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Genetic diversity and structure of the threatened anti-cancerous plant *Nothapodytes nimmoniana* as revealed by ISSR analysis

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Abstract

Inter simple sequence repeat markers were used to assess the genetic diversity and population genetic structure in 12 populations of *Nothapodytes nimmoniana* from Western Ghats of India. A total of 16 selected primers produced 103 discernible bands, with 76 (73.7%) being polymorphic. The Nei's gene diversity (*h*) ranged from 0.1166 to 0.2124, with an average of 0.1518 at the population level and 0.2965 at the species level indicating high genetic diversity. The Shannon's index (*I*) was estimated to be 0.2189 within populations (range 0.1703–0.2947) and 0.4352 at the species level. The analysis of molecular variance showed that the genetic variation was found mainly within populations (73%), but variance among populations was only 27% and its value, $\Phi_{PT} = 0.271$, $P < 0.001$, implied that high genetic differentiation among populations. In addition, Nei's differentiation coefficient (G_{ST}) was found to be high (0.4882) and the gene flow (N_m) was low (0.5242), confirming the high population genetic differentiation. The unweighted pair-group method using arithmetic average clustering elicited similar results. Based on this, we propose conservation strategy for this plant species.

Keywords: anti-cancer; camptothecin; genetic differentiation; genetic diversity; ISSR; *Nothapodytes nimmoniana*; population structure; Western Ghats

Introduction

Over the long term, the ability of a population to respond adaptively to environmental changes depends on the level of genetic variability or diversity that it contains (Ayala and Kiger, 1984). Understanding the relative importance of processes that structure diversity within and among populations can provide both a means to assess future risk of erosion of diversity and a means to design effective conservation strategies for rare taxa (Neel and Ellstrand, 2003). Information on genetic diversity pattern of economically important, rare and threatened plant species is a prime concern to develop strategies for sustainable harvesting of secondary metabolites and, where appropriate, reintroduction to its natural habitat (Neel and Cummings, 2003). *Nothapodytes nimmoniana* Graham (Icacinaceae) syn. *Mappia foetida* Miers is one such threatened species having great medicinal value.

Nothapodytes nimmoniana, commonly known as 'stinking tree', is a medium-sized tree distributed in

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the evergreen, semi-evergreen and deciduous forests of Western Ghats of India, North East India, Srilanka, Myanmar and Thailand (Hombegowda *et al.*, 2002). It is a rich source of anti-cancer alkaloids camptothecin (CPT) and 9-methoxy CPT (9-mCPT) (Govindachari and Viswanathan, 1972). CPT is regarded as one of the most promising anti-cancer drugs of the 21st century (Li and Adair, 1994). It is shown to be effective in the complete remission of lungs, breast (Takeuchi *et al.*, 1991) and uterine cervical cancers (Potmesil, 1994). Its primary cellular target is topoisomerase I, and the anti-tumour activity is attributed to the inhibition of topoisomerase I (Hsiang *et al.*, 1985). Irinotecan and Topotecan, two water-soluble derivatives of CPT, have been approved by the FDA of the USA for treating colorectal and ovarian cancers (Vladu, 2000). In recent years, because of the enormous demand for the chemical worldwide, there has been an indiscriminate felling of *N. nimmoniana* from Western Ghats. In fact, it is estimated that in the last decade alone, there has been at least a 20% decline in the population leading to the red listing of the species as vulnerable (Ved, 1997; Ravikumar and Ved, 2000). The species is now confined only to the remnant of forest pockets. Thus, an understanding of the genetic diversity and structure of *N. nimmoniana* is important for developing effective conservation strategies for this species in Western Ghats of India, which has not been investigated so far.

DNA markers are able to detect the genetic variation beyond coding loci and to provide broader information on the amount of genetic variation and the genetic divergence among populations (Winter and Kahl, 1995). Dominantly expressed multilocus DNA markers such as inter simple sequence repeat (ISSR) have been successfully used to provide valuable information about the genetic diversity and structure of natural plant populations including medicinal plants (Souza and Lovato, 2010; Wu *et al.*, 2010). It amplifies DNA sequence between the two adjacent, inversely oriented repeats of microsatellites (Zietkiewicz *et al.*, 1994). In comparison with molecular assays such as amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism, ISSR is cost-efficient, overcomes hazards of radioactivity and requires lesser amounts of DNA (25–50 ng). Furthermore, ISSR markers have higher reproducibility than random amplified polymorphic DNAs (RAPDs) (Meyer *et al.*, 1993; Fang *et al.*, 1997), are more informative, (Nagaoka and Ogihara, 1997), require no prior sequence information and hence were the choice markers for this study. This study consists of exploration and collection of *N. nimmoniana* from Western Ghats of India, mapping its distribution using DIVA GIS software (Hijmans *et al.*, 2005) and analysing its population genetic diversity and genetic structure using ISSR markers.

Materials and methods

Exploration and collection

An extensive floristic survey was conducted to collect *N. nimmoniana* from Western Ghats of India. The study area covered four states of South India, namely Kerala, Tamil Nadu, Karnataka and Maharashtra and 12 populations. The geographic location data (latitude, longitude and altitude) for 57 accessions from the 12 populations of *N. nimmoniana* were collected using global positioning system. The latitudes of the collection sites ranged from 09°34′39.2″ to 16°48′26.0″ and the altitudes ranged from 243 m (Charmadi Ghat, Karnataka) to 2169 m (Ooty, Tamil Nadu). Details of the accessions collected and analysed were given in Table 1. DIVA GIS software was used to map geographical distribution of the species (Fig. 1).

DNA extraction and ISSR-PCR analysis

Total genomic DNA was isolated from the fresh leaves (0.5 g) of *N. nimmoniana* using modified cetyltrimethyl ammonium bromide method (Doyle and Doyle, 1987). Quantity and quality of the DNA samples were estimated by comparing band intensities on a 0.8% agarose gel and using a spectrophotometer. The components of the ISSR-PCR were optimized with varying concentrations of template DNA, dNTPs, Taq DNA polymerase and annealing temperature. The reaction was performed in a volume of 10 µl containing 50 ng template DNA, 0.5 mM dNTPs (Chromous Biotech, Bangalore, India), 0.15U Taq DNA polymerase (Chromous Biotech), 0.5 mM ISSR primers of UBC set # 9 (The Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada) and 1X PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, 3 mM $MgCl_2$) (Merck, Darmstadt, Germany). A total of 16 reproducible primers were selected from 100 ISSR primers for this study (Table 2). A control PCR tube containing all components but no genomic DNA was run with each primer to check any contamination. The reactions were carried out in a DNA thermocycler (Eppendorf, Hamburg, Germany). The PCR program consisted of an initial denaturation step of 94°C for 4 min, followed by 34 cycles at 94°C for 1 min, 45 s at the specific annealing temperature of each primer (45–69°C), 72°C for 1 min, and a final extension at 72°C for 8 min and a hold temperature of 4°C at the end. After amplification, the reaction products were subjected to electrophoresis in 1.5% agarose gels in 1 × Tris Acetate EDTA (40 mM Tris-acetate, 1 mM EDTA, pH 8.3) buffer stained with 5 µg/ml ethidium bromide and photographed under UV light with the help of a gel documentation system (Bio-Rad, Hercules, CA, USA). A 1 kb molecular ladder was used as a marker to

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Table 1. Details of *N. nimmoniana* populations taken for inter simple sequence repeat analysis

Population code	Locality	Latitude (N) and longitude (E)	Altitude (m)	Approximate population size	Sample size
SR	Sirsi, Karnataka	N: 14°41′45.3″ E: 74°54′55.5″	531–668	55	4
CM	Chickamagalur, Karnataka	N: 13°25′50.5″ E: 75°45′01.1″	1401–1615	135	12
CO	Coorg, Karnataka	N: 12°08′037.1″ E: 75°55′048″	780–1109	113	10
AG	Agumbe, Karnataka	N: 13°29′51.3″ E: 75°04′55.4″	249–637	83	4
CG	Charmadi Ghat, Karnataka	N: 13°07′06.4″ E: 75°30′14.7″	243–920	15	2
JT	Jambotty, Karnataka	N: 15°43′15.4″ E: 74°22′93.9″	748–852	33	3
AB	Amboli, Maharashtra	N: 15°52′20.2″ E: 73°56′43.6″	713–738	43	3
KP	Kolhapur, Maharashtra	N: 16°53′40.8″ E: 73°46′77.4″	360–784	55	4
KT	Kalpetta, Kerala	N: 11°35′24.9″ E: 76°06′13.7″	828–1099	15	4
ID	Idukki, Kerala	N: 09°44′54.9″ E: 77°08′13.1″	869–1187	35	6
OT	Ooty, Tamil Nadu	N: 11°27′36.9″ E: 76°36′59.1″	2092–2169	34	4
KD	Kodaikanal, Tamil Nadu	N: 10°14′79.5″ E: 77°31′59.7″	2046–2160	44	2

know the size of the fragments. All the PCR results were tested for reproducibility at least three times. Bands that did not show fidelity were eliminated.

Data analysis

Amplified fragments for the 16 ISSR primers, which were reproducible and consistent in performance, were scored for band presence (1) or absence (0) and a binary qualitative data matrix was constructed. Percentage of polymorphic bands (PPB) was calculated by dividing the number of polymorphic bands by the total number of bands surveyed. The binary matrix was used to determine the genetic diversity, genetic differentiation and gene flow using the program PopGene 32 version 1.31 (Yeh *et al.*, 1999). Genetic diversity within and among populations was measured by the PPB, effective number of alleles (n_e), observed number of alleles (n_a), Nei's (1973) gene diversity (h) and Shannon's information index (I). At the specieswide level, total genetic diversity (H_T), genetic diversity within populations (H_S) and Nei's coefficient of genetic differentiation among populations ($G_{ST} = (H_T - H_S)/H_T$) were calculated. Corresponding estimates of gene flow (N_m), i.e. the average number of migrants per generation exchanged among populations, was calculated using the formula $N_m = 0.5(1 - G_{ST})/G_{ST}$ (Nei, 1978). Analysis of Molecular Variance (AMOVA) (Excoffier *et al.*, 1992) was performed to calculate the partitioning of genetic variance among and within population using GenAlEx version 6.41 (Peakall and Smouse, 2006). The permutation number for significance testing was set to 999 for all the analysis. Given that the above estimation of allele frequencies from dominant markers requires the assumption

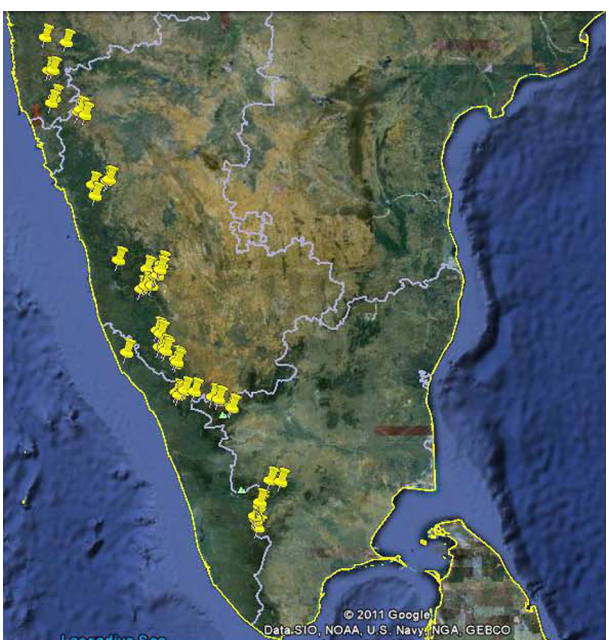

Fig. 1. Geographical distribution map of *N. nimmoniana*. A colour version of this figure can be found online at journals.cambridge.org/pgr.

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Table 2. Inter simple sequence repeat primers used for PCR amplification of *N. nimmoniana* and total number of amplified fragments generated from 57 individuals[a]

UBC primer set # 9	Primer sequence (5′ → 3′)	Total no. of bands	No. of polymorphic bands	Polymorphic bands (%)
822	TCT CTC TCT CTC TCT CA	4	3	75
827	ACA CAC ACA CAC ACA CG	5	4	80
834	AGA GAG AGA GAG AGA GYT	8	4	50
844	CTC TCT CTC TCT CTC TRC	4	1	25
845	CTC TCT CTC TCT CTC TRG	6	5	83.3
854	TCT CTC TCT CTC TCT CRG	10	7	70
857	ACA CAC ACA CAC ACA CYG	9	6	66.6
866	CTC CTC CTC CTC CTC CTC	7	7	100
873	GAC AGA CAG ACA GAC A	5	3	60
874	CCC TCC CTC CCT CCC T	5	3	60
876	GAT AGA TAG ACA GAC A	7	6	85.7
881	GGG TGG GGT GGG GTG	8	8	100
887	DVD TCT CTC TCT CTC TC	5	4	80
895	AGA GTT GGT AGC TCT TGA TC	8	6	75
902	CTC GT GT GT GT GT GT GT GT	4	3	75
906	CCA CCA CCA CCA CCA	8	6	75

UBC, University of British Colombia.
[a] Y, (C, T); D, (A, G, T); V, (A, C, G); R, (A, G).

of the Hardy–Weinberg equilibrium. For elucidating the relationship between the populations, unweighted pair-group method with arithmetic averages dendrogram was constructed based on Nei's (1978) unbiased genetic diversity. Bootstrap analysis of 1000 replicates was used to assess the statistical support of each branch by using the TFPGA program version 1.3 (Miller, 1997).

Results

Exploration and collection of N. nimmoniana

A total of 12 distinct populations of *N. nimmoniana* were located and 57 representation accessions were collected during our exploration and collection missions along the Western Ghats of India. It has been observed that the distribution of the population was discontinuous and the number of individuals in most of the populations was few. Due to the anthropogenic activities and unsustainable harvesting of CPT, most of the trees were cut. The flowering time varied from June to January and ripened fruits were available from October to June in the different populations of Western Ghats. This variation in flowering and fruiting time is due to different agroclimatic conditions prevalent along the Western Ghats.

Genetic diversity

Using the 16 ISSR-University of British Colombia (UBC) primers that showed the best resolution in the amplification profiles, 103 clearly identifiable bands were obtained from 57 accessions of 12 populations from four states. Of these, 76 (73.7%) bands were polymorphic and remaining 27 (26.2%) were monomorphic bands. But, at the population level, the PPB ranged from 28.16 to 48.54%, with an average of 35.84% (Table 3). Each primer yielded four to ten bands with an average of 6.44 bands per primer. The sizes of bands ranged from 350 to 2000 bp (Fig. 2). From UBC 866 and UBC 881, 100% polymorphic bands were recorded. Since ISSR markers are dominant, each band represents the phenotype at a single biallelic locus. The ISSR primers identified in this study will be used for further genetic analysis of *N. nimmoniana*.

Assuming the Hardy–Weinberg equilibrium, the average Nei's (1973) gene diversity (h) ranged from 0.1166 ($\pm$0.1872) to 0.2124 ($\pm$0.2334), with an average of 0.1518 ($\pm$0.0246) at the population level and 0.2965 ($\pm$0.0419) at the species level. Shannon's index (I) ranged from 0.1703 ($\pm$0.2733) to 0.2947 ($\pm$0.3184), with an average of 0.2189 at the population level and 0.4352 ($\pm$0.2829) at the species level. Among the 12 populations, CM population exhibited the highest level of genetic diversity (PPB = 48.54%, $h = 0.2124$ and $I = 0.2947$), whereas CG population showed the lowest variability (PPB = 28.16%, $h = 0.1166$ and $I = 0.1703$). The genetic diversity of populations from high to low ranked as follows: CM > CO > ID > KP > AG > SR > KT > OT > JT > KD > AB > CG. The same results were also obtained by observing the number of alleles (n_a) and effective number of alleles (n_e) (Table 3). When calculated across the populations, the n_a and n_e values equalled 1.7379 and 1.5410, respectively.

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Table 3. Genetic variability parameters of *N. nimmoniana* based on inter simple sequence repeat markers

S. no.	Population	Observed no. of alleles (n_a)	Effective no. of alleles (n_e)	Nei's diversity index (h)	Shannon's information index (I)	Percentage of polymorphic loci (% PPB)
1	SR	1.34 ± 0.047	1.25 ± 0.037	0.1401 ± 0.02	0.2035 ± 0.029	33.98
2	CM	1.49 ± 0.050	1.38 ± 0.042	0.2052 ± 0.22	0.2947 ± 0.031	48.54
3	CO	1.48 ± 0.049	1.40 ± 0.045	0.2124 ± 0.023	0.3016 ± 0.032	47.57
4	AG	1.36 ± 0.048	1.30 ± 0.042	0.1607 ± 0.022	0.2288 ± 0.031	35.92
5	CG	1.28 ± 0.045	1.20 ± 0.032	0.1166 ± 0.018	0.1703 ± 0.027	28.16
6	JT	1.32 ± 0.046	1.24 ± 0.037	0.1326 ± 0.02	0.1924 ± 0.028	32.04
7	AB	1.29 ± 0.045	1.24 ± 0.039	0.1293 ± 0.020	0.1845 ± 0.029	29.13
8	KP	1.37 ± 0.048	1.29 ± 0.041	0.1571 ± 0.021	0.2259 ± 0.030	36.89
9	KT	1.34 ± 0.048	1.25 ± 0.037	0.1385 ± 0.02	0.2018 ± 0.029	33.98
10	ID	1.41 ± 0.049	1.29 ± 0.038	0.1642 ± 0.020	0.2398 ± 0.03	40.78
11	OT	1.32 ± 0.047	1.24 ± 0.038	0.1356 ± 0.021	0.1959 ± 0.029	32.04
12	KD	1.31 ± 0.046	1.23 ± 0.033	0.1287 ± 0.019	0.1879 ± 0.028	31.07
	Mean	1.37 ± 0.02	1.28 ± 0.018	0.1518 ± 0.009	0.2189 ± 0.012	35.84
	Species level	1.7379 ± 0.044	1.5410 ± 0.039	0.2997 ± 0.020	0.4352 ± 0.028	73.79

Population genetic structure

Across the 12 populations of *N. nimmoniana* surveyed for ISSR variation, Nei's estimator of population substructure (G_{ST}) indicated a fairly high level of population differentiation ($G_{ST} = 0.4882$). These G_{ST} values translated into correspondingly low levels of gene flow (N_m), with 0.5242 migrants exchanged between populations (on average) in each generation. The AMOVA also revealed highly significant genetic differences among the 12 populations of *N. nimmoniana* (Table S1, available online only at http://journals.cambridge.org). The analysis revealed that a larger part of the genetic variation exists within the populations (73%) than among the populations (27%) ($\Phi_{PT} = 0.271$, $P < 0.001$). The dendrogram obtained using the UPGMA algorithm based on Nei's (1978) genetic distance is presented in Fig. 3. The analysis of groupings showed the formation of two groups, one comprising the samples of the SR, CM, CO, AG, CG and JT, and the other the populations of AB, KP, KT, ID, OT and KD. As observed by the mean values of similarity, the populations of AB and KP were genetically close related, while populations CG and ID were more distantly related. Genetic distances between the populations ranged from 0.0252 (between AB and KP) to 0.3230 (between CG and ID), and the Nei's genetic identity ranged from 0.7240 to 0.9751 (Table S2, available online only at http://journals.cambridge.org).

Discussion

Threatened medicinal plant species have become the focus of world attention, because they represent a vanishing and decreasing flora in need of protection and conservation and their role as an essential commodity for health care cannot be neglected (Kala, 2005).

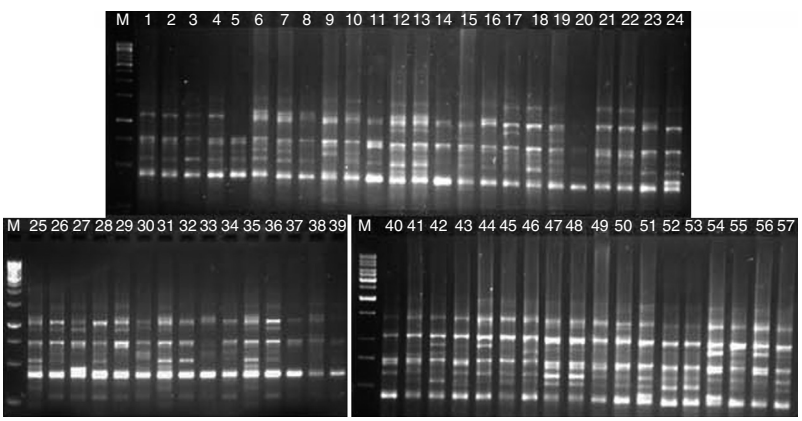

Fig. 2. ISSR fingerprint of 57 accessions of *N. nimmoniana* using primer University of British Colombia 857. Lane M, 1 kb marker.

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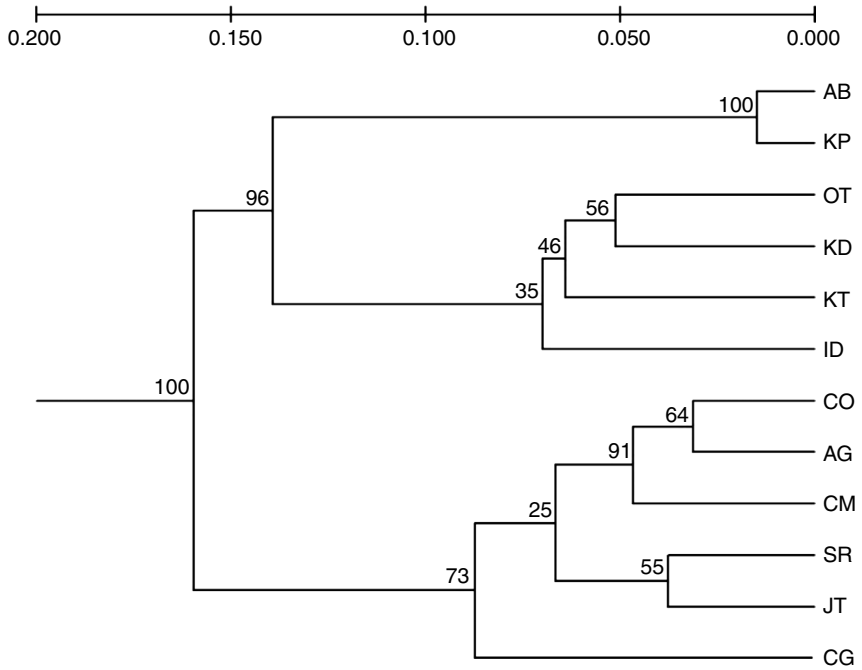

Fig. 3. UPGMA dendrogram illustrating the genetic relationships among 12 populations of *N. nimmoniana,* based on Nei's (1978) unbiased genetic diversity. Numbers on branches indicate bootstrap values from 1000 replicates.

Scientific approaches for conservation and utilization of plant resources require accurate assessment of the amount and distribution of genetic variation within and among populations (Shah *et al.*, 2008). Several aspects of conservation biology, such as loss of genetic diversity and restoration of threatened populations, can only be addressed by detailed population genetics studies (Hamrick and Godt, 1989). A species without enough genetic diversity is thought to be unable to cope with changing environments and demographic fluctuations, both in the short and long term (Reisch *et al.*, 2003).

This study clearly showed that relatively high level of genetic variation (PPB = 73.7%; $h = 0.3001$; $I = 0.4353$) exists among *N. nimmoniana.* Similar result was reported in *Rheum tanguticum*, an endangered perennial medicinal herb in China, ($h = 0.2689$ and $I = 0.4163$) using ISSR markers (Hu *et al.*, 2010). Rare and endangered species are generally known to have low genetic variability. However, some endangered species also showed high levels of genetic variation even within extremely narrow distributions such as *Apterosperma oblate* ($h = 0.275$) (Su *et al.*, 2008) and *Heptacodium miconioides* ($h = 0.2469$) (Jin and Li, 2007), both were assessed by ISSR markers.

Restricted gene flow and genetic drift might have influenced the extent of differentiation among *N. nimmoniana* populations. The G_{ST}-derived N_m value of 0.4709 is indicative of restricted gene flow among natural populations, and this value is actually below the level ($N_m \approx 1$) needed to counteract genetic drift (Slatkin, 1993). Equivalently the high G_{ST} value of 0.515 ($Gs_T > 0.15$) also implied the rapid genetic differentiation among the populations. The high genetic differentiation was also confirmed with the AMOVA ($\Phi_{PT} = 0.271$). Similar results were obtained in *Dysosma pleiantha,* a threatened medicinal plant, (AMOVA: $\Phi_{ST} = 0.500$; Nei's genetic diversity: $G_{ST} = 0.465$) by using ISSR markers (Zong *et al.*, 2008). The data on genetic structure of *N. nimmoniana* obtained in this study showed that the among-population differentiation coefficients ($\Phi_{PT} = 0.271$and $G_{ST} = 0.4882$) were almost similar to, but a little higher than, the perennial endangered plants ($\Phi_{ST} = 0.290$ and $G_{ST} = 0.3585$) (Hu *et al.*, 2010). The result was in contrary to the earlier report on higher genetic variance (69.55%) among the populations than that within the populations (30.45%) of *N. nimmoniana* of Taiwan as assessed by AFLP markers (Qiuying *et al.*, 2005). The reason for the contradiction between these two studies might be the entirely different geographical populations investigated.

It is widely accepted that genetic diversity and population genetic structure are influenced by factors such as historical events, breeding system, genetic drift and natural selection (Barrett, 1992). *Nothapodytes nimmoniana* propagates naturally by seeds. Seed germination in natural habitat is very less and due to the lack of appropriate germination conditions and hard seed-coat. In a

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study conducted for seed germination behaviour of *N. nimmoniana* in our laboratory, the recalcitrant behaviour of this seed was revealed as they showed a tendency to lose viability quickly at room temperature (data not shown). The low seed germination percentage might be one of the reasons for discontinuous and restricted distribution of this species. It was observed that very less flowering and fruit setting in some of the years (Sharma *et al.*, 2010), may be due to physiological reasons, might also be contributed to the vulnerable status of this species. It is important to note that the species is polygamous in nature (Hombegowda *et al.*, 2002) and is reported to be self-pollinated (26.7%) along with the widely observed cross-pollination (Sharma *et al.*, 2010). It is obvious that self-pollination will reduce the genetic diversity among the populations and will lead to homozygosity. But it has been observed that transition of one sex type to another every year in some of the physically disturbed individuals of *N. nimmoniana* resulted in 'sex lability' (Sharma *et al.*, 2010). Some of the polygamous individuals, which produced male, female and bisexual flowers during the first year, were reported to become male in the second year and the female individuals changed to polygamous. But male trees remained male across the years. This phenomenon of rotation of sexes has got great significance for genetic diversity of this species on the special context of self-pollination. It is believed that the 'sex lability' may reduce the risk of erosion of genetic diversity developed due to self-pollination.

The size of populations has a great effect on the long-term persistence of a species. A population with large number of individuals is proposed to have more genetic diversity, which increases their ability to adapt to changing environmental conditions (Vrijenhoek *et al.*, 1985). On the other hand, reduction in population size usually results in loss of genetic diversity and allelic richness, inbreeding and increased extinction risk (Frankham *et al.*, 2002). In this study, the populations of CM and CO showed relatively more number of individuals and high population abundance than the other populations, which indicated that the agroclimatic conditions prevalent in these regions are the most favourable for this species. Due to the enormous demand for the CPT worldwide, habitat destruction of *N. nimmoniana* has been increased. During our survey along the Western Ghats, we could observe a drastic decrease in the number of wild populations and individuals of the species. Its habitat has been extremely threatened, mainly by overcollection, and its distribution has been reduced to a very restricted area. The consequent reductions in its distribution and population size may have promoted genetic differentiation among isolated populations (Ellstrand and Elam, 1993).

Implication for conservation and sustainable utilization

Knowledge of genetic variation within and among populations provides essential information in the formulation of appropriate management strategies for conservation (Francisco-Ortega *et al.*, 2000). As this study revealed, the high population differentiation and discontinuous distribution along with sharply decreasing numbers of populations and individuals remain a serious threat to *N. nimmoniana*. Our findings would provide ample genetic information for developing conservation strategies and sustainable harvesting of this important medicinal plant. Conservation goals may be achieved by protection of all extant populations, *ex situ* conservation of seeds and re-introduction of saplings to its natural populations as well in new sites. To increase an artificial gene flow among populations, we propose to collect the seeds from different populations and exchange the saplings among the populations. Translocation and establishment of new populations are frequently used to curtail the extinction risk of rare plant species (Holl and Hayes, 2006). For those populations with high levels of genetic variation and abundance such as CM and CO, we suggest that their habitats be protected and overexploitation be forbidden. Promoting domestication and cultivation of this species is necessary to satisfy market demand and protecting the wild resource. We have observed that *N. nimmoniana* could be successfully grown as an intercrop in coffee and cardamom plantations. We also suggest to adapt non-destructive harvesting of CPT without cutting the entire tree as we proposed earlier (Kavitha *et al.*, 2010). Our results indicate the existence of high genetic diversity among *N. nimmoniana* populations of Western Ghats along with high population differentiation, which enlighten the necessity to adapt conservation strategies and sustainable exploitation.

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