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Inter and Intrapopulation Genetic Diversity of *Rauvolfia serpentina* (L.) Benth. ex Kurz, an Endangered Medicinal Plant, by RAPD Analysis

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ABSTRACT

The present study is the first report of inter and intrapopulation diversity analysis using RAPD markers in *Rauvolfia serpentina*, an endangered, commercially and medicinally very important plant, collected from six locations of Andhra Pradesh (AP), India. In the interpopulation analysis screening with forty primers revealed 263 scorable polymorphic markers out of the 379 total markers. A high proportion of polymorphism i.e., about 70% was found with 23 unique markers. Cluster analysis based on the Dice coefficient showed two major groups indicating that in cross-pollinated plants, a high level of differentiation occurs among existing accessions. The grouping of these accessions was independent of the geographical distance. The significant variation in the accession collected from Sukumamidi when compared to other accessions needs to be further investigated. Intrapopulation diversity in *R. serpentina* collected from Dulapally mostly exhibited monomorphism, which proved the maintenance of homogeneity. Hence the results of the present study can be seen as a starting point for future research on the population and evolutionary genetics of this species.

Keywords: conservation, genetic distance, genetic drift, polymorphism, Random Amplified Polymorphic DNA, variation
Abbreviations: AP, Andhra Pradesh; **CTAB**, cetyltrimethylammonium bromide; **GD**, genetic distance; **RAPD**, Random Amplified Polymorphic DNA

INTRODUCTION

Rauvolfia serpentina (L.) Benth. ex Kurz (Apocynaceae), commonly known as "Sarpagandha", one of the important medicinal plants is categorized as globally endangered (Jadhav *et al*. 2001), distributed in India, Malaysia, Sub-Himalayas, Pakistan, Bangladesh, Sri Lanka, Myanmar, Thailand and Java. In India it is present in moist deciduous forests in Andhra Pradesh (AP), Karnataka, Kerala and Tamil Nadu (Gupta 1986). It prefers tropical and subtropical areas for its natural growth. Overexploitation for comercial use is the major cause of threat, which has brought the species to the brink of extinction in the wild (Jain and Sastry 1980). Roots contain an active principle reserpine, an alkaloid that is medicinally very important apart from ajmaline and ajmalicine, which have been extensively researched in India, Europe and North America. The Ayurvedic preparations of *R. serpentina* are "Sarpagandha ghanavati", "Sarpagandha yoga", "Sarpagandha churna" and "Maheshvari vati", among others (Vaidya 2005).

To understand the effective management of plant genetic diversity from a conservation point of view it is essential to consider variation as richness and distribution at intra- and interspecific levels. Depending on the state of our heritable understanding of a taxon genetic diversity may be considered at different organizational levels: the genepool, population, individual genome, locus and DNA based sequence. RAPD markers have been employed as an alternative for morphological and biochemical markers in *Hordeum spontanum*, *Paeonia zuffruticoza* and *Dacydium pierrei* (Dawson *et al*. 1993; Yoon and Glawe 1993; Pei *et al*. 1995; Wolfe and Liston 1998; Su *et al*. 1999; Esselman *et al*. 2000). Both inter and intraspecific genetic diversity can be explained by calculation of polymorphism and displayed using a cluster diagram, which also provides equivalent levels of resolution for determining genetic relationships (Santo *et al*. 1994). Reliability of RAPDs among closely related taxa and the limitation of RAPD data for producing expected associations among more divergent taxa was observed in *Pisum* species (Hoey *et al*. 1996).

Although earlier studies on *R. serpentina* have reported results of research on phytochemical analysis and on micropropagation, studies on genetic diversity was unclear (Roja *et al*. 1984; Warzecha *et al*. 2000). Thus the present study is focused on determining the interpopulation diversity between six accessions of *R. serpentina* collected from AP and on the intrapopulation diversity of plants collected from Dulapally (Medak District) to check the level of homogeneity. An analysis of such variation may help to preserve the biodiversity of *R. serpentina* in AP, which is under threat and also will provide a framework for future efforts to incorporate wild germplasm into rootstock breeding and hybridization programmes.

MATERIALS AND METHODS

Plant source

Plants of *R. serpentina* were collected from six different locations in AP, which include Maredumilli (RSMI-East Godavari), Araku (RSAR-Vishakapatnam), Mallur (RSMA-Warangal), Dulapally (RSDY-Medak), Hyderabad (RSHC-Hyderabad) and Sukumamidi (RSSI-Khammam) (**Fig. 1**). The samples were collected at random. The latitudes and longitudes of each location are mentioned in **Table 1**. For intrapopulation studies, eight plants (RS1, RS2, RS3, RS4, RS5, RS6, RS7 and RS8) from Dulapally were selected for analysis. The samples were collected not singly but rather in bulk so that DNA samples could be pooled at the end and repeated three times for data consistency. District names are mentioned in the parenthesis. Locations are further mentioned in the form of abbrev-

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iations. As the plant is endangered and distributed in very few locations as collected, the accession number could not be confirmed.

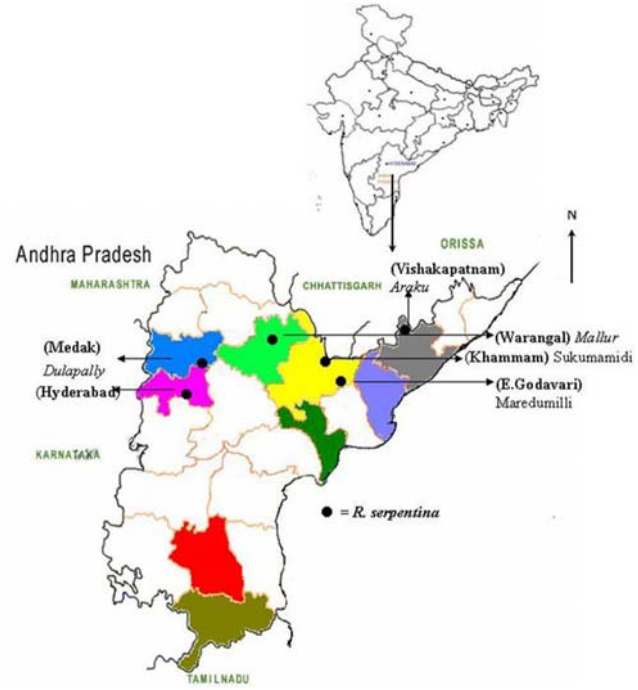

Fig. 1 Various locations in Andhra Pradesh from where the germplasm of *Rauvolfia serpentina was* collected.

Table 1 *Rauvolfia serpentina* germplasm accessions collected from different locations of AP used for molecular diversity analysis.

Accession code	Location	District	Latitude	Longitude
RSMA	Mallur	Warangal	18° 15′	80° 31′
RSAR	Araku	Vishakapatnam	19° 08′	19° 08′
RSHC	Hyderabad	Hyderabad	27° 20′	78° 20′
RSDY	Dulapally	Medak	27° 20′	78° 20′
RSSI	Sukumamidi	Khammam	17° 45′	81° 50′
RSMI	Maredumilli	East Godavari	19° 35′	80° 48′

Genomic DNA isolation

DNA was isolated by using the CTAB (Sigma Aldrich, USA) protocol developed by Doyle and Doyle (1987) with slight modifications. Young leaf tissue (3 g) was ground into a fine powder in liquid nitrogen along with 0.1 g of polyvinylpolypyrrolidone (PVPP) (Sigma Aldrich, USA) and transferred to preheated extraction buffer (2% CTAB, 100 mM Tris HCl (Qualigens Chemicals, India), pH 8.0, 20 mM EDTA (Qualigens Chemicals, India), 1.4 M NaCl (Qualigens Chemicals, India) containing 10 mM β-mercaptoethanol (Qualigens Chemicals, India) per gram of tissue. The slurry was incubated for 90 min at 65°C in a water bath. An equal volume of chloroform:isoamyl alcohol (24:1) (Qualigens Chemicals, India) was added to the extract prior to centrifugation at 12,000 rpm for 15 min. To the supernatant equal volumes of ice-cold isopropanol (100%) was added and incubated at –20°C for a period of minimum 30 min followed by centrifugation at 12,000 rpm for 15 min. The pellet was washed with 70% ethanol, centrifuged at 10,000 rpm for 8 min. The pellet was dried and redissolved in 100 µl of Tris EDTA (TE) buffer. In order to eliminate RNA contamination the sample was treated with 5.0 µl of RNase A (10 µg/µl) incubated at 37°C for minimum of 3 hrs or overnight. This was followed by phenol:chloroform:isoamyl alcohol (25:24:1, v/v/v) extraction by centrifuging at 8000 rpm for 15 min. To the supernatant equal volumes of chloroform:isoamyl-alcohol (24:1) was added and centrifuged at 12,000 rpm for 15 min. To the supernatant $1/10^{th}$ volume of 3 M sodium acetate and equal volumes of ice-cold isopropanol was added and left for 30 min or overnight at –20°C to precipitate DNA followed by centrifugation at 12,000 rpm for 15 min. The DNA was washed by adding 70% ethanol and centrifuged at 10,000 rpm for 10 min at 4°C. After complete drying the pellet was dissolved in TE buffer and was stored at 4°C for future use.

Qualitative and quantitative extraction of DNA

To test the quality of DNA the OD values were recorded at 260 and 280 nm and the ratio of OD_{260} to OD_{280} was calculated to check the purity of each DNA sample that should be between 1.6-1.8. Further purity of DNA was tested by gel electrophoresis using 0.8% TBE-agarose (Himedia Chemicals, India; Sigma Aldrich, USA). Gels were stained with ethidium bromide and viewed on a UV transilluminator, photographed with the help of a gel documentation system (LTF Labortechnik, Germany). The concentrated DNA was diluted with Ultrapure Milli Q (Milli Q academic, USA) sterile water to 50 ng/µl (Sambrook *et al*. 1989).

RAPD Analysis

Forty decamer primers of arbitrary sequence (Kits OPA and OPC provided by Operon Technologies Inc, Alameda, CA) were tested for PCR amplification among six accessions of *R. serpentina* to check the interpopulation diversity whereas intrapopulation diversity of eight plants of *R. serpentina* collected from Dulapally was carried out by using a total of 14 primers belonging to OPA and OPC series. PCR reactions were carried out in a DNA Thermocycler (MJ Research Inc., USA). PCR conditions and the programme were followed according to Padmalatha and Prasad (2006). Negative controls were also run without template DNA to ensure amplification. PCR products were electrophoresed on 2% (w/v) agarose gels, in 1× TBE Buffer at 50 V for 3 hrs and then stained with ethidium bromide (0.5 µg/ml). Gels with amplification fragments were visualized and photographed under UV light. Lambda DNA *Eco*RI *Hind*III double digest was used as a molecular marker (Bangalore Genei, New Delhi, India) to identify the fragment sizes.

Data scoring and analysis

PCR reactions and electrophoresis were repeated thrice to ascertain the reproducibility of the bands. For each accession, polymorphism was scored as 1 for the presence and 0 for absence of a band. RAPD data generated with forty primers were used to compile a binary matrix for cluster analysis using the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System, Bio-statistics, New York, USA, software version 2.02j package; Rohlf 1998). The Dice coefficient was used to construct a dendrogram using the UPGMA (Unweighted Pairwise Group Method with Arithmetical average) through NTSYS-package.

RESULTS

Analysis of six accessions of *R. serpentina* revealed 70% polymorphism. The number of scorable polymorphic markers generated was 263 out of 379 total markers (**Table 2**). High polymorphism i.e., 100% was observed with 16 primers and monomorphism with 2 primers except in the accession collected from Sukumamidi, which was found to be a variant when compared to the other accessions (**Fig. 2**). Levels of genetic diversity within accessions i.e., the genetic distance (GD) ranged from 0.596 to 0.928. The minimum GD of 0.596 was exhibited between the accessions collected from Sukumamidi and Maredumilli, whereas the accessions that exhibited a maximum GD of 0.928 belonged to plants collected from Dulapally and Hyderabad. The mean value of GD among the accessions was 0.820. The accessions (**Fig. 3**) grouped together following cluster analysis irrespective of the geographical distances were those collected from Araku and Hyderabad, and showed a GD of 0.905 (**Fig. 4**). The primer with the maximum and minimum number of polymorphic bands, respectively was OPA-18 (17 bands) and OPA-19 (1 band) and the primer which exhibited monomorphism was OPC-07. The range of the number of bands generated for all the primers falls between 0-17. The GC% of all the primers ranged from 60-70%, and did not show any effect on amplification. The average number of poly-

Table 2 Polymorphism among six accessions of *R. serpentina*.

Primer Code	Primer sequence (5′-3′)	Total Bands	Polymorphic Bands	% Polymorphism
OPA-01	CAGGCCCTTC	6	6	100
OPA-02	TGCCGAGCTG	2	2	100
OPA-03	AGTCAGCCAC	16	16	100
OPA-04	AATCGGGCTG	9	9	100
OPA-05	AGGGGTCTTG	12	12	100
OPA-06	GGTCCCTGAC	12	5	41.6
OPA-07	GAAACGGGTG	18	16	88.8
OPA-08	GTGACGTAGG	11	11	100
OPA-09	GGGTAACGCC	11	11	100
OPA-10	GTGATCGCAG	7	7	100
OPA-11	CAATCGCCGT	8	8	100
OPA-12	TCGGCGATAG	8	8	100
OPA-13	CAGCACCCAC	14	13	92.8
OPA-14	TCTGTGCTGG	11	11	100
OPA-15	TTCCGAACCC	7	7	100
OPA-16	AGCCAGCGAA	6	6	100
OPA-17	GACCGCTTGT	8	8	100
OPA-18	AGGTGACCGT	17	17	100
OPA-19	CAAACGTCGG	7	1	14.2
OPA-20	GTTGCGATCC	11	2	18.1
OPC-01	TTCGAGCCAG	8	7	87.5
OPC-02	GTGAGGCGTC	14	13	92.8
OPC-03	GGGGGTCTTT	12	4	33.3
OPC-04	CCGCATCTAC	6	3	50
OPC-05	GATGACCGCC	11	7	63.6
OPC-06	GAACGGACTC	11	4	36.3
OPC-07	GTCCCGACGA	6	0	0
OPC-08	TGGACCGGTG	13	4	30.7
OPC-09	CTCACCGTCC	5	3	60.0
OPC-10	TGTCTGGGTG	6	6	100
OPC-11	AAAGCTGCGG	12	6	50.0
OPC-12	TGTCATCCCC	9	4	44.4
OPC-13	AAGCCTCGTC	9	4	44.4
OPC-14	TGCGTGCTTG	11	7	63.6
OPC-15	GACGGATCAG	8	3	37.5
OPC-16	CACACTCCAG	7	2	28.5
OPC-17	TTCCCCCCAG	2	0	0
OPC-18	TGAGTGGGTG	8	3	37.5
OPC-19	GTTGCCAGCC	7	2	28.5
OPC-20	ACTTCGCCAC	13	7	53.8

morphic bands generated per primer was 6.5 out of the total number of bands of 9.4. The primers OPA-01, OPA-02, OPA-03, OPA-04, OPA-05 OPA-08, OPA-09, OPA-10, OPA-11, OPA-12, OPA-14, OPA-15, OPA-16, OPA-17, OPA-18 and OPC-10 exhibited 100% polymorphism with all the accessions. The size of the amplified fragments ranged from 300-3500 bp.

Intrapopulation diversity of *R. serpentina* collected from Dulapally

Analysis of eight plants of *R. serpentina* (Dulapally) revealed 77% polymorphism. The number of scorable polymorphic markers generated was 86 out of 111 total markers (**Table 3**) and individual plant specific polymorphism was observed. The levels of genetic diversity within eight different plants i.e., the GD, ranged from 0.604 to 0.895 (**Figs. 5, 6**). A minimum GD of 0.604 was exhibited between the plants RS1 and RS4 whereas a maximum GD of 0.805 was found between RS1 and RS3. The mean value of GD among the plants is 0.77. The primer with the maximum and minimum number of polymorphic bands was OPA-10 (13 bands) and OPA-6 (3 bands). Hence the range of the bands generated for all the primers fell between 3 and 13. The average number of polymorphic bands per primer generated was 6.1 out of the total number of bands i.e., 7.9. The primers that exhibited 100% polymorphism were OPA-1, OPA-10, OPA-13, OPA-14, OPC-4, and OPC-19. The size of the amplified fragments ranged from 300 to 3500 bp.

Table 3 Intrapopulation variation in eight plants of *R. serpentina* collected from Dulapally, Ranga Reddy District of Andhra Pradesh.

Primer code	Primer sequence (5′-3′)	Total Bands	Polymorphic Bands	% Polymorphism
OPA-01	CAGGCCCTTC	7	7	100
OPA-02	TGCCGAGCTG	9	4	44.4
OPA-03	AGTCAGCCAC	9	4	44.4
OPA-06	GGTCCCTGAC	9	3	33.3
OPA-09	GGGTAACGCC	6	3	50
OPA-10	GTGATCGCAG	13	13	100
OPA-11	CAATCGCCGT	6	5	83.3
OPA-13	CAGCACCCAC	12	12	100
OPA-14	TCTGTGCTGG	7	7	100
OPC-02	GTGAGGCGTC	2	0	0
OPC-03	GGGGGTCTTT	6	4	66.6
OPC-04	CCGCATCTAC	7	7	100
OPC-16	CACACTCCAG	7	6	85
OPC-19	GTTGCCAGCC	11	11	100

DISCUSSION

Estimating the genetic differentiation coefficient among populations using RAPDs has been problematic due to their dominance, and analytical methods usually rely on knowledge of the selfing rate or assume Hardy-Weinberg equilibrium (Lynch and Milligan 1994). This assumption does not hold when accessions of many plant species exhibit fixed heterozygosity, hence in our studies an alternative method i.e., the Dice coefficient (Dice 1945) was used to partition the genetic diversity which is supposed to be in accordance with the RAPD data which was found to be consistent in showing the variation within accessions by the aid of a dendrogram obtained after analysis.

The genetic diversity of *R. serpentina* (plant species) could be partly explained as a result of abiotic (geographical, e.g., hydrographic connections or climactic differentia-

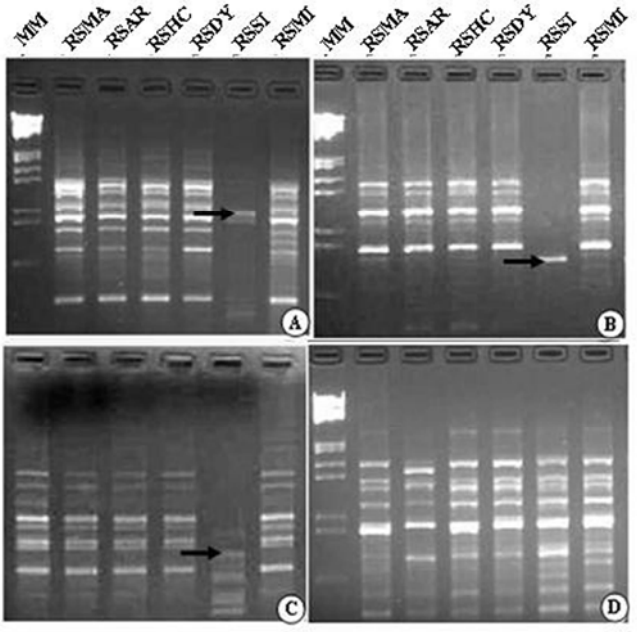

Fig. 2 (**A**) RAPD profile using primer OPA-03 (5′-AGTCAGCCAC-3′). Monomorphic markers are generated with all the accessions of *R. serpentina* except in the lane Si (RSSI), where an accession-specific diagnostic marker was generated, as indicated by an arrow. (**B**) RAPD profile using primer OPA-08 (5′-GTGACGTAGG-3′). Monomorphic markers were generated with all the accessions of *R. serpentina* except in the lane Si (RSSI), where an accession-specific diagnostic marker was generated, as indicated by an arrow. (**C**) RAPD profile using primer OPA-14 (5′-TCTGTGCTGG-3′). Monomorphic markers were generated with all the accessions of *R. serpentina* except in the lane Si (RSSI), where polymorphic markers are depicted as indicated by the arrow. (**D**) RAPD profile using primer OPC-20 (5′-ACTTCGCCAC-3′). Polymorphic markers were generated in *R. serpentina* among all the accessions.

	1	2	3	4	5	6
RSMR	1.00					
RSAU	0.90	1.00				
RSHC	0.90	0.90	1.00			
RSDY	0.86	0.90	0.92	1.00		
RSSI	0.59	0.64	0.63	0.61	1.00	
RSMI	0.86	0.87	0.88	0.87	0.61	1.00

Fig. 3 Similarity matrix of interpopulation studies in *R. serpentina* generated from the Dice estimate of similarity based on the number of shared fragments.

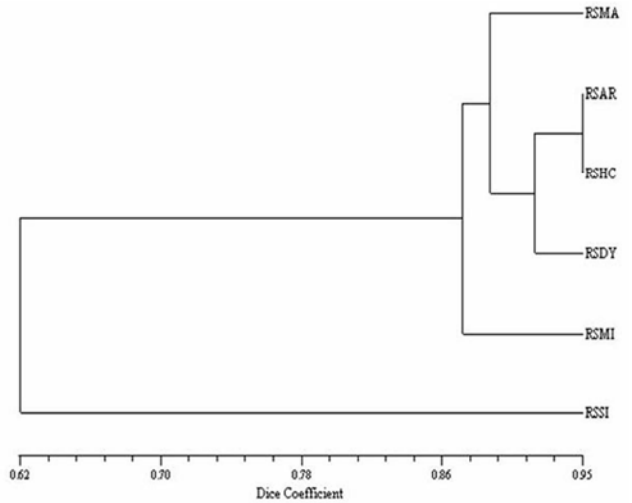

Fig. 4 Cluster diagram of interpopulation studies (six accessions) of *R. serpentina* based on Dice's genetic identity.

	1	2	3	4	5	6	7	8
RS1	1							
RS2	0.827	1						
RS3	0.895	0.923	1					
RS4	0.604	0.713	0.673	1				
RS5	0.821	0.810	0.824	0.666	1			
RS6	0.786	0.858	0.858	0.703	0.802	1		
RS7	0.733	0.830	0.831	0.709	0.784	0.873	1	
RS8	0.658	0.781	0.746	0.758	0.717	0.717	0.8082	1

Fig. 5 Similarity matrix of intrapopulation studies in *R. serpentina* generated from the Dice estimate of similarity based on the number of shared fragments (Dulapally - RSDY).

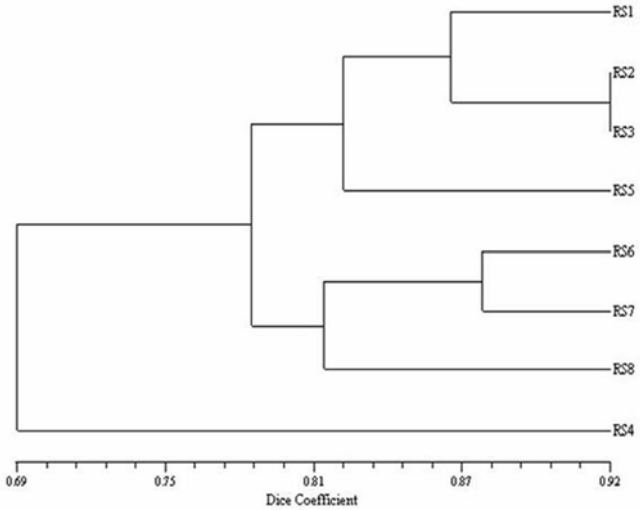

Fig. 6 Cluster diagram of intrapopulation studies in *R. serpentina* based on Dice genetic identity (Dulapally - RSDY).

tion, e.g. annual rainfall differences) and biotic (pollination, seed dispersal, etc.) factors, sampling frequency, pollen and gene flow, genetic drift, local selection pressures, obligate outcrossing, inbreeding depression and strict self-incompatibility which is reported accordingly in many other plant species (Wolff *et al*. 1994; Apostol *et al*. 1996).

As interpopulation studies showed an overall percentage of polymorphism i.e., 70 % and 100% polymorphism with 16 primers, suggests that sampling from a few localities for either breeding or conservation could capture a large proportion of the variation within the species. The range of genetic diversity calculated in terms of GD was 0.596-0.928, evidence that the accessions from different geographical locations exhibited a wide range of GD which did not show any correlation with geographical distances between the collection sites, negating a simple isolation by distance mode.

In the dendrogram the accession collected from Sukumamidi showed 62% genetic similarity with other accessions and a considerable degree of variation was observed from the distinctive banding pattern and also the separate grouping in the dendrogram (**Figs. 2, 4**) which has to be investigated further. Accessions from Araku and Hyderabad showed a 95% similarity though they were geographically very distant. A similar association has been found previously in *Hordeum spontaneum* populations by Dawson *et al*. (1993). This situation arises only in the case of natural populations of many cross-pollinated plant species where there is a free/random pollen flow and fertilization. On the other hand the vast genetic variation may serve for its evolution. Pollen can be dispersed over large distances and this long-term reciprocal movement of pollen must also have contributed to the variation. Experiments using pollen traps have shown that oak pollen can migrate over several kilometers (Lahtinen *et al*. 1996).

It can be inferred that in the accessions, which are clustered in similar groups, there is an effective gene flow in those locations whereas in the accession collected from Sukumamidi, which may be a variant, the gene flow is less and hence they are extremely divergent, comparatively speaking (**Figs. 3, 4**). This may be due to the highly cross-pollinated nature of the plant which results in higher levels of heterozygosity and occurrence of some mutations which may include a base change, deletions and insertions within the priming site sequence that changes the size of a DNA fragment without preventing its amplification (Williams *et al*. 1990; Wolff *et al*. 1994).

Gene flow in higher plants is accomplished by dispersal of seeds and pollen as well as by vegetative mobility Gene flow by pollen dispersal is often low in herbaceous plants (Handel 1985; Parker and Hamrick 1992). In *R. serpentina* none of the accessions collected for our study have less than 50 km distance between them. Hence, the genetic structure of any of these accessions is stable and free from any gene flow into them. Thus there is a wide range of genetic differentiation. The genetic variation is related to the distances of pollen and seed dispersal. As *R. serpentina* is insect pollinated there are more chances of dispersal resulting in a broad range of variations. The gene flow homogenizes population structure and counteracts the effects of drift and diversifying selection. It may also be detrimental to small populations because under certain conditions it may reduce local variation, prevent local adaptive differentiation and reduce fitness. Thus populations can undergo genetic differentiation from one generation to another (Slatkin 1987). Further amplification of such cross-hybridized seeds through dissemination by natural modes like wind is possible. This is probably the reason why accessions are closely related at the genetic level, although, are geographically from distinct locations of AP. Results from RAPD analysis indicates that a drastic genetic drift might have occurred among the accessions thereby producing differentiation among accessions. The main reason i.e., human activity that damaged their habitats and their excessive use for medicine make the species population

decrease in size and habitat. With a larger population area, the probability of crossing among the individuals increases, which results in the retention of genetic variation. Though many individuals of these species were reported earlier (Pullaiah and Chennaiah 1998), they gradually disappeared in due course along with environmental changes in their habitat. For decades much attention has focused on the genetic risks associated with small population size not only from inbreeding and genetic drift but also from gene flow. Local selection pressures may be due to the effects of environmental factors and due to a struggle for existence in nature. The widespread occurrence of wind pollination and breeding systems that promotes out-crossing may have led to a higher genetic diversity.

Apart from genetic drift, inbreeding depression may also be one of the factors which may lead to genetic variation (Sherwin and Moritz 2000). An understanding of these genetic processes is required in order to fully evaluate the consequences of fragmentation and its relationship to genetic variation. One would therefore expect rare and endangered species of a small population size to be genetically depauperate and often be associated with increased inbreeding and genetic drift, processes that lead to loss of genetic variation (Gaston and Kunin 1997a; Loeffler and Morden 2003).

Although the results of the present study provide evidence for genetic loss, information is required on the implications of reduced genetic variation for survival and fertility. The high degree of genetic variation or differentiation recorded by the transfer of germplasm between different locations should be avoided, to ensure that the genetic material is adapted to local conditions (Ennos 1998). The genetic analyses presented here could be used for the development of conservation strategies for the species**,** for example through the definition of appropriate units of management (Newton *et al*. 1999). In the case where gene flow between neighboring populations is not limited, populations of longer geographical distances will generally show greater diversification.

It is believed that mutations, genetic drift due to a finite population size and natural selection will lead to the genetic diversification of local populations and that the movement of gametes and individuals (gene flow) will oppose that diversification. The lack of gene flow and the effect of genetic drift due to restricted population size might have caused the accessions of selected species to differentiate genetically among themselves.

Additional information is also required on the patterns of variation in quantitative genetic traits, on which plans for conservation action should ideally be based (Ennos 1998). The probability of detection reveals how the alleles are associated to make up the diploid genotypes. For a given number of alleles at given frequencies, the probability of detection is minimal under complete homozygosity, since at most times a new allele can be found per homozygous individual, as opposed to two in a heterozygous one (Gregorius 1980). As stated earlier due to the unpredictable level of homozygosity and heterozygosity all accessions were collected at random. Alternatively, if wild populations exist as heterogeneous mixtures of several inbreds, genetic erosion in the gene bank could also occur.

In *R. serpentina* substantial heterozygosity is present which may be lost over time. In studying genetic diversity it is desired to maximize the preservation of alleles. Geographical, climatic or reproductive variables explain the partitioning of the diversity observed which may aid in improving the strategies for maximizing the efficiency of germplasm collection and preservation. Diploidy and self-incompatibility presumably generate high levels of heterozygosity and therefore greater vulnerability to loss of diversity in plants (Loveless and Hamrick 1984).

Therefore the differences found in the dendrogram could be partially explained by different number of PCR products analyzed reinforcing the number of loci and their coverage of the overall genome, in obtaining reliable estimates of genetic relationships among the plant species of *R. serpentina* (**Figs. 3, 4**).

Intrapopulation variation in *R. serpentina* collected from Dulapally

Intrapopulation diversity in *R. serpentina* showed that one of the plants was highly variant and fell into an entirely different group, which may be due to the highly cross-pollinated nature of that particular plant; there might also be few internal rearrangements occurring in the genome. The monomorphism exhibited by different plants collected from the same location indicates the occurrence of self-pollination in all the plants where homogeneity is being maintained. When intrapopulation variation analysis was carried out, one of the plants was found to have a very low level of genetic variation, presumably as a consequence of the techniques applied to seed production, responsible for genetic drift (**Table 3**).

CONCLUSIONS

R. serpentina populations are seen to disappear completely for periods of time only to be replaced by large numbers of vigorous new recruits presumably from buried seed reserves. Extant living plants are thus only part of the gene pool and a large genetic reserve lies in buried seed banks, the size and long term viability of which is unknown. This is not restricted to the study taxa but is a feature of many rare and restricted populations of *R. serpentina.* There are many natural stands which are being lost due to many factors, therefore efforts should be made to conserve the remaining stands mostly under *ex situ* conditions. This stresses the need to address breeders to apply appropriate techniques for seed sampling. It also underlines the need for further monitoring of the genetic and demographic status of populations, if they decrease too much in size, they will become critically stochastic events. However a detailed study is desirable to understand all the aspects related to variations. Hence further investigation is required on patterns of gene flow within and between populations, and its effects on reproductive and demographic processes, to be able to assess its impact on population viability.

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