Journal of Medicinal Plants Research Vol. 6(3), pp. 405-415, 23 January, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1064
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Genetic diversity among natural populations of *Rhodiola imbricata* Edgew. from trans- Himalayan cold arid desert using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers

Sunil Gupta[1], Manish S. Bhoyar [1]*, Jitendra Kumar[1], Ashish R. Warghat[1], Prabodh K. Bajpai[1], Muzamil Rasool[2], Gyan P. Mishra[1], Pradeep K. Naik[3] and Ravi B. Srivastava[1]

[1]Defence Institute of High Altitude Research, DRDO, C/o 56 APO, Leh, India, 901 205, India.
[2]Department of Floriculture SKUAST_Kashmir, Shalimar, Srinagar, India, 190001, India.
[3]Jaypee University of Information Technology, Waknaghat, Solan, India, 173 215, India.

Accepted 6 October, 2011

***Rhodiola imbricata* Edgew. (Crassulaceae), well acknowledged medicinal plant is widely distributed in trans-Himalayan region of India. It has multiple uses in cuisine, forage, health care and ornamental worth. In the present investigation, 70 wildly grown *Rhodiola* genotypes, collected from three different sampling sites in trans-Himalayan region of Ladakh were analyzed using 40 DNA-based markers (20 random amplified polymorphic DNAs (RAPDs) and 20 inter simple sequence repeats (ISSRs). RAPD analysis yielded 134 fragments, of which 130 were polymorphic, with an average of 6.5 polymorphic fragments per primer. Of the 20 ISSR primer screened, only 8 were amplified in the present investigation. These amplified primers produced 59 bands, of which 57 were polymorphic with an average of 7.12 polymorphic fragments per primer. RAPD markers were found more efficient with regards to polymorphism detection, as they detected 98.28% as compared to 97.55% for ISSR markers. Clustering of genotypes within groups was remained more or less same when RAPD, ISSR and RAPD + ISSR derived dendrograms were compared. The results of principal coordinates analysis (PCA) analysis were corresponding to the dendrogram analysis. These analyses allowed us to identify the groups corresponding to the three *Rhodiola* collection sites. Analysis of molecular variance (AMOVA) showed that genetic variation within the populations was found maximum than among populations in all the three cases. It may because of high level of differentiation within populations due to geographical and genetic isolation of populations in a harsh mountainous environment. With reference to the management of *Rhodiola*, the high genetic separation of population indicates the requisite of conserving the extreme possible number of populations from diverse parts of trans-Himalayas.**

Key words*: Rhodiola imbricata*, genetic diversity, random amplified polymorphic DNA (RAPD), intergenic simple sequence repeat polymorphism (ISSR), analysis of molecular variance (AMOVA).

INTRODUCTION

Rhodiola imbricata Edgew. (*Crassulaceae*) is a highly valuable medicinal plant used in Chinese phytotherapy and Amchi System of Medicine for centuries in attempt to maintain body health and to treat various diseases (Zhao et al., 1998; Rohloff, 2002). It is generally known as Golden Root or Himalayan stone crop and locally known as *Shrolo marpo*, distributed in the high cold region of the Northern Hemisphere (Lei et al., 2003) and in high Arctic latitudes and mountain regions of Europe and Asia (Saratikov and Krasnov, 1987). In India, it is mainly

*Corresponding author. E-mail: mann.bs1007@gmail.com.

Table 1. Three populations of *R. imbricata* collected from different altitudes covering 70 individuals.

Name of valley	Individual No. (villages)	Individual No.	Sampling site	Altitude (ft)
	Khardung-la	KDL 1-25	Khardung-la	18,080
Indus	Khaltse	KL 26-45	Khaltse	10,490
	Chang-la	CGL 46-70	Chang-la	17,360

distributed in the trans-Himalaya between 14000 to 18500 ft above MSL which includes rocky slopes, wet places and higher passes of Ladakh region (Chaurasia et al., 2007).

Rhodiola is a succulent, dioecious and herbaceous perennial plant with yellow or red flower. In Ladakh, it is used as wild edible, fodder and ornamental plant, the young shoots are boiled and then washed thoroughly with water then mixed with yogurt by which a delicious dish "Tantur" is prepared (Chaurasia and Singh, 1996). As a traditional herbal remedy, *Rhodiola* have been used for the treatment of a variety of conditions such as clearing heat in the lungs, eliminating toxins from the body, treating various epidemic diseases, edema of limbs, traumatic injuries and burns. Beside these properties, it reduces the effects of anoxia, microwave radiation and fatigue**.** Other pharmacological properties of *Rhodiola* have been reported to include anti-aging, anticancer, antistress, antihypoxia, radioprotective, immunostimulant, adaptogenic, anti-fatigue, anti-toxic, anti-radiation and anti-tumour properties and many others. Many *Rhodiola* species have been used as traditional medicines for the treatment of long term illness and weakness due to infection in Tibet and other regions for over 1000 years (Xiong, 1995; Zhao et al., 1998; Ohsugi et al., 1999; Rohloff, 2002). In addition, *Rhodiola* roots and rhizomes contain different antioxidant compounds such as salidrosides, cynnamyl alcohol glycosides (rosine, rosavine, rosarine), flavonoids, (rhodionine, rhodiosine, rhodioline), terpenoids, tyrosol, organic acids (gallic, caffeic, chlorogenic acid) etc.

In Amchi and Tibetian system of traditional medicine, the roots are used in medicine against lung problems, cold, cough, fever, loss of energy, pulmonary complaints and contagious diseases (Chaurasia et al., 2007). Earlier, *Rhodiola* leaves were used for culinary and roots and rhizome for medicinal purposes by laypeople, but recently commerce and demand have increased. Heavy exploitation of roots from the wild, loss of habitat by deforestation and excessive grazing at high altitude pasture in the entire Ladakh region now threaten its survival.

In recent years, several breeding and registration of medicinal plant cultivars are being reported from different countries (Bernath, 2002). Modification of the accumulation level of biologically active ingredients are the most important goals of any medicinal plant breeding programme. The variation on the morphological and pharmaceutical characters has been attributed to environmental or genetic factors. In recent years, DNA-based molecular markers have been used for assessment of the genetic diversity between germplasm in many plant species. DNA-based molecular markers are free from environmental modulations. Random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers have proved to be very useful tool providing a convenient and rapid assessment of the genetic difference between genotypes (Williams et al., 1990; Zietkiewicz et al., 1994). Moreover, these markers use arbitrary primers that provide a large number of multilocus markers and can be applied to analyze almost any organism, even those which no previous genetic or molecular information are available.

Since ages, *Rhodiola* has been exploited by local people from every part of Ladakh region for culinary and medicinal purpose but till date no research has been conducted on this plant with conservation point of view. The aim of the present investigation was to study genetic diversity of different trans-Himalayan accessions of *R. imbricata* based on RAPD and ISSR markers in order to support breeding programme. To our knowledge, no report has been published on genetic characterization among the *R. imbricata* populations from Ladakh region using RAPD and ISSR markers and its implications on future *Rhodiola* conservation and management programs.

MATERIALS AND METHODS

Seventy wildly grown individual plants collected from three different locations in trans-Himalayan region of Ladakh with an altitude ranging from 18,080 ft (Khardung-la), 17,360 ft (Chang-la) to 10,490 ft (Khaltse) (Table 1). The interval between samples was 100 to 500 m, the pair wise distance between populations was 80-120 km.

Total genomic DNA was extracted from frozen leaves (5 g) by the cetyltriammonium bromide (CTAB) method (Saghai-Maroof et al., 1984) with minor modifications. Twenty (20) random decamer primers from IDT Tech, USA (Table 2) were used for RAPD amplification following the protocol of Williams et al. (1990). Amplification reaction were performed in volumes of 25 µl containing 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 50 mM KCl, 200 µM of each dNTPs, 0.4 µM primer, 20 ng template DNA and 0.5 unit of *Taq* polymerase ('Sigma-Aldrich, USA'). The first cycle consisted of denaturation of template DNA at 94°C for 5 min, primer annealing at 37°C for 1 min., and primer extension at 72°C for 2 min. In the next 40 cycles, the period of denaturation was reduced to 1 min at 92°C. The last cycle consisted of only primer extension (72°C) for 7 min.

Table 2. List of primers used for RAPD amplification, GC content, total number of loci, the level of polymorphism and resolving power.

Primer	Primer sequence (5' – 3')	GC (%)	Tm (°C)	Total no. of loci	Number of polymorphic loci	Percentage of polymorphic loci	Total number of fragments amplified	Resolving power
S1	CAGGCCCTT C	70	36.4	7	6	85.7	362	10.34
S2	TGCCGAGCT G	70	40.7	10	10	100	358	10.22
S3	AGTCAGCCA C	60	34.3	7	7	100	310	8.85
S4	AATCAGCCA C	50	30.1	7	7	100	359	10.25
S5	AGGGGTCTT G	60	32.6	10	10	100	524	14.97
S6	GGTCCCTGA C	70	35.2	10	10	100	471	13.45
S7	GAAACGGGT G	60	33.2	10	10	100	454	12.97
S8	GTGACGTAG G	60	31.1	6	6	100	231	6.6
S9	GGGTAACGC C	70	37.4	7	7	100	346	9.88
S10	GTGATCGCA G	60	33.1	7	7	100	367	5.24
S11	CAATCGCCG T	60	36.7	10	10	100	448	12.8
S12	TCGGCGATA G	60	34.0	10	6	100	428	12.22
S13	CAGCACCCA C	70	37.7	7	7	100	369	10.54
S14	TCTGTGCTG G	60	34.3	10	10	100	368	10.52
S15	TTCCGAACC C	60	34.2	10	10	100	400	11.42
S16	AGCCAGCGA A	60	38.3	11	10	100	509	14.54
S17	GACCGCTTG T	60	35.7	10	9	90.0	467	13.34
S18	AGGTGACCG T	60	36.2	8	8	100	322	9.2
S19	CAAACGTCG G	60	34.2	10	9	90.0	378	10.8
S20	GTTGCGATC C	60	33.5	6	6	100	272	7.77
Total				134	130	98.28%	7743	-

In case of ISSR, the primers were obtained from 'Applied Biosciences, India' (Table 3) and polymerase chain reaction (PCR) amplification was performed in reaction cocktail similar to RAPD. Initial denaturation for 5 min at 94°C was followed by 40 cycles of 1 min at 94°C, 1 min at specific annealing temperature (± 5°C of Tm), 2 min at 72°C and a 10 min final extension step at 72°C. Amplification products were electrophoresed on 1.5% agarose gel (Life Science Technologies, USA) and run at constant voltage (50 V) in 1X TBE for approximately 2 h. The gels were documented on a gel documentation system (Alpha Innotech, Alphaimager, USA). Molecular size of amplicons was estimated using a 100 bp and 1 Kb DNA ladders ('Bangalore Genei, India'). The banding patterns obtained from RAPD and ISSR were scored as present (1) or absent (0), each of which was treated as an independent character. Jaccard's similarity coefficient (J) was used to calculate similarity between pairs of individuals.

The similarity matrix was subjected to cluster analysis by unweighted pair-group method analysis (UPGMA) and a dendrogram was generated using the program NTSYS-pc (Rohlf, 1992). POPGENE software was used to calculate Nei's unbiased genetic distance among different individuals with all markers. Data for Nei's genetic diversity (H), Shannon's information index (I), number of polymorphic loci (NPL) and percentage polymorphic loci (PPL) across all the three populations were also analyzed (Zhao et al., 2006). Within species diversity (Hs) and total genetic diversity (Ht) (Nei, 1978) were calculated within the species and within major groups (as per their collection site) using POPGENE software. RAPD and ISSR data were subjected to a hierarchical analysis of molecular variance (AMOVA) (Excoffier et al., 1992), using two hierarchical levels; within and among populations. GenAlEx software was used to calculate a principal coordinates analysis (PCA) that plots the relationship between distance matrix elements based on their first two principal coordinates (Peakall and Smouse, 2001). According to Prevost and Wilkinson (1999), the resolving power (Rp) of a primer is: $Rp = \Sigma IB$ where IB (band informativeness) takes the value of: $1–[2*(0.5–P)]$, P being the proportion of the 70 individuals

Table 3. List of primers used for ISSR amplification, sequence, GC content, total number of loci, the level of polymorphism, size range of fragments and resolving power. Where, (Y = C, T; R = A,G).

Primer	Primer sequence (5' – 3')	GC (%)	Tm (°C)	Total number of loci	Number of polymorphic loci	Percentage of polymorphic loci	Total number of fragment s amplified	Resolving power
ISSR 1	(AG)8 T	47	47.0	8	8	100	330	9.42
ISSR 2	(GA)8 T	47	45.4	7	7	100	364	10.4
ISSR 3	(AC)8 T	47	51.4	9	8	88.8	316	9.02
ISSR 4	(TG)8 A	47	51.3	4	4	100	214	6.11
ISSR 5	(AG)8YT	47.2	49.2	12	11	91.6	480	13.71
ISSR 6	(GA)8YT	47.2	47.4	7	7	100	251	7.17
ISSR 7	(CT)8 RA	47.2	47.1	6	6	100	98	2.8
ISSR 8	(GT)8 YC	52.7	52.7	6	6	100	147	4.2
	Total			59	57	97.55	2200	-

ISSR 9-20 did not amplify with the individuals used in the present investigation.

containing the band.

RESULTS

Genetic variability details from random amplified polymorphic DNA (RAPD) markers

Twenty (20) RAPD primers produced 7743 fragments which varied in size from 200 to 2,500 bp with the number of amplified fragments ranging from 6 (S8 and S20) to 11 (S16). Of these 134 amplified bands, 130 were found polymorphic with an average number of bands per primer and average numbers of polymorphic bands per primer as 6.7 and 6.5, respectively (Table 2). The other primer amplification details are also shown in the same Table. When H, I, Ht, NPL and PPL were studied for sites (that is, Khardung-la, Chang-la and Khaltse) then most of the respective values were found higher for Chang-la individuals and least for Khaltse individuals indicating more variability in Chang-la and least in Khaltse individuals (Table 4). The respective values for overall genetic variability for H, I, Ht, and Hs across all the 70 individuals were given in Table 5. AMOVA showed significant (p<0.001) genetic variation within population. Most of the total variation (56.00%) was found within population, whereas only (44.00%) occur among population (Table 6). This is helpful in making strategy for germplasm collection and evaluation. The UPGMA analysis was performed based on genetic similarity (Jaccard's similarity coefficient). All 70 individuals were divided into three major clusters (I-III) with Jaccard's similarity coefficient ranging from 0.49 to 0.98 (Figure 1a). Cluster I contains individuals from Khardung-la, cluster II represents individuals from Chang-la. However, cluster III contains individuals from Khaltse. The results of PCA analysis were comparable to the cluster analysis.

Genetic variability details from inter simple sequence repeat (ISSR) markers

Of the 20 ISSR primers used, out of which only 8 primers amplified in the present investigation. These amplified primers on an average generated 59 bands across 70 individuals, of which 57 bands were found polymorphic, accounting for 97.55% polymorphism. Number of amplified fragments varied from 4 (ISSR4) to 12 (ISSR5) which varied in size from 200 to 2,500 bp. Average numbers of bands and polymorphic bands per primer was found to be 7.37 and 7.12, respectively. The other primer amplification details are shown in Table 3. When H, I, Ht, NPL and PPL were studied for all three sites (that is, Khardung-la, Chang-la and Khaltse) then, all these respective values were found higher for Khaltse and least for Khardung-la individuals indicating more variability in Khaltse and least in Khardung-la individuals (Table 4). The details of overall genetic variability across 70 individuals were given in Table 5. Molecular variance indicates significant diversity among population (22.00%) and within the population (78.00%) indicating more variations within the population (Table 6). Dendrogram based on UPGMA analysis and Jaccard's similarity

Table 4. Summary of genetic variation statistics for all loci of RAPD, ISSR and RAPD + ISSR among the *Rhodiola* populations with respect to their distributions.

Valley	Sample size	Na	Ne	H	I	Ht	NPL	PPL
RAPD								
Khardung-la	25	1.786±0.411	1.455±0.365	0.266±0.188	0.400±0.260	0.266±0.035	136	78.61
Chang-la	25	1.878±0.327	1.467±0.345	0.279±0.172	0.424±0.230	0.279±0.029	152	87.86
Khaltse	20	1.786±0.411	1.415±0.361	0.247±0.184	0.377±0.254	0.247±0.034	136	78.61
ISSR								
Khardung-la	25	1.814±0.393	1.432±0.379	0.252±0.193	0.382±0.262	0.252±0.037	48	81.36
Chang-la	25	1.882±0.326	1.548±0.346	0.317±0.170	0.472±0.229	0.317±0.028	52	88.14
Khaltse	20	1.966±0.183	1.613±0.337	0.348±0.153	0.517±0.192	0.348±0.023	57	96.61
RAPD + ISSR								
Khardung-la	25	1.793±0.406	1.449±0.368	0.262±0.189	0.395±0.261	0.263±0.036	184	79.31
Chang-la	25	1.879±0.326	1.487±0.346	0.288±0.171	0.436±0.228	0.288±0.029	204	87.93
Khaltse	20	1.832±0.374	1.465±0.364	0.273±0.182	0.413±0.247	0.273±0.033	193	83.19

Na, Observed number of alleles; Ne, effective number of alleles; H, Nei's gene diversity; I, Shannon's Information index; Ht, total genetic diversity; Hs, genetic diversity in population; Gst, genetic diversity between population; NPL, number of polymorphic loci; PPL, percentage of polymorphic loci.

Table 5. Overall genetic variability across all the 70 genotypes of *Rhodiola* species based on RAPD, ISSR and RAPD+ISSR analysis.

Marker type	Na	Ne	H	I	Ht	Hs	Gst	NPL	PPL	Nm
RAPD	1.988 (0.107)	1.618 (0.288)	0.359 (0.132)	0.533 (0.164)	0.359 (0.017)	0.265 (0.015)	0.261	171	98.84	1.416
ISSR	2.000 (0.000)	1.645 (0.279)	0.371 (0.126)	0.548 (0.154)	0.372 (0.016)	0.302 (0.014)	0.184	59	100	2.204
RAPD+ISSR	1.992 (0.093)	1.626 (0.284)	0.363 (0.130)	0.538 (0.161)	0.363 (0.017)	0.275 (0.015)	0.241	230	99.14	1.574

Nm, Estimate of gene flow from Gst; Nm, 0.25 (1-Gst)/Gst; DI, diversity index; EMR, effective multiplex ratio; MI, marker index.

coefficient ranged from 0.53 to 1.0 (Figure 1b). The individuals were grouped into three major clusters (I to III). Where, cluster I represents the individuals from Khardung-la whereas; cluster II contains individuals from Chang-la. Khaltse individuals were clustered together in III. The results of PCA analysis were comparable to the cluster analysis.

Genetic variability details from random amplified polymorphic DNA + inter simple sequence repeat polymorphism (RAPD+ISSR) combined data

When H, I, Ht, Hs, NPL and PPL parameters were analyzed for site divisions, then most of the respective values were found higher for Chang-la individuals and least for Khardung-la individuals (Table 4). The details of overall genetic variability of 70 individuals were given in Table 5.

AMOVA helps in partitioning of the overall RAPD variations among populations and within the population. Molecular variance for among population (29.00%) and within the population (71.00%) indicated more variations within the population (Table 6).

The UPGMA dendrogram obtained from the cluster analysis of RAPD and ISSR combined data provided similar clustering pattern, with Jaccard's similarity coefficient ranging from 0.56 to 0.95 (Figure 1c). All the individuals were clustered into three major clusters (I to III) as grouped in RAPD and ISSR individual dendrograms, where cluster I represents individuals from Khardung-la, while cluster II contains all the individuals from Chang-la. Individuals from Khaltse were clustered in cluster III. Both RAPD and ISSR clusters showed complete similarity with combined data of RAPD+ISSR. The results of PCA analysis were comparable to the cluster analysis (Figure 2).

Table 6. Summary of nested AMOVA based on RAPD, ISSR individually and in combination, among the *Rhodiola* populations. Levels of significance are based on 1000 iteration steps.

Source of variation df Marker	Among population			Within population		
	2			67		
	RAPD	ISSR	RAPD+ISSR	RAPD	ISSR	RAPD+ISSR
S.S.D	21.366	141.874	709.387	37.920	625.540	2248.670
Variance component	0.436	2.654	13.833	0.566	9.336	33.562
Percentage	44	22	29	56	78	71
P-value	0.001	0.001	0.001	0.001	0.001	0.001

d.f., Degree of freedom; S.S.D, sum of square deviation; P-value, probability of null distribution.

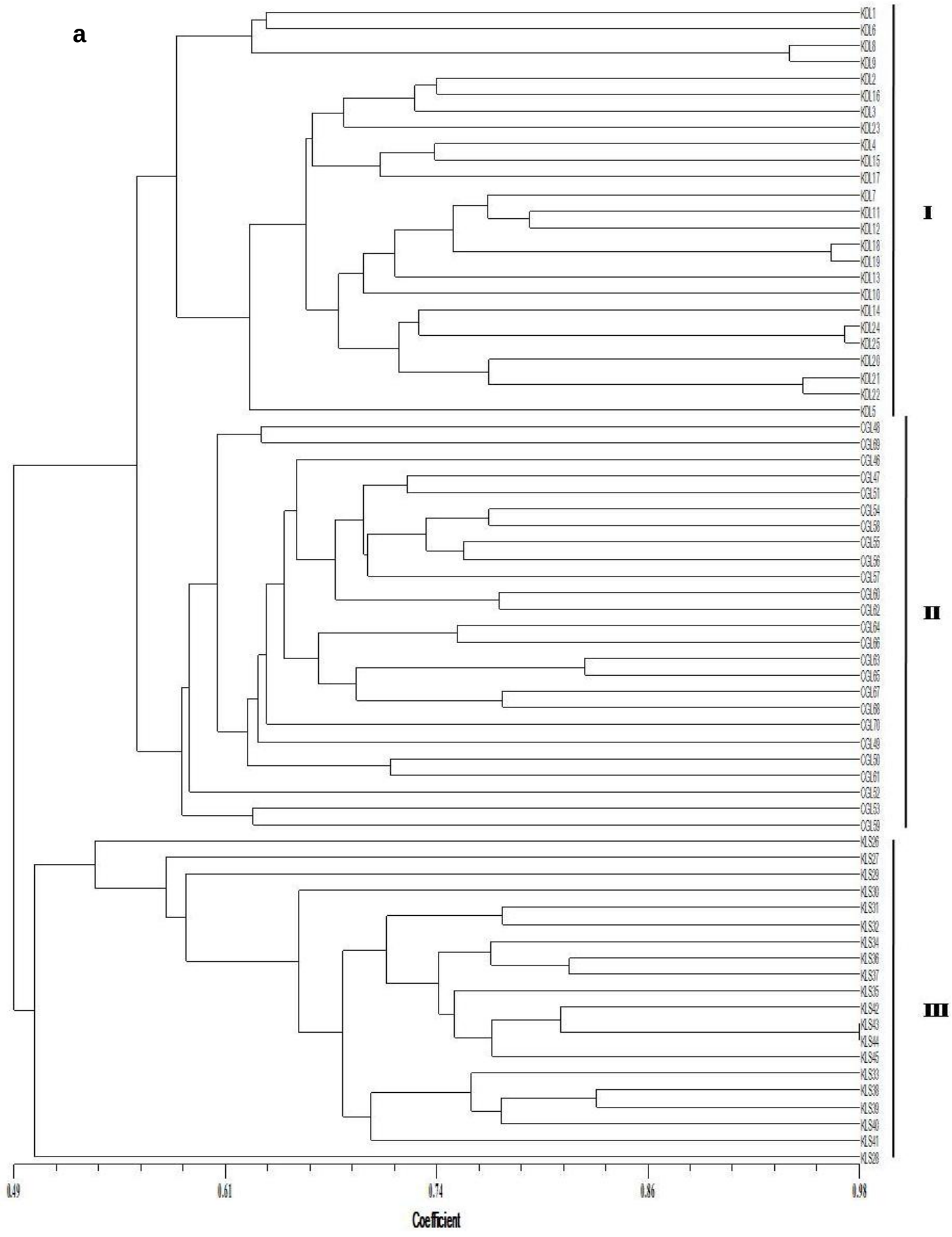
DISCUSSION

RAPD and ISSR markers were applied to characterize and compare genetic diversity among three populations of *R. imbricata*. All RAPD, ISSR and RAPD+ISSR combined analysis showed same results. RAPD markers were found little efficient with regards to polymorphism detection, as they detected 98.28% as compared to 97.55% for ISSR markers. But the diversity index, marker index, H, I, Ht and Hs are somewhat more for ISSR than for RAPD markers (Table 5). This is in agreement with the results as obtained for several other plant species like wheat (Nagaoka and Ogihara, 1997) and *Vigna* (Ajibade et al., 2000).

Dendrograms in the present study showed very clear pattern of clustering for among population samples as well as within population in all the three cases. Similar results were obtained in azukibean (Fernandez et al., 2002) and apricot (Kumar et al., 2009). The genetic closeness among the Khardung-la and Chang-la cultivars can be explained by the high degree of commonness in their individuals which is same as observed in blackgram (Gaffor et al., 2001). The genetic similarity of these individuals is probably associated with their similarity in the genomic and amplified region. The differences found among the dendrograms generated by RAPDs and ISSRs could be partially explained by the different number of PCR products analyzed (7743 for RAPDs and 2200 for ISSRs) reinforcing again the importance of the number of loci and their coverage of the overall genome, in obtaining reliable estimates of genetic relationships among *Rhodiola* cultivars as observed by Kumar et al. (2009) in Apricot.

AMOVA for RAPD, ISSR and RAPD+ISSR combined markers illustrated significant (p<0.001) genetic variation within population than among populations. In case of RAPD, 56.00% variation was found within population, whereas only 44.00% occur among population, molecular variance for ISSR showed 78.00% diversity within population while among popu-lation were 22.00% only and RAPD+ISSR combined data indicating 71.00% variability within population and only 29.00% among population. The occurrence of more genetic variance of *R. imbricata* within population as reported in our study is pattern often described for mountain plants (Cotrim et al., 2003), for endemic plants from Tibet (Chen et al., 2005) and from central Asian desert plants (GE et al., 2003) and several other studies of out crossing endemic species (Jaquemyn et al., 2004; Juan et al., 2004). Similar reports have been made in ISSR studies of populations of *R. crenulata* from Hengduan Mountain Region, China where the within population diversity was 55.14%, while among population diversity 44.8% (Lei et al., 2006). The genetic diversity analysis of *R. chrysanthemifolia* and *R. asia* from Tibetan Plateau using ISSR markers also reported more genetic variation between populations and less among populations (Xia et al., 2005, 2007) which may be due to severe isolation of populations. Yan et al. (1999) in *R. sachalinensis* observed that genetic diversity within population at high altitude was greater than that of populations at lower altitude. Same conclusion was drawn in the present investigation where, AMOVA analysis revealed that total variation within the population was maximum, which can be attributed to high inter-population gene flow.

In trans-Himalayan region of Ladakh, at more than 3000 m above mean sea level, there is an array of factors which leads to expectations on partitioning of total genetic variation of a plant species which deviates from the general pattern, such as short vegetation period (about 120 days), the low temperature, the insufficient content of oxygen and high ultraviolet (UV) radiations, so the chance of seeding recruitment may be a rather rare event. It is observed that, the geographical conditions were one of the most important factors for dispersal of seeds based on seed dispersal pattern and germination of *Rhodiola* at Ladakh and no regular dispersal pattern was found because of the effect of wind and habitats. We also found that populations from different sites are poorly differentiated. The most reasonable interpretation of these data a very short time has been available for the genetic divergence of the species *R. imbricata* after the uplift of trans-Himalayan range In conclusion, we can recommend that RAPD and ISSR markers were extremely constructive for studying the genetic relationships of *Rhodiola* individuals from the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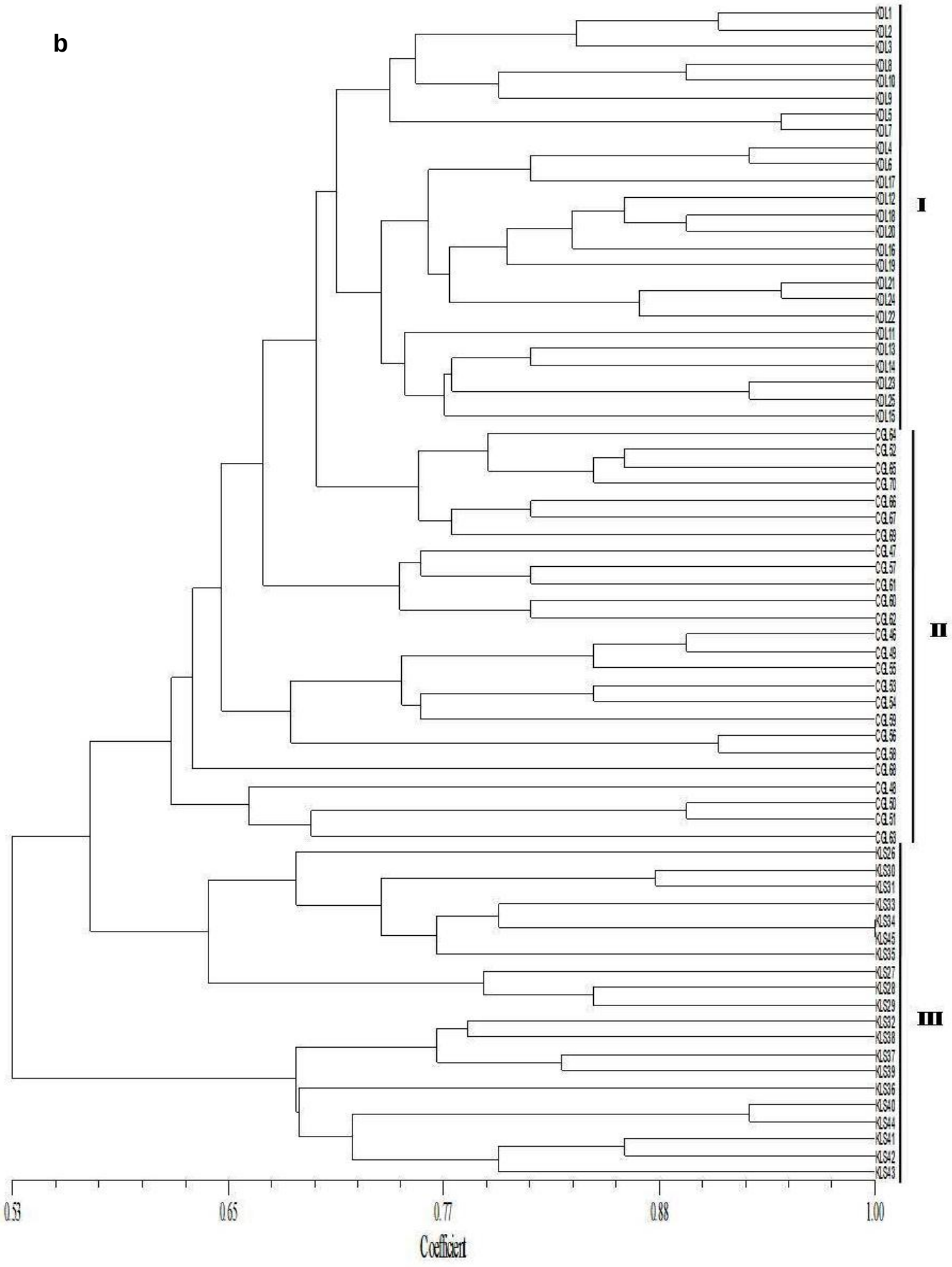
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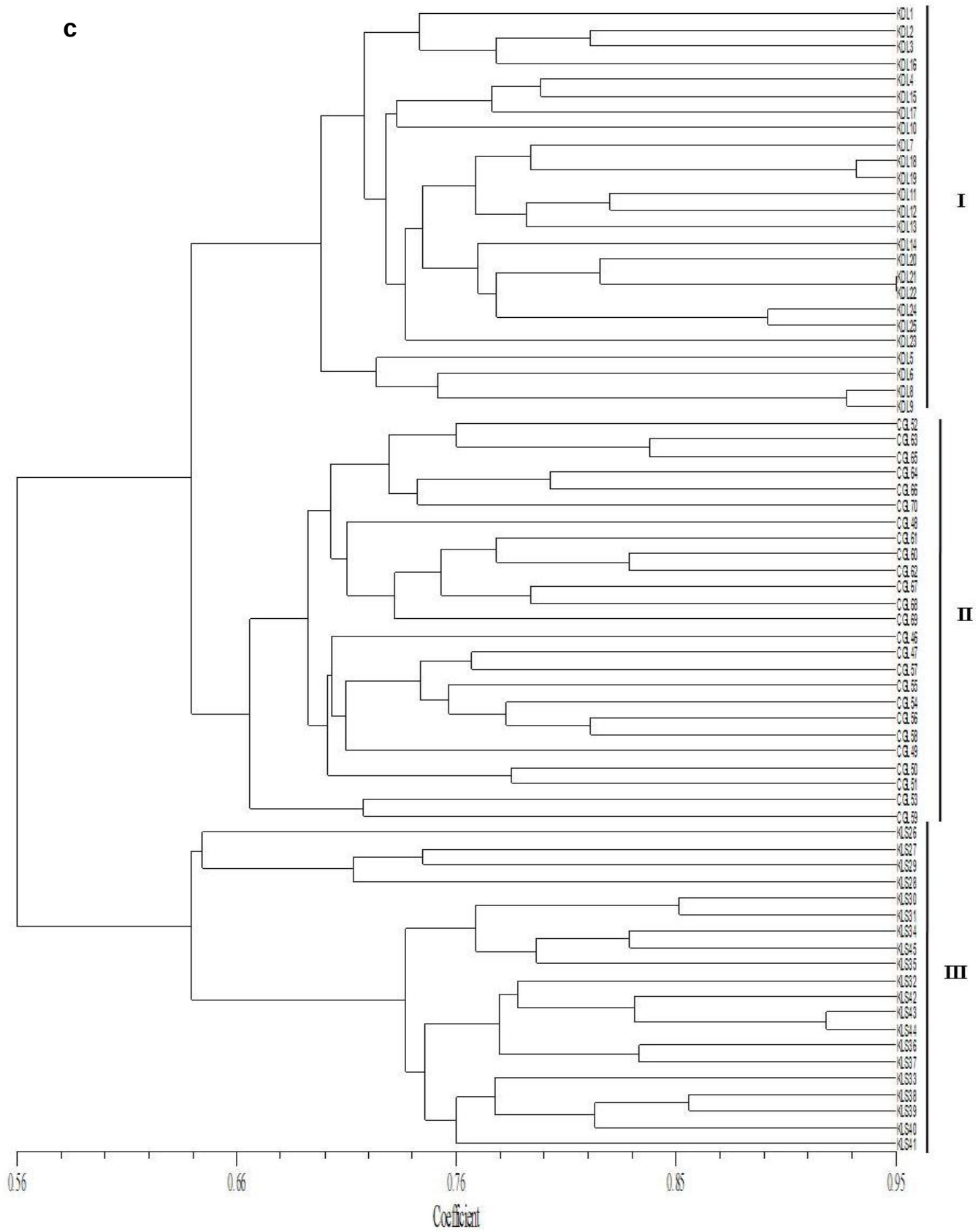

Figure 1. Dendrograms generated using UPGMA showing relationships between 70 *Rhodiola* individuals (a), RAPD; (b), ISSR; (c), ISSR+RAPD.

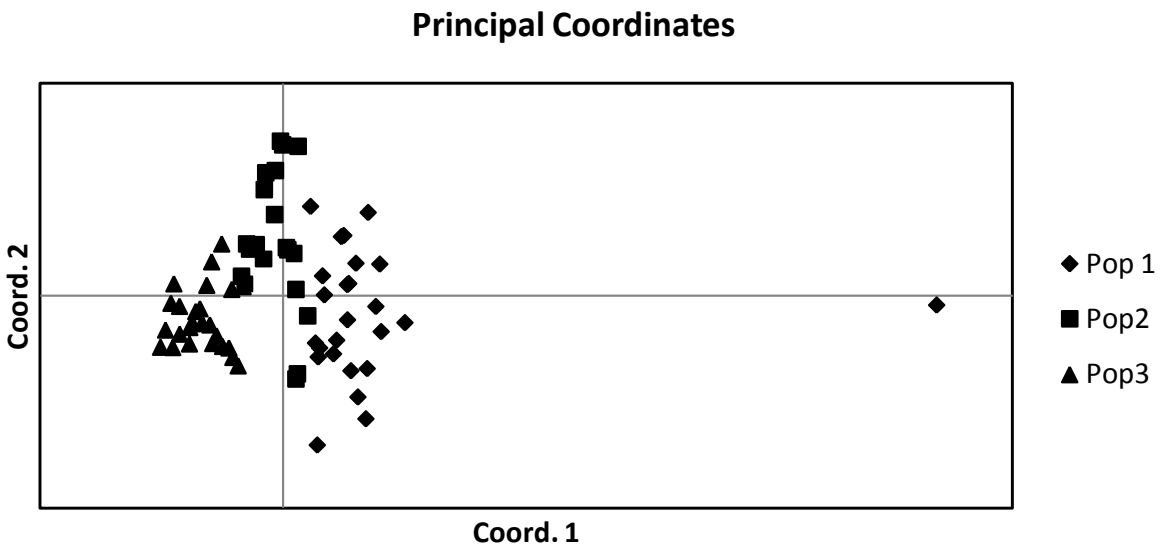

Figure 2. Two-dimensional plot of principle component analysis of seventy *R.imbricata* individuals using RAPD+ISSR analysis. The numbers plotted represents individual cultivars. Where, Pop 1, *Khardung-la*; Pop 2, *Chang-la*; Pop 3, *Khaltse*).

trans-Himalayan region of Ladakh. In the present study, Molecular variances for within population were more than among population, which implied the need to conservation of more individuals in any population. In addition, it would be wise to save populations in different regions in order to limit population decline caused by large scale environmental catastrophes.

Abbreviations: RAPD, Random amplified polymorphic DNA; **ISSR,** inter simple sequence repeat polymorphism; **AMOVA,** Analysis of molecular variance; **PCA,** principal coordinates analysis; **PCR,** polymerase chain reaction; **UPGMA,** unweighted pair-group method analysis; **H,** Nei's genetic diversity; **I,** Shannon's information index; **NPL,** number of polymorphic loci; **PPL,** percentage polymorphic loci; **Ht,** total genetic diversity.

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