI and Hind III was used (Banlalore genei, India) on the gel as standard size marker.

Data analysis

PCR of each sample was repeated three times. Only reproducible and unambiguous fragment were scored as (1) for presence or (0) for absence of a band after electrophoresis. A fragment was considered polymorphic if both the presence and absence of that fragment were observed in the same species and monomorphic if it was present among all individual within a species. To reduce the possibility of comparing non-homologous bands, a positive control (an individual possessing the band to be scored) was included on each agarose-gel electrophoresis. Analysis of RAPD markers was based on the following three assumptions: (1) each RAPD marker represented a single locus comprising two alleles, a marker allele (amplified product) and a non-marker alleles (null allele); (2) RAPD marker is inherited in a dominant fashion with the marker allele dominant to the non-marker allele; (3) co-migrating bands from different populations present homologous amplified products (Allan et al., 1997; Hadrys et al., 1992).

The genetic associations among *B. lycium* were evaluated by calculating the jaccard similarity coefficient for pair-wise comparisons based on the proportion of shared bands (alleles) produced by primer. Similarity matrices were generated using 'Simqual subprogram, similarity coefficients were used for cluster analysis of accessions performed using the 'SHAN' sub program, dendrogram were built by the un-weighted Pair Group Method with Arithmetic average (UPGMA) Figure 3. The computer program NTSYS-pc Version 2.02 was used (Rohlf, 2000).

The polymorphic information content (PIC) was calculated by applying the formula given by Powell et al. (1996) and Smith et al. (1997):

$$PIC = 1-\sum_{i=1}^{n} f_i^2$$

Where f i is the frequency of the ith alleles and the summation extends over n alleles.

RESULTS AND DISCUSSION

RAPD marker system has been used for the molecular characterization of *B. lycium* complex. A total of thirty-two accessions of *B. lycium* var. *lycium*, five accessions of *B.s lycium* var. *simlensis*, eleven accessions of *B. lycium* var. *subfascicularis* and two accessions of *B. lycium* var. *subvirescens* were included in this study. Eighty primers were used to study molecular genetic diversity. Most of the primers did not amplify with *B.lycium* DNA. Only 28 primers yielded scorable amplification pattern (Figure 1) and rest primers gave unreadable and smear band pattern. A total of 11,683 amplicon generated 50 accessions of *B. lycium* with 28 primers. 332 amplification

Table 1. Collected *Berberis lycium* Royle complex accessions, accession name and their location.

Sl. No	Accessions No.	Accessions code	Accessions name	Location
Berberis lycium Royle var. *lycium*				
1.	223184	BLL1	*Berberis lycium* var. *lycium*	Himachal Pradesh, India
2.	223193	BLL2	*Berberis lycium* var. *lycium*	Himachal Pradesh, India
3.	223164	BLL3	*Berberis lycium* var. *lycium*	Uttarakhand, India
4.	219974	BLL4	*Berberis lycium* var. *lycium*	Uttarakhand, India
5.	223185	BLL5	*Berberis lycium* var. *lycium*	Himachal Pradesh, India
6.	219975	BLL6	*Berberis lycium* var. *lycium*	Uttarakhand, India
7.	223161	BLL7	*Berberis lycium* var. *lycium*	Uttarakhand, India
8.	223196	BLL8	*Berberis lycium* var. *lycium*	Uttarakhand, India
9.	223190	BLL9	*Berberis lycium* var. *lycium*	Himachal Pradesh, India
10.	223186	BLL10	*Berberis lycium* var. *lycium*	Himachal Pradesh, India
11.	223160	BLL11	*Berberis lycium* var. *lycium*	Uttarakhand, India
12.	223108	BLL12	*Berberis lycium* var. *lycium*	Uttarakhand, India
13.	223119	BLL13	*Berberis lycium* var. *lycium*	Uttarakhand, India
14.	219980	BLL14	*Berberis lycium* var. *lycium*	Uttarakhand, India
15.	219981	BLL15	*Berberis lycium* var. *lycium*	Uttarakhand, India
16.	219982	BLL16	*Berberis lycium* var. *lycium*	Uttarakhand, India
17.	219983	BLL17	*Berberis lycium* var. *lycium*	Uttarakhand, India
18.	219966	BLL18	*Berberis lycium* var. *lycium*	Uttarakhand, India
19.	219989	BLL19	*Berberis lycium* var. *lycium*	Uttarakhand, India
20	219992	BLL20	*Berberis lycium* var. *lycium*	Uttarakhand, India
21.	219983	BLL21	*Berberis lycium* var. *lycium*	Uttarakhand, India
22.	219996	BLL22	*Berberis lycium* var. *lycium*	Uttarakhand, India
23.	219997	BLL23	*Berberis lycium* var. *lycium*	Uttarakhand, India
24.	219998	BLL24	*Berberis lycium* var. *lycium*	Uttarakhand, India
25.	222399	BLL25	*Berberis lycium* var. *lycium*	Uttarakhand, India
26.	223298	BLL26	*Berberis lycium* var. *lycium*	Uttarakhand, India
27.	222397	BLL27	*Berberis lycium* var. *lycium*	Uttarakhand, India
28.	A	BLL28	*Berberis lycium* var. *lycium*	BSI, Dehradun, India
29.	B	BLL29	*Berberis lycium* var. *lycium*	BSI*, Dehradun, India
30.	C	BLL30	*Berberis lycium* var. *lycium*	BSI*, Dehradun, India
31.	D	BLL31	*Berberis lycium* var. *lycium*	BSI*, Dehradun, India
32	E	BLL33	*Berberis lycium* var. *lycium*	BSI*, Dehradun, India
Berberis lycium var. *simlensis* Ahrendt				
33.	223159	BLS1	*Berberis lycium* var. *simlensis*	Uttarakhand, India
34.	223181	BLS2	*Berberis lycium* var. *simlensis*	Himachal Pradesh, India
35.	223182	BLS3	*Berberis lycium* var. *simlensis*	Himachal Pradesh, India
36.	223189	BLS4	*Berberis lycium* var. *simlensis*	Himachal Pradesh, India
37.	223183	BLS5	*Berberis lycium* var. *simlensis*	Himachal Pradesh, India

Table 1. Contd.

Berberis lycium var. *subfascicularis* Ahrendt				
38.	223167	BLS6	*Berberis lycium* var. *subfascicularis*	Uttarakhand, India
39.	223173	BLS7	*Berberis lycium* var. *subfascicularis*	Uttarakhand, India
40.	223162	BLS8	*Berberis lycium* var. *subfascicularis*	Uttarakhand, India
41.	223171	BLS9	*Berberis lycium* var. *subfascicularis*	Uttarakhand, India
42.	223180	BLS10	*Berberis lycium* var. *subfascicularis*	Himachal Pradesh, India
43.	219988	BLS11	*Berberis lycium* var. *subfascicularis*	Himachal Pradesh, India
44.	223179	BLS12	*Berberis lycium* var. *subfascicularis*	Himachal Pradesh, India
45.	219978	BLS13	*Berberis lycium* var. *subfascicularis*	Uttarakhand, India
46.	219977	BLS14	*Berberis lycium* var. *subfascicularis*	Uttarakhand, India
47.	219984	BLS15	*Berberis lycium* var. *subfascicularis*	Uttarakhand, India
48.	219987	BLS16	*Berberis lycium* var. *subfascicularis*	Uttarakhand, India
Berberis lycium var. *subvirescens* Ahrendt				
49.	223157	BLS17	*Berberis lycium* var. *subvirescens*	Uttarakhand, India
50.	219979	BLS18	*Berberis lycium* var. *subvirescens*	Uttarakhand, India

BSI*- botanical survey of India.

products were scored and 284 (85%) were polymorphic and the rest, monomorphic (Table 3). Maximum numbers of 21 amplification product were obtained with primer OPAP-3 and 20 products with OPB-4. Minimum numbers of 6 RAPD products were generated with primer OPAP-14. Average numbers of 11.8 bands were obtained per primer and amplification ranged from 100 bp to 4.5 kb, and after study, no primer gave single band among all 50 accessions (Table 4). Highest similarity (0.97%) was identified between BLL10 or BLL11 accessions and least (0.23%) genetic similarity in BLL10 or BLS6 (Table 2). Polymorphic information content (PIC) scores represented gene diversity for specific locus. PIC scores for the RAPD primers ranged from 0.013 to 0.52 with an average of 0.12.

The relationship amongst the *B. lycium* complex obtained by RAPD method differs from the mostly cited classification of *B. lycium* Royle complex-reference. Cluster I included one accession of *B. lycium* and come out separately from rest of the species. Cluster II included 46 accessions of *B. lycium* var. *lycium, B. lycium* var. *simlensis, B. lycium* var. *subfascicularis* and *B. lycium* var. *subvirescens.*

The genetic relatedness among the *B. lycium* Royle complex, showed large diversity when confirmed by the principal component analysis (PCA). In Figure 2, the *B. lycium* cultivars were dispersed on PCA graph, which is a reflection of narrow genetic base of this genus. The results of PCA show a clear-cut separation of 50 *B. lycium* cultivars into five clusters. The present study concluded that molecular evidence is not supporting previous taxonomic classification of *B. lycium* Royle complex (Figure 3).

The evolution of varieties in distinct climatic location demonstrates significant levels of variation in response to the selection pressure in the location (Millan et al., 1996). It is therefore not surprising to find significant levels of polymorphism among 50 accessions of *B. lycium* and related varieties in RAPD (85%). The success of our study in identifying polymorphism is due to the use of a number of randomly selected, prescreened, and highly informative primers. Geographically isolated population accumulates genetic differences as they adapt to different environment. All 50 accessions in this study revealed a unique profile with the 28 primers and thus can be used for the DNA-RAPD fingerprinting. Generally, a large chromosome size and more repetitive sequences provided greater chances for the primers to find homology and to give more and differently sized amplified fragment.

Several doubts have been raised regarding the suitability of RAPD for variety identification and diversity studies, the most important one being that co-migrating bands may not be allelic or composed of similar sequences (Swaboda and Bhalla, 1997). However, the homology of co migrating RAPD bands has been demonstrated in some species of *Glycine* and *Allium* (Williams et al., 1993; Wilkie et al., 1993). In addition, conformity of phylogenetic grouping, based on RAPD data to these, based on conventional approaches like-morphological and cytology analysis, is in itself indirect, but significant evidence in support of the allelism of co migrating RAPD bands.The use of a large number of polymorphic markers would minimize the skewing of result due to non-allilism.

Another problem often encountered in RAPD analysis is that of reproducibility of bands pattern between different PCR reactions. These aspects can be overcome by using a thoroughly optimized PCR protocol and by scoring only reproducible bands. The RAPD method has been employed in the past, successfully for varieties relationship in mango (Srivastava et al., 2006), *cassia*

Table 2. Genetic relationships among the *Berberis lycium* and varieties.

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1												
2	0.516	1											
3	0.651	0.829	1										
4	0.693	0.434	0.569	1									
5	0.622	0.554	0.64	0.794	1								
6	0.642	0.831	0.535	0.648	0.8	1							
7	0.644	0.824	0.562	0.887	0.656	0.789	1						
8	0.628	0.799	0.481	0.843	0.855	0.673	0.792	1					
9	0.603	0.775	0.504	0.788	0.809	0.803	0.681	0.797	1				
10	0.635	0.775	0.808	0.882	0.853	0.231	0.653	0.796	0.639	1			
11	0.817	0.513	0.813	0.861	0.866	0.841	0.897	0.634	0.774	0.97	1		
12	0.767	0.543	0.772	0.814	0.809	0.786	0.849	0.874	0.523	0.661	0.637	1	
13	0.709	0.691	0.636	0.672	0.633	0.672	0.628	0.649	0.672	0.658	0.575	0.739	1
14	0.676	0.725	0.627	0.708	0.766	0.722	0.696	0.753	0.742	0.727	0.812	0.607	0.791
15	0.642	0.782	0.549	0.776	0.836	0.802	0.776	0.837	0.851	0.796	0.706	0.797	0.612
16	0.647	0.784	0.542	0.765	0.826	0.811	0.809	0.848	0.809	0.777	0.675	0.797	0.822
17	0.692	0.796	0.572	0.767	0.831	0.802	0.842	0.831	0.799	0.694	0.772	0.832	0.863
18	0.787	0.564	0.779	0.837	0.793	0.796	0.828	0.814	0.763	0.672	0.789	0.831	0.864
19	0.791	0.572	0.793	0.817	0.777	0.787	0.816	0.794	0.768	0.688	0.741	0.792	0.801
20	0.673	0.714	0.625	0.652	0.605	0.626	0.641	0.634	0.592	0.767	0.77	0.657	0.685
21	0.732	0.518	0.712	0.747	0.716	0.713	0.721	0.722	0.728	0.594	0.659	0.711	0.707
22	0.761	0.635	0.737	0.748	0.494	0.565	0.816	0.711	0.472	0.597	0.607	0.606	0.728
23	0.631	0.609	0.568	0.612	0.592	0.614	0.665	0.558	0.592	0.622	0.541	0.578	0.645
24	0.706	0.625	0.676	0.767	0.615	0.769	0.554	0.766	0.799	0.768	0.762	0.785	0.788
25	0.781	0.768	0.651	0.734	0.745	0.716	0.805	0.761	0.534	0.683	0.745	0.478	0.548
26	0.618	0.621	0.633	0.656	0.617	0.694	0.667	0.636	0.633	0.674	0.643	0.665	0.711
27	0.421	0.635	0.641	0.698	0.665	0.738	0.649	0.622	0.768	0.641	0.775	0.583	0.762
28	0.654	0.733	0.781	0.779	0.641	0.749	0.764	0.725	0.753	0.821	0.558	0.753	0.781
29	0.748	0.694	0.713	0.744	0.729	0.724	0.684	0.734	0.729	0.673	0.721	0.735	0.723
30	0.737	0.761	0.719	0.645	0.721	0.685	0.823	0.584	0.674	0.646	0.695	0.636	0.672
31	0.648	0.598	0.611	0.637	0.726	0.575	0.692	0.735	0.731	0.764	0.731	0.628	0.759
32	0.631	0.666	0.686	0.741	0.712	0.631	0.654	0.664	0.663	0.686	0.591	0.669	0.696
33	0.742	0.824	0.551	0.644	0.625	0.677	0.645	0.652	0.681	0.599	0.644	0.666	0.659
34	0.731	0.742	0.443	0.632	0.647	0.706	0.662	0.682	0.651	0.727	0.738	0.757	0.735
35	0.589	0.686	0.746	0.728	0.611	0.712	0.721	0.679	0.731	0.729	0.549	0.644	0.688
36	0.639	0.668	0.695	0.652	0.699	0.688	0.697	0.684	0.793	0.791	0.739	0.712	0.742

Table 2. Contd.

37	0.692	0.711	0.756	0.783	0.762	0.741	0.699	0.503	0.635	0.585	0.604	0.623	0.582
38	0.527	0.667	0.657	0.467	0.591	0.667	0.683	0.675	0.644	0.602	0.704	0.655	0.678
39	0.628	0.691	0.753	0.729	0.615	0.679	0.692	0.663	0.713	0.745	0.531	0.665	0.731
40	0.749	0.749	0.739	0.638	0.707	0.786	0.749	0.757	0.711	0.721	0.639	0.651	0.752
41	0.771	0.734	0.605	0.732	0.748	0.694	0.713	0.744	0.729	0.724	0.684	0.734	0.729
42	0.674	0.646	0.695	0.636	0.672	0.681	0.648	0.675	0.695	0.686	0.695	0.711	0.801
43	0.524	0.647	0.611	0.669	0.631	0.622	0.648	0.598	0.611	0.637	0.631	0.666	0.686
44	0.824	0.551	0.644	0.625	0.677	0.645	0.652	0.681	0.599	0.644	0.666	0.659	0.667
45	0.771	0.618	0.711	0.547	0.745	0.499	0.719	0.589	0.686	0.746	0.728	0.611	0.711
46	0.684	0.793	0.791	0.739	0.711	0.741	0.712	0.735	0.755	0.642	0.709	0.706	0.651
47	0.624	0.611	0.638	0.687	0.69	0.644	0.633	0.635	0.641	0.672	0.565	0.639	0.643
48	0.749	0.761	0.724	0.632	0.667	0.758	0.725	0.751	0.713	0.741	0.628	0.691	0.753
49	0.751	0.749	0.749	0.739	0.638	0.707	0.786	0.749	0.757	0.711	0.721	0.639	0.652
50	0.599	0.644	0.666	0.659	0.667	0.691	0.691	0.664	0.643	0.664	0.663	0.693	0.712

14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1															
	1														
0.794															
0.648	0.817	1													
0.639	0.789	0.673	1												
0.862	0.639	0.793	0.657	1											
0.839	0.825	0.545	0.658	0.642	1										
0.675	0.699	0.697	0.579	0.706	0.523	1									
0.704	0.708	0.715	0.635	0.665	0.811	0.835	1								
0.571	0.602	0.551	0.534	0.584	0.587	0.597	0.686	1							
0.631	0.574	0.601	0.475	0.728	0.652	0.442	0.612	0.608	1						
0.755	0.641	0.728	0.778	0.735	0.763	0.762	0.756	0.639	0.723	1					
0.787	0.652	0.796	0.582	0.499	0.662	0.617	0.677	0.668	0.623	0.633	1				
0.633	0.669	0.672	0.581	0.631	0.667	0.657	0.629	0.652	0.465	0.698	0.715	1			
0.788	0.755	0.728	0.802	0.813	0.798	0.654	0.742	0.775	0.742	0.781	0.791	0.768	1		
0.498	0.561	0.742	0.702	0.758	0.613	0.768	0.687	0.597	0.731	0.656	0.734	0.605	0.732	1	
0.686	0.691	0.765	0.763	0.598	0.695	0.733	0.706	0.721	0.794	0.522	0.775	0.779	0.471	0.596	1
0.681	0.648	0.675	0.695	0.686	0.695	0.711	0.801	0.767	0.698	0.698	0.693	0.683	0.699	0.653	0.727
0.753	0.499	0.599	0.599	0.732	0.778	0.642	0.693	0.695	0.741	0.808	0.524	0.647	0.612	0.669	0.632

Table 2. Contd.

0.548	0.623	0.711	0.657	0.733	0.692	0.598	0.768	0.706	0.486	0.591	0.686	0.736	0.726	0.667	0.631
0.667	0.691	0.694	0.664	0.643	0.664	0.663	0.693	0.712	0.622	0.697	0.692	0.559	0.653	0.668	0.664
0.771	0.618	0.711	0.547	0.745	0.499	0.742	0.766	0.739	0.752	0.762	0.741	0.742	0.607	0.658	0.752
0.697	0.684	0.793	0.791	0.739	0.711	0.742	0.711	0.735	0.755	0.642	0.709	0.706	0.651	0.682	0.722
0.711	0.735	0.755	0.642	0.709	0.706	0.651	0.682	0.721	0.732	0.727	0.707	0.564	0.758	0.738	0.456
0.608	0.567	0.625	0.622	0.624	0.611	0.638	0.687	0.691	0.644	0.633	0.635	0.641	0.672	0.565	0.639
0.746	0.726	0.712	0.613	0.735	0.616	0.744	0.595	0.757	0.541	0.744	0.763	0.712	0.777	0.749	0.761
0.403	0.535	0.742	0.665	0.714	0.597	0.749	0.683	0.741	0.757	0.716	0.646	0.687	0.742	0.706	0.711
0.728	0.597	0.692	0.711	0.717	0.816	0.735	0.552	0.691	0.712	0.453	0.537	0.776	0.654	0.804	0.576
0.673	0.721	0.735	0.723	0.686	0.691	0.765	0.763	0.598	0.695	0.733	0.706	0.721	0.794	0.522	0.775
0.767	0.698	0.698	0.693	0.683	0.695	0.653	0.727	0.726	0.575	0.692	0.735	0.731	0.764	0.731	0.628
0.741	0.711	0.632	0.654	0.664	0.663	0.686	0.591	0.669	0.696	0.548	0.623	0.711	0.657	0.733	0.691
0.691	0.664	0.643	0.664	0.663	0.693	0.712	0.622	0.697	0.692	0.559	0.651	0.668	0.664	0.701	0.756
0.721	0.679	0.731	0.729	0.549	0.644	0.696	0.423	0.528	0.737	0.616	0.756	0.513	0.745	0.596	0.741
0.682	0.723	0.732	0.727	0.707	0.564	0.758	0.738	0.456	0.637	0.688	0.714	0.757	0.668	0.681	0.692
0.534	0.589	0.649	0.614	0.689	0.638	0.527	0.667	0.657	0.467	0.591	0.667	0.683	0.675	0.644	0.602
0.729	0.615	0.679	0.692	0.663	0.713	0.745	0.531	0.665	0.731	0.403	0.535	0.742	0.665	0.714	0.597
0.752	0.728	0.597	0.692	0.711	0.717	0.686	0.591	0.669	0.696	0.548	0.623	0.711	0.657	0.733	0.691
0.622	0.697	0.692	0.559	0.651	0.668	0.664	0.701	0.756	0.521	0.731	0.741	0.443	0.631	0.647	0.706
29	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**
1															
0.727	1														
0.632	0.621	1													
0.631	0.652	0.692	1												
0.664	0.701	0.756	0.521	1											
0.752	0.726	0.752	0.725	0.719	1										
0.722	0.732	0.636	0.644	0.752	0.712	1									
0.456	0.637	0.688	0.714	0.757	0.668	0.681	1								
0.639	0.643	0.534	0.589	0.649	0.614	0.689	0.638	1							
0.761	0.724	0.632	0.667	0.758	0.725	0.751	0.713	0.741	1						
0.711	0.614	0.734	0.578	0.736	0.561	0.717	0.747	0.765	0.751	1					
0.576	0.762	0.624	0.737	0.687	0.732	0.689	0.635	0.791	0.693	0.656	1				
0.775	0.779	0.471	0.596	0.737	0.76	0.719	0.645	0.721	0.685	0.823	0.584	1			
0.628	0.759	0.753	0.499	0.599	0.732	0.731	0.778	0.642	0.693	0.695	0.741	0.808	1		
0.691	0.598	0.768	0.706	0.486	0.591	0.686	0.736	0.726	0.667	0.631	0.652	0.692	0.742	1	

Table 2. Contd.

0.756	0.521	0.731	0.742	0.443	0.631	0.647	0.706	0.662	0.682	0.652	0.727	0.738	0.757	0.735	1
0.741	0.692	0.687	0.636	0.644	0.575	0.711	0.687	0.712	0.639	0.668	0.695	0.651	0.699	0.688	0.697
0.692	0.711	0.756	0.783	0.762	0.741	0.699	0.503	0.635	0.585	0.604	0.623	0.582	0.608	0.567	0.625
0.602	0.704	0.655	0.678	0.746	0.726	0.712	0.613	0.735	0.616	0.744	0.595	0.757	0.541	0.744	0.763
0.597	0.749	0.683	0.741	0.757	0.716	0.646	0.687	0.742	0.706	0.711	0.614	0.734	0.578	0.736	0.561
0.691	0.598	0.768	0.706	0.486	0.591	0.686	0.736	0.726	0.631	0.652	0.692	0.742	0.824	0.551	0.644
0.706	0.735	0.771	0.618	0.547	0.745	0.499	0.742	0.766	0.739	0.752	0.762	0.741	0.742	0.607	0.658

45	46	47	48	49	50
1					
0.622	1				
0.712	0.777	1			
0.717	0.747	0.765	1		
0.625	0.677	0.645	0.652	1	
0.752	0.726	0.751	0.725	0.719	1

(Whity et al., 1994), rice (Takeuchi, 1994), mustard (Lin et al., 1996) and soybean (FuJishiro and Sasakuma., 1994). We analyzed genetic relationship between genotypes of B. lycium varieties. It was apparent that RAPD marker is capable of differentiating between closely related varieties. Similar results have been found in case of peanut and blackgram where they could differentiate the species with RAPD (Raina et al., 2000; Souframanian et al., 2004).

The present study showed that *B. lycium* is quite different from the rest of the varieties and did not show any close relationships. Four varieties of *B. lycium* did not arrange into four clusters. Within species, genetic diversity existed among the accessions but not in the case of *B. lycium* complex. Ahrendt (1941) described four varieties (*B. lycium* var. *lycium, B. lycium* var. *simlensis, B. lycium* var. *subfascicularis* and *B. lycium* var. *subvirescens*) under *B. lycium* complex. Uniyal and Rao (1993) observed that *B. lycium* var. *simlensis* can be separated from other species by pubescent nature of the stem but this is not true as all the other varieties of *B. lycium* also have pubescent stem either at young or mature stage (Rao et al., 1998). This result is not supportive of previous taxonomic classification of *B. lycium* Royle complex proposed by Ahrendt (1941), Rao et al. (1998) and Uniyal and Rao (1993).

It was concluded from the present study that to obtain identification, tracing genetic relationships and characterization of the *B. lycium* accessions, the molecular approach based on RAPD profile is a powerful technique. RAPD markers amplified from few primers could identify the *B. lycium* cultivars. The information obtained will facilitate the choosing of appropriate breeding program, genome mapping, and tagging numerous traits of economic importance. It was also suggested that RAPD technique would be more useful for identification of cultivars and for estimating genetic relationship in *B. lycium* complex. The present study analyzed the genetic relationships at molecular level utilizing RAPD assay. RAPD markers have been used earlier to study taxonomic and phylogenetic relationships Demeke et al., 1992; Millan et al., 1996). Virk et al. (1995) have analyzed the germplasm collection of rice accessions by RAPD markers and classified the unclassified rice accessions as *indica* and *japonica* types. Similarly, Howell and Newburg (1994) have used RAPD for identifying and classifying *Musa* germplasm. Pipe et al. (1995) supported the separation of two groups of *Opiostoma piceae* into two species based on the clear-cut divergence revealed by RAPD. In another case, the genus *Scaevola*, which was initially misclassified by Linnaneus (1753), and further rearranged several times by other scientist (Bentham, 1868; Krauze, 1912; Carolin, (1992), has now been reclassified, resolving the previous confusions through RAPD analysis (Swoboda et al., 1997). Phylogenetic relationships investigated

Table 3. Analysis of polymorphism among *Berberis lycium* complex obtained with random primers. The pair wise similarity indices RAPD band data among 50 accessions of *Berberis lycium*.

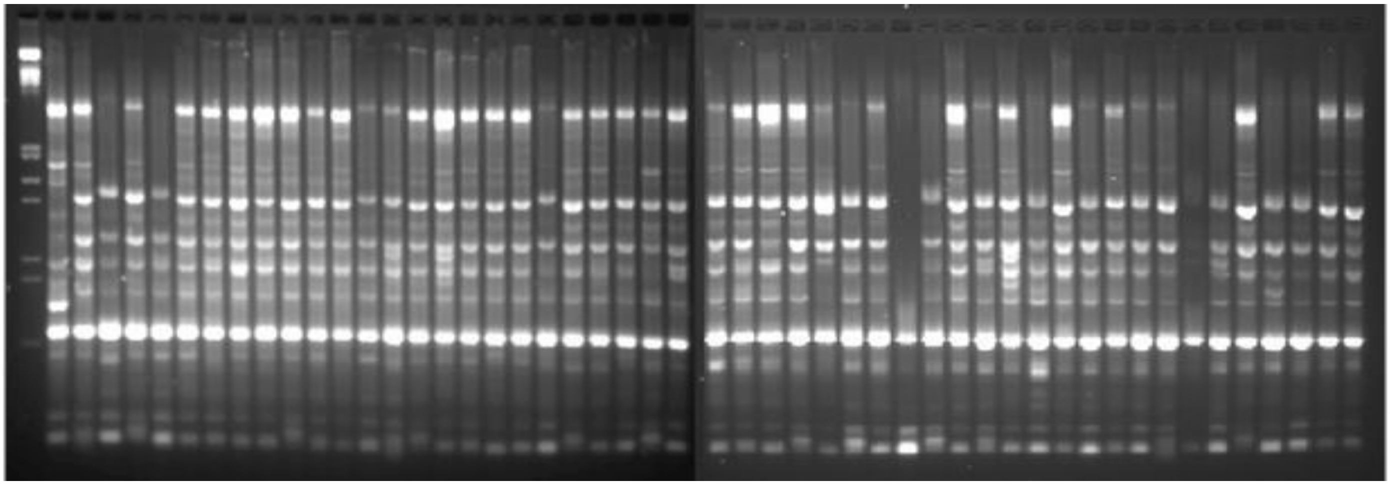
Primer no.	Total no. of amplicon	Total no. of bands	Polymorphic bands	Monomorphic bands	PIC Values	Average	Average no. of bands	Size range of amplified product
AP-1	589	17	13	4	0.086	0-0.47	11.7	125-4000
AP-2	467	12	8	4	0.055	0.11-0.49	9.3	200-4268
AP-3	646	21	21	0	0.097	0.03-0.5	13	125-4000
AP-4	460	12	12	0	0.045	0.03-0.41	9.2	125-3000
AP-5	258	9	8	1	0.03	0.11-0.49	5.1	400-4000
AP-6	460	12	12	0	0.045	0.03-0.41	9.2	125-3000
AP-7	565	17	15	2	0.077	0.07-0.48	11.3	250-2000
AP-8	433	12	10	2	0.046	0.11-0.49	8.6	125-1500
AP-9	439	13	13	0	0.066	0.07-0.49	8.7	400-3530
AP-10	314	10	9	1	0.40	0.03-0.47	6.2	500-3000
AP-11	541	14	14	0	0.51	0.03-0.5	10.8	125-4268
AP-12	287	8	7	1	0.034	0.21-0.48	5.7	500-4000
AP-13	373	10	8	2	0.54	0.37-0.47	7.4	300-4000
AP-14	279	6	3	3	0.013	0.03-0.37	5.5	150-4268
AP-15	367	10	7	3	0.038	0.03-0.42	7.3	300-4500
B-1	312	10	6	4	0.043	0.11-0.5	6.2	100-2027
B-4	561	20	18	2	0.083	0.03-0.48	11.2	125-4000
B-5	436	12	12	0	0.07	0.11-0.5	8.7	250-3100
B-17	388	12	12	0	0.52	0.03-0.41	7.7	125-300
C-1	450	11	7	4	0.334	0.07-0.34	9	250-3530
C-2	361	9	7	2	0.036	0.07-0.5	7.5	200-3500
C-5	394	11	8	3	0.047	0.11-0.49	7.8	200-1375
C-18	282	7	6	1	0.020	0.03-0.41	5.6	250-3600
C-19	334	7	7	0	0.012	0.03-0.41	6.6	200-1900
U-3	307	11	8	3	0.041	0.11-0.49	6.1	150-3000
U-8	462	15	12	3	0.052	0.07-0.38	4.2	200-3530
U-6	386	11	8	3	0.041	0.07-0.49	7.7	500-3700
U-13	532	13	13	0	0.039	0.03-0.48	10.6	350-3000
28	11,683	332	284	48	0.122		Max=13, Min=4.2	

Table 4. Summary of detection of RAPD marker in *Berberis lycium* complex.

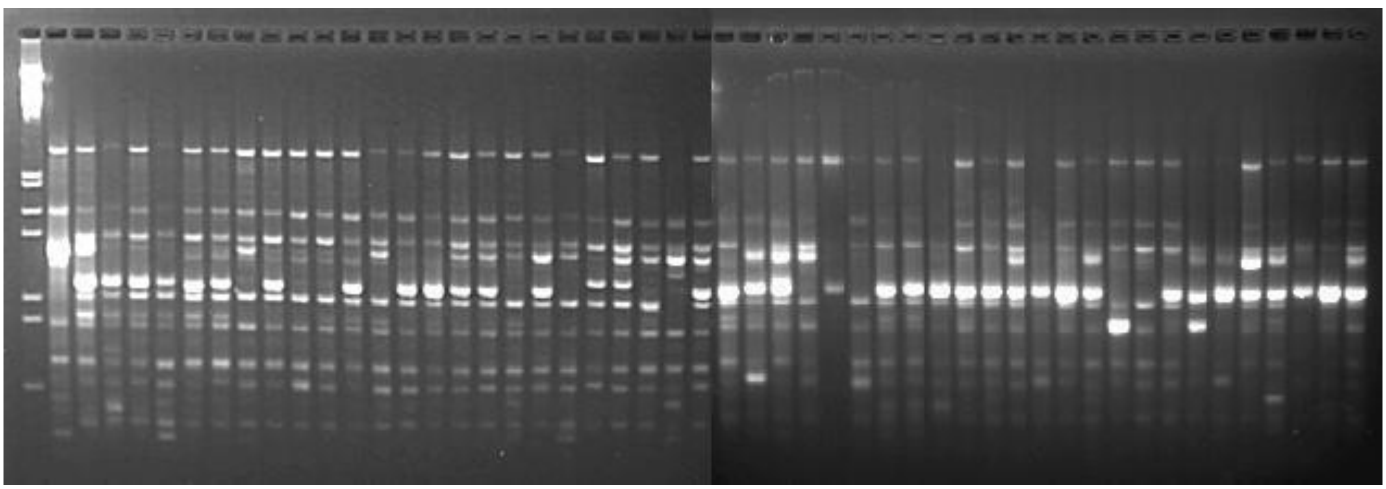
Total no. of primer	80
No. of polymorphic primers	28
Total no. of bands amplified product	332
Size range of amplified product	100-4500 bp
Average no. of bands per polymorphic primer	11.5
Total no. of polymorphic bands identified	284
Total no. of monomorphic bands	48
Average no. of RAPD per polymorphic primer	10.1
Percentage of total polymorphic bands	85.5

using RAPD analysis among the *Rosa* species accessions proved useful in assigning unclassified accessions to specific taxonomic groups (Millan et al., 1996). RAPD analysis of Tibetan wheat, common wheat

M 1 2 3 4 5 6 7 8 9 1011 1213141516171819 202122232425 262728 29303132 3334353637 38394041424344546 4748 4950



M 1 2 3 4 5 6 7 8 9 1011 1213141516171819 202122232425 262728 29303132 3334353637 38394041424344546 4748 4950



M 1 2 3 4 5 6 7 8 9 1011 1213141516171819 202122232425 262728 29303132 3334353637 38394041424344546 4748 4950

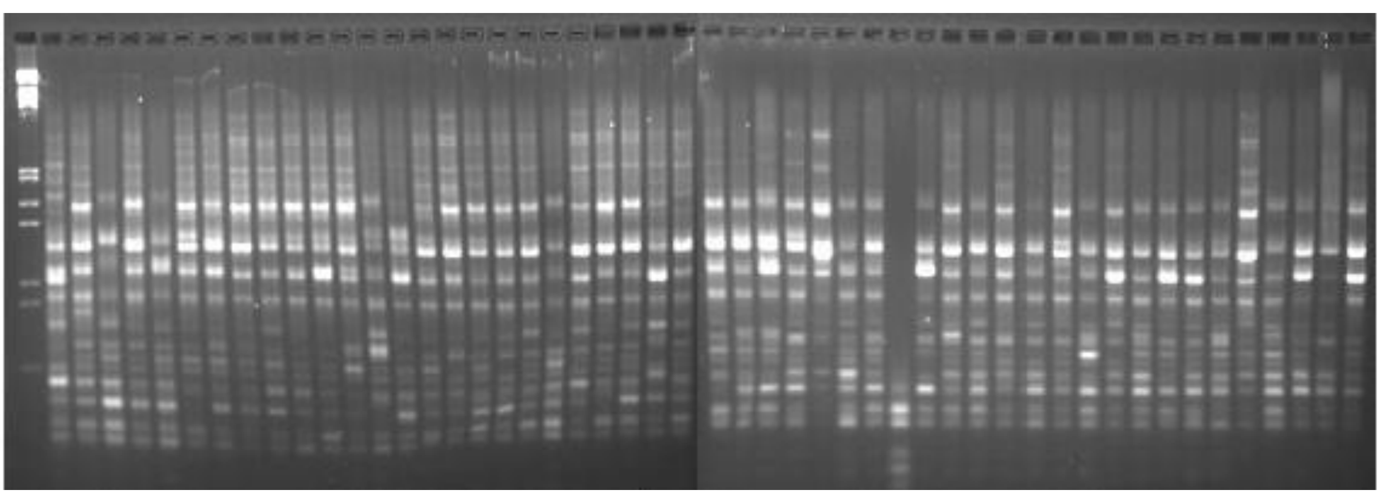


Figure 1. Lane 1, Eco RI and Hind III digested DNA; lane 1–50, amplified pattern. different accessions of *Berberis lycium* Royle complex with primer OP AP-3, OP U-3 and OP U-8.

Lane-1 Eco RI and Hind III digested λDNA.

Lane-2-51 Amplification pattern different accessions of *Berberis lycium* Royle complex with Primer OP AP-3, OP U-3 and OP U-8.

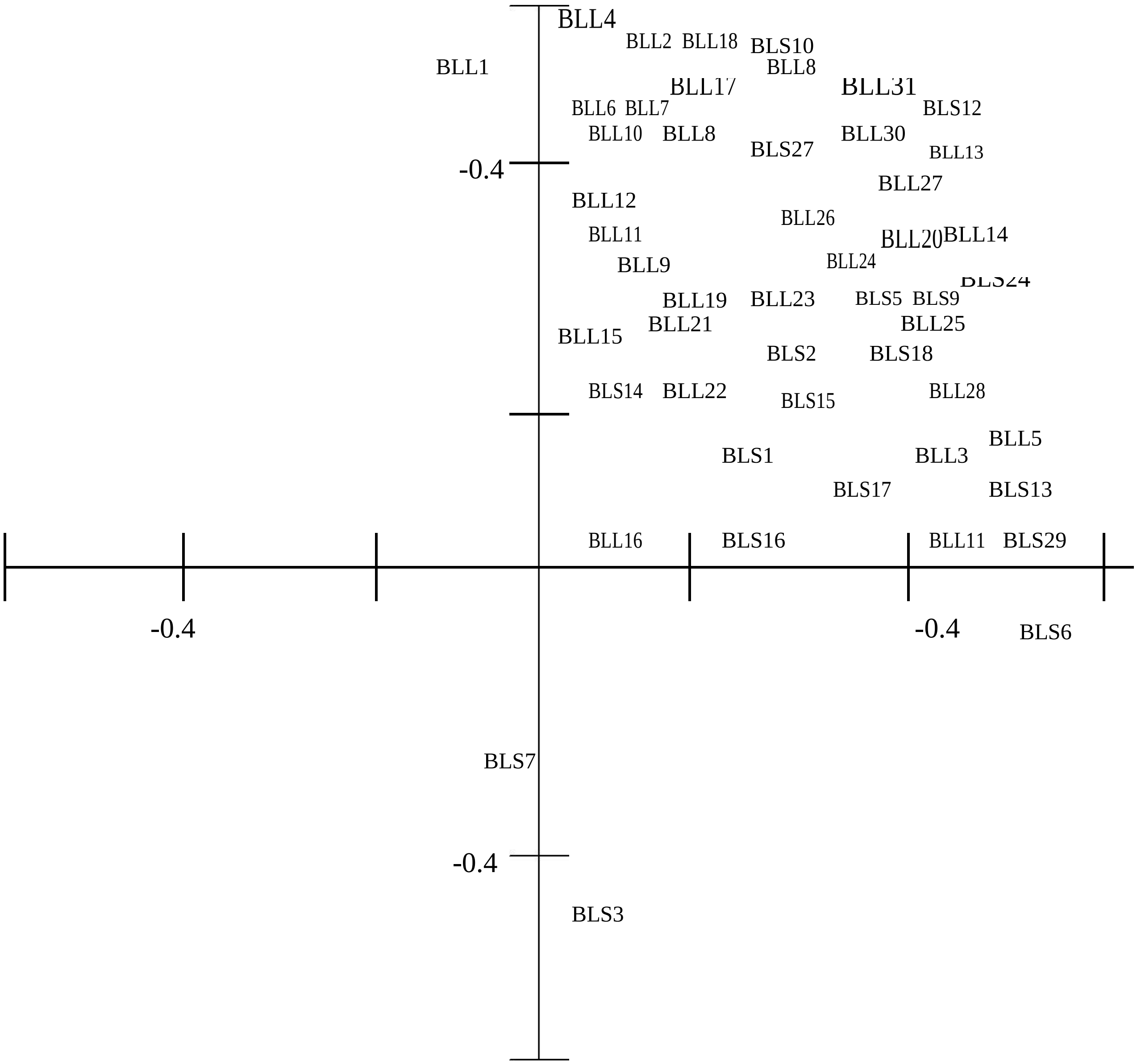


Figure 2. Principle co-ordinate analysis of RAPDs products from *Berberis lycium* complex. The accessions are separated into five groups.

and European spelt wheat supported the previous classification of Tibetan wheat as a subspecies of common wheat (Sun et al., 1998). Recently, Singh et al. (2004) classified *Ocimum* species using RAPD markers.

In day-to-day management of the germplasm collection, RAPD allow identification of redundancy and provide additional cultivars verification method. The genetic diversity analysis within the *B. lycium* germplasm collection may provide useful information for proper management and its future utilization in basic and applied studies. To our knowledge, this study is the first attempt in using molecular markers for the varietial identification and genetic diversity assessment of *B. lycium* accessions. This study will be helpful for the breeding, conservation and germplasm management of *B. lycium*.

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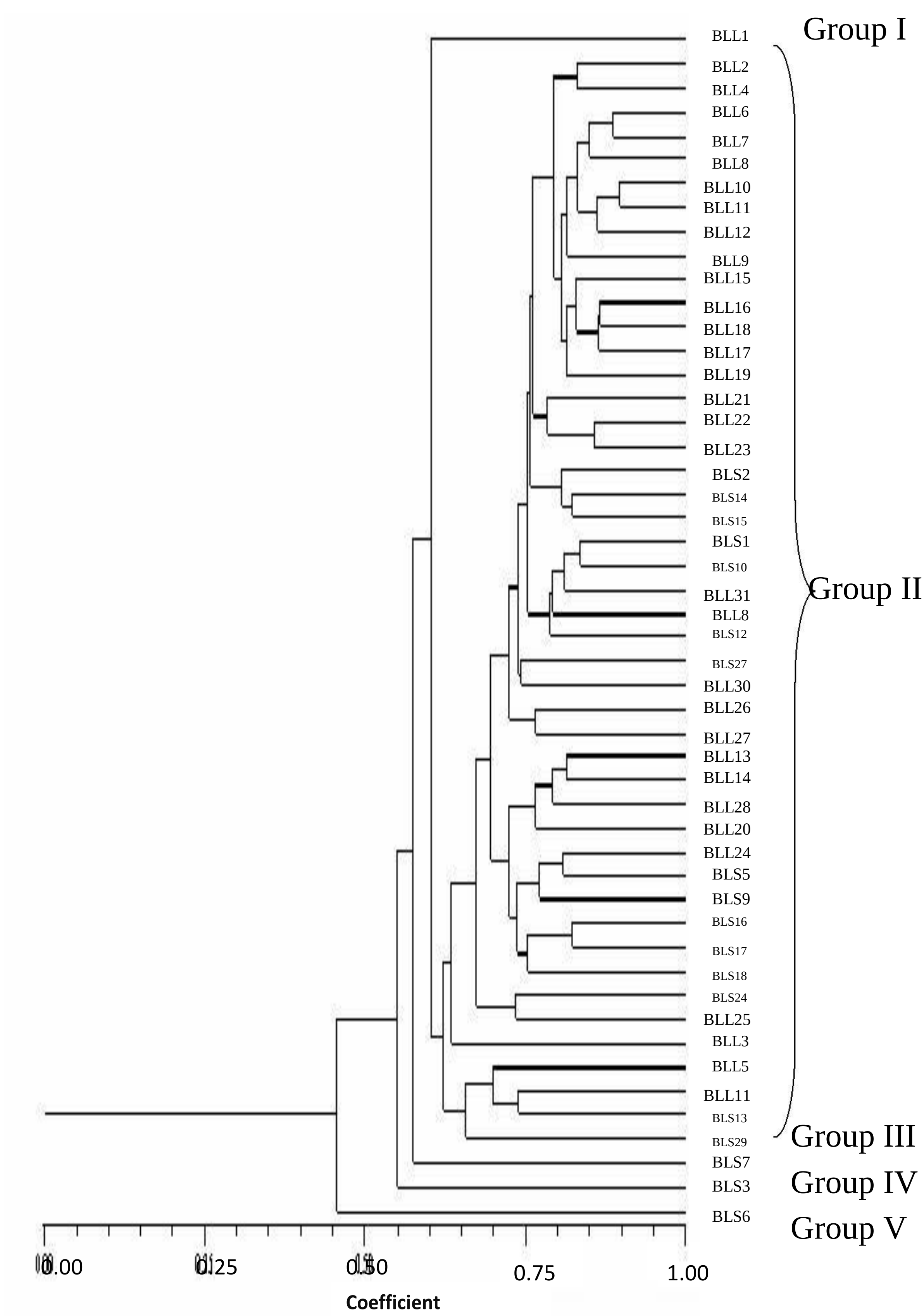

Figure 3. Dendogram showing the relationship among different species of *Berberis lycium* complex based on UPGMA and sequential agglomerative hierarchical nested.

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