academicJournals

Vol. 12(20), pp. 2816-2821, 15 May, 2013
DOI: 10.5897/AJB12.9717
ISSN 1684-5315 ©2013 Academic Journals
http://www.academicjournals.org/AJB

African Journal of Biotechnology

*Full Length Research Paper*

# Genetic diversity analysis of *Nardostachys jatamansi* DC, an endangered medicinal plant of Central Himalaya, using random amplified polymorphic DNA (RAPD) markers

**Uma M. Singh[1], Dinesh Yadav[2], M. K. Tripathi[3], Anil Kumar[4] and Manoj K. Yadav[1]***

[1]Department of Biotechnology, SVP University of Agriculture and Technology, Meerut 250 110, India.
[2]Department of Biotechnology, DDU University, Gorakhpur, India.
[3]Department of Biochemistry, Central Institute of Agricultural Engineering, Bhopal, India.
[4]Department of MBGE, GB Pant University of Agriculture and Technology, Pantnagar, India.

Accepted 18 April, 2013

**The genetic diversity analysis of eight populations of *Nardostachys jatamansi* DC. collected from different altitude of Central Himalaya has been attempted using 24 sets of random amplified polymorphic DNA (RAPD) primers. These sets of RAPD marker generated a total of 346 discernible and reproducible bands across the analysed population with 267 polymorphic and 75 monomorphic bands. The unweighted pair group method with arithmetic average (UPGMA) cluster analysis revealed three distinct clusters: I, II and III. The cluster I was represented by *N. jatamansi* population collected from Panwali Kantha (3200 m asl) and Kedarnath (3584 m asl), India together with Jumla (2562 m asl) from Nepal. Cluster II included collections from Har Ki Doon (3400 m asl) and Tungnath (3600 m asl) from India while Cluster III was represented by collections from Munsiyari (2380 m asl), Dayara (3500 m asl) and Valley of Flowers (3400 m asl) from India. The clustering of these populations was independent of variations in altitude and geographical locations. The genetic variations observed in different populations of Jatamansi might be due to environmental influences (biotic and abiotic), rather than altitude level differences. The abiotic (geographical or climatic differentiation) and biotic (pollination between population and seed dispersal) factors might be responsible for the genetic variations among these accessions of Jatamansi.**

**Key words:** Genetic diversity, random amplified polymorphic DNA (RAPD), *Nardostachys jatamansi*, Central Himalaya, unweighted pair group method with arithmetic average (UPGMA).

## INTRODUCTION

*Nardostachys jatamansi* DC. is a small, perennial herb commonly known as jatamansi, Indian nard, balchar or spikenard. It is a dwarf, hairy, rhizomatous medicinal herb growing in steep, moist, rocky, undisturbed grassy slopes of India, Nepal, China, Tibet and Bhutan (Weberling, 1975; Anon, 1970) from 2200 to 5000 m asl in random forms (Ghimire et al., 2005). *Nardostachys grandiflora* DC, *Nardostachys chinensis* Batalin and *Nardostachys gracilis* Kitamura are synonyms of *N. jatamansi* (Weberling, 1975, 1978; Mulliken and Crofton, 2008). The

*Corresponding author. E-mail: mkyadav711@gmail.com. Tel: +91-9412403615.

**Table 1.** List of different populations of *N. jatamansi* with their geographical locations.

| Sample code | Location | District | State/Country |
|---|---|---|---|
| PK | Panwali Kantha (3200 m asl) | Tehri Garhwal | Uttarakhand, India |
| JM | Jumla (2562 m asl) | Jumla | Karnali, Nepal |
| KN | Kedarnath (3584 m asl) | Rudraprayag | Uttarakhand, India |
| HKD | Har Ka Doon (3400m asl) | Uttarkashi | Uttarakhand, India |
| TN | Tungnath (3600m asl) | Rudraprayag | Uttarakhand, India |
| MS | Munsiyari (2380 m asl) | Pithoragarh | Uttarakhand, India |
| DR | Dayara (3500 m asl) | Uttarkashi | Uttarakhand, India |
| VF | Valley of Flowers (3400 m asl) | Chamouli | Uttarakhand, India |

plant is valued for its antioxidant, anticonvulsant, hypolipidemic, hypoglycemic, hepatoprotective, sedative, tranquilizing antihypertensive, antiarrhythmic, anticonvulsant, anti-asthmatic and anti-estrogenic activity (Singh et al., 2013). It is used for the treatment of hair loss, greyness, growth and luster, hysteria, epilepsy, neurosis, insomnia, excitation, habitual constipation, scorpion stings, alzheimer's disease, learning and memory disorders (Yadav et al., 2011; Rahman et al., 2011; Singh et al., 2013). The species is used in both the Unani and Ayurvedic systems of medicine. In traditional medicinal system, it is historically used in Ayurveda as Medhya (brain tonic), Rasayana (Rejuvenative to the mind), Nidrajnana (promotes sleep) and Manasrogaghna (alleviates mental diseases) (Sharma et al., 2001). It is also widely used in the modern medicine industry (Chauhan and Nautiyal, 2005). It also has antibacterial, antifungal, nematicidal, mosquito repellent and anti-malarial activity (Singh et al., 2013).

Reports suggest that the species has become endangered due to over-exploitation of rhizomes for its high medicinal value, habitat degradation and other biotic interferences in its distribution ranges. There is an immediate need for conservations of multitude utility medicinal herbs. Conservation Assessment and Management Plan (CAMP) workshops in India reported substantial reduction of these population from 75 to 80% and classified *N. jatamansi* as endangered (Arunachal Pradesh, Sikkim and Himachal Pradesh) and critically endangered (Uttarakhand) plant (Mulliken and Crofton, 2008; Airi et al., 2000). *N. jatamansi* was assessed to be vulnerable at a CAMP workshop in Nepal in 2001 (Anon, 2001). Due to the high level of threat, Convention on International Trade of Endangered Species (CITES) has notified *N. jatamansi* DC for its conservation. Jatamansi are essentially cross pollinated species, which results in a high degree of variation and offers ample scope to undertake genetic diversity study. Genetic variation studies of different crops are relevant for developing efficient strategies for its conservation.

Nowadays, fingerprinting system based on random amplified polymorphic DNA (RAPD) analysis have been increasingly utilized for detecting polymorphism in those genera which has no prior sequence information. Due to technical simplicity and speed, RAPD methodology has been used for diversity analysis in many plant species (Tonk et al., 2011). RAPD markers have several advantages over other polymorphism detection techniques including RFLP. These include quickness, relatively easy assay, requirement for small amount of template DNA, no requirement of DNA sequence information and use of fluorescence. Because of these advantages, RAPD are commonly used to characterize variability (Vijay et al., 2009). There have been substantial reports of genetic diversity analysis of medicinal plants using RAPD markers (Tonk et al., 2011; Laribi et al., 2011; Vijay et al., 2009; Hnia et al., 2013).

To the best of our knowledge, this is the first report of RAPD analysis of different population of *N. jatamansi* DC collected from different altitudes of Central Himalaya of India and Nepal.

## MATERIALS AND METHODS

### Plant material and DNA isolation

The samples of *N. jatamansi* DC were collected from different populations in the variable altitudes of Central Himalaya of India and Nepal (Table 1). Fresh leaves of *N. jatamansi* were collected in a silica gel containing plastic bag during May 2008. Leaf samples were used for genomic DNA extraction by the standard CTAB method with minor modifications (Doyle and Doyle, 1987).

Leaf tissue were ground in liquid $N_2$ and mixed in 8 ml of prechilled CTAB extraction buffer (0.1 M TrisCl (pH 8.0), 25 mM EDTA, 1.5 M NaCl, 2.5% CTAB, 0.2% β-merceptoethanol (v/v) with 2% PVP (w/v) added immediately prior to use and incubated at 65°C for 1 h. The content was mixed with equal volume of chloroform : isoamyl alcohol (24:1) and subjected to centrifugation for 10 min at 10,000 rpm at 4°C. The supernatant was recovered and mixed with 3 ml of NaCl (5M) followed by addition of 0.7 volume of isopropanol and left at -20°C for 1 to 2 h. The mixture was again centrifuged at 10,000 rpm for 10 min at 4°C and supernatant was decanted carefully. The pellet formed was dried completely and resuspended in 1 ml of TE buffer with 100 µg/ml of RNase solution and incubated at 37°C for 30 min. This was followed by addition of equal amount of phenol : chloroform : isoamyl alcohol (25:24:1) and centrifugation at 8,000 rpm for 15 min at room temperature (RT). The supernatant was transferred to sterile tube and equal volume of chilled absolute ethanol was added

**Table 2.** Profile of polymorphism among eight accession of *N. Jatamansi* resulting from twenty RAPD markers.

| Primer | Total number of band | Number of polymorphic band | Polymorphism (%) |
|---|---|---|---|
| NJ-1 | 19 | 17 | 89.4 |
| NJ-3 | 18 | 14 | 77.7 |
| NJ-4 | 13 | 10 | 76.9 |
| NJ-5 | 14 | 11 | 78.5 |
| NJ-10 | 12 | 8 | 66.6 |
| NJ-11 | 13 | 10 | 76.9 |
| NJ-12 | 11 | 5 | 45.4 |
| NJ-13 | 17 | 13 | 76.4 |
| NJ-14 | 13 | 9 | 69.2 |
| NJ-17 | 12 | 10 | 83.3 |
| NJ-19 | 5 | 4 | 80.0 |
| NJ-20 | 12 | 10 | 83.3 |
| NJ-30 | 13 | 11 | 84.6 |
| NJ-33 | 10 | 7 | 70.0 |
| NJ-34 | 10 | 7 | 70.0 |
| NJ-37 | 10 | 8 | 80.0 |
| NJ-41 | 18 | 14 | 77.7 |
| NJ-42 | 16 | 11 | 68.7 |
| NJ-43 | 20 | 16 | 80.0 |
| NJ-45 | 21 | 18 | 85.7 |
| NJ-46 | 19 | 16 | 84.2 |
| NJ-47 | 16 | 12 | 75.0 |
| NJ-49 | 20 | 16 | 80.0 |
| NJ-53 | 14 | 10 | 71.4 |

and incubated at -20°C for 30 min followed by centrifugation at 10,000 rpm for 10 min at 4°C. The resulting pellet was washed with 70% ethanol and resuspended in sterile double distilled water. The quantity and quality of genomic DNA were analyzed by UV-spectrophotometer (Eppendorf Biophotometer) at 260 nm and by agarose (0.8%) gel electrophoresis, respectively.

#### PCR amplification using RAPD primers

The PCR was performed in a thermal cycler (Eppendorf, USA) using 24 sets of RAPD markers (Table 2) synthesized GeNei™ by Merck Specialities Private Limited, Mumbai, India. The PCR reaction set up for 25 µl reaction comprised of genomic DNA (50 ng), 1.5 mM $MgCl_2$, 1.2 mM dNTPs, 1.0 U *Taq* DNA Polymerase and 30 ng primer. The cycling conditions included initial cycle of denaturation at 94°C for 5 min followed by repeated 38 cycles of denaturation at 94°C for 45 s, annealing at 30°C for 45 s and extension at 72°C for 80 min. After completion of 38 cycles, a final extension at 72°C for 7 min was carried out and finally held at 4°C (Table 2). The amplified products resulting from different primers were resolved on 1.6% agarose gel and analyzed by standard ethidium bromide staining and finally photographed using Gel documentation system (Alfa Ease™ USA). The software Gene Profiler 4.2 was used for further analysis of stored gels. The PCR amplification was performed twice with each primers and band scoring was done accordingly.

#### Scoring of bands

The bands resulting from different sets of RAPD primers for different populations of *N. jatamansi* were scored on agarose gel photograph in Gel documentation system for its presence and absence across the populations collected (Figure 1). The image profiles of banding patterns were recorded and molecular weight of each bands were determined by Alpha View Software. The results were analysed based on the principle that a band is considered to be polymorphic if it is absent in at least one individuals or accessions. Similarity index of bands which were common between two accessions was estimated by Nei and Li (1979). The final RAPD data generated with 24 primers were used to calculate pair wise similarity coefficients using Jaccard's coefficient of similarity (Jaccard, 1908). The cluster analysis was performed by Tree Con™ (Scanalytics Inc, USA) software for generating phylogenetic tree using the unweighted pair group method with arithmetic average (UPGMA) method (Nie and Li, 1979). Boot strap values were calculated which proves a confidence level for each branch point and indicate the number of times that the position of the branch point occurred during the iterative bootstrap process.

## RESULTS

### RAPD analysis

A total of 24 primers as listed in Table 2 were used to analyze the genetic diversity among different populations of *N. jatamansi* collected from different altitudes of Central Himalaya of India and Nepal. The number of bands varied from 5 by primer NJ-19 to a maximum of 21

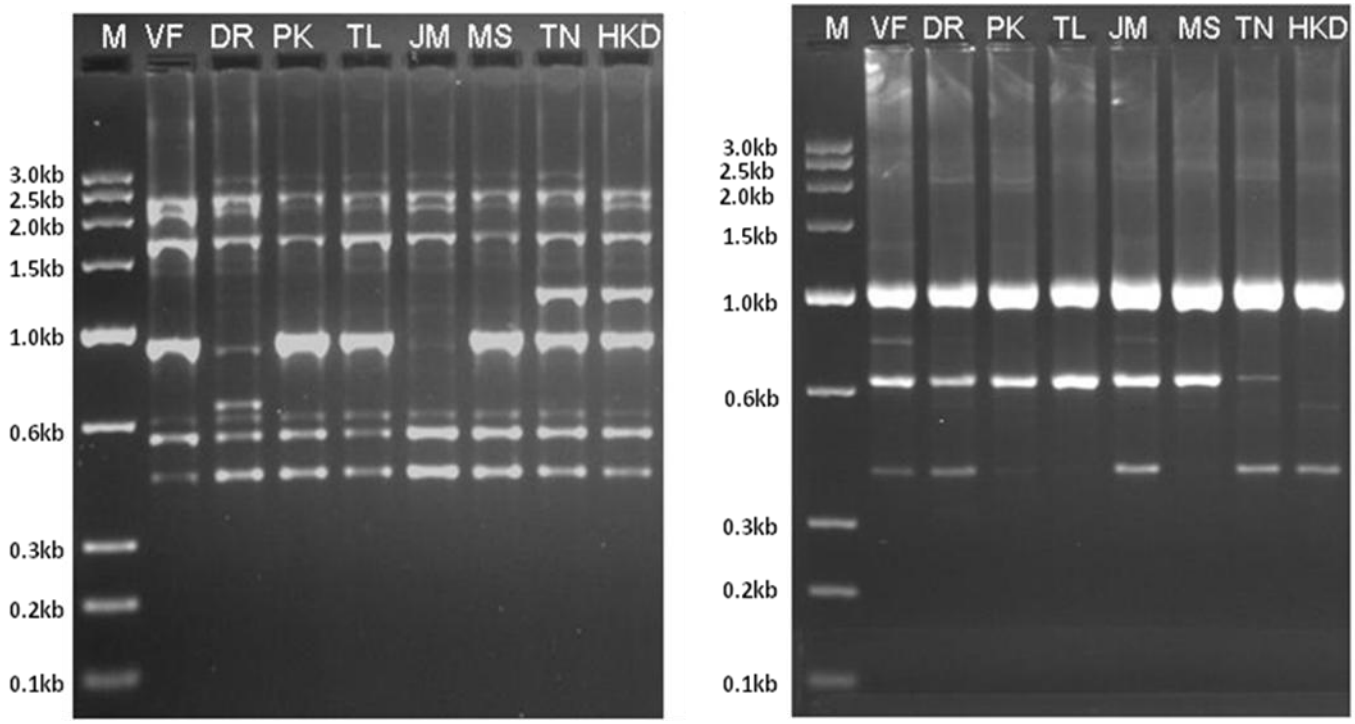


**Figure 1.** RAPD profile of different accessions of *N. jatamansi*: Marker (M) NJ-12 and NJ-19; (1 Kb). See Table 1 for sample codes.

**Table 3.** Average similarity coefficient among different accessions of *N. jatamansi*.

| | VF | JM | DR | TN | PK | HKD | KN | MS |
|---|---|---|---|---|---|---|---|---|
| VF | 1.00 | | | | | | | |
| JM | 0.44 | 1.00 | | | | | | |
| DR | 0.49 | 0.44 | 1.00 | | | | | |
| TN | 0.38 | 0.45 | 0.43 | 1.00 | | | | |
| PK | 0.43 | 0.48 | 0.48 | 0.47 | 1.00 | | | |
| HKD | 0.38 | 0.38 | 0.43 | 0.52 | 0.49 | 1.00 | | |
| KN | 0.41 | 0.51 | 0.47 | 0.43 | 0.50 | 0.47 | 1.00 | |
| MS | 0.41 | 0.45 | 0.45 | 0.43 | 0.41 | 0.39 | 0.39 | 1.00 |

See Table 1 for sample codes.

bands by primer NJ-45. The PCR amplicons sizes ranged from 0.36 to 4.3 Kb. These sets of primers revealed intra-specific variations. A total of 346 bands were scored with 267 polymorphic bands and 75 monomorphic (Table 2).

## Genetic distance and UPGMA analysis

The primers showing reproducible, scorable and clear bands were only considered for final analysis (Figure 1). Based on RAPD markers, the similarity index value were estimated. The similarity index value ranged from 0.38 to 0.52 (Table 3). The minimum genetic similarities (0.38) were exhibited between the accessions collected from VF and TN, VF and HKD, JM and HKD whereas, maximum genetic similarity of 0.52 was observed for accessions collected from TN and HKD. The dendrogram was constructed using Unweighted Pair Group Method with Arithmetic average (UPGMA) as shown in Figure 2. Three distinct clusters labelled as I, II and III were observed. The cluster I comprised of all the accessions collected from Panwali Kantha (PK, 3200 m asl), Jumla (JM, 3150 m asl) and Kedar Nath (KN, 3584 m asl). Within this cluster, the two accessions collected from Panwali Kantha and Jumla showed maximum similarity with similarity coefficient of 0.48. The cluster II had accessions from Har Ka Doon (HKD, 3400m asl) and Tungnath (TN, 3600m asl) with similarity coefficient of

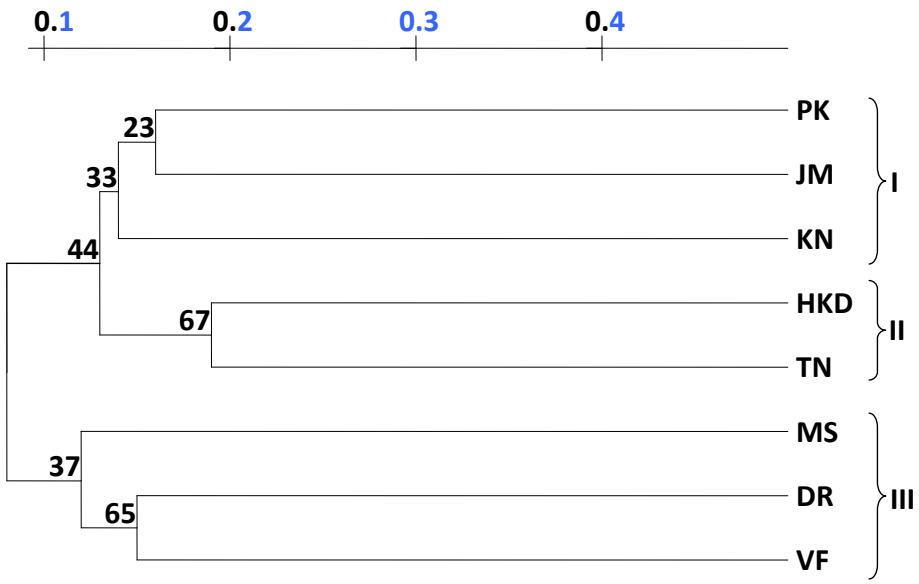


**Figure 2.** Dendrogram of *N. jatamansi* accessions based on RAPD analysis with the help of TreeCon software using UPGMA cluster analysis. See Table 1 for sample codes.

0.52. The cluster III comprised of three accession from Munsiyari (MS, 2380 m asl), Dayara (DR, 3500 m asl) and Valley of Flowers (VF, 3400m asl).

## DISCUSSION

A total of 346 bands were obtained with twenty four RAPD primers in eight populations of *Nardostachys jatamansi*. The majority of bands were polymorphic (267) and only 75 bands were monomorphic. The percent of polymorphism ranged from 45.4 (NJ12) to 89.4% (NJ1). Out of the twenty four primers used, twenty three primers showed more than 65% polymorphism. The similarity coefficient value among different populations of *N. jatamansi* ranged from 0.38 to 1.0. Out of eight populations studied, five population of jatamansi had similarity index value less than 0.5. The population collected from HKD, TN and PK had relatively more similarity and its value is greater than 0.5. The similarity coefficient values between HKD and TN, and KN and JM were relatively more, that is, 0.52 and 0.51, respectively. Similarity matrix and the dendrogram clearly depicts that they are genetically similar because the altitude from sea level and distance between them are also close. However, the altitude and condition of PK (India) and JM (India) differed significantly but they showed resemblance with each other.

The geographical conditions and altitude of accessions VF and DR are nearly similar, that is, 3500 and 3400 m asl, respectively, showed more resemblance with each other. The reason for higher diversity (less similarity value) in different accessions of *N. jatamansi* might be due to reproductive isolation, entomophillous pollination, and dispersal of hairy seeds by wind, habitat changes and small population size in different locations of Himalaya. Similar results were also reported in some other endangered plant for example *Gaultheria fragrantissima* (68.52%) (Bantawa et al., 2011), *Torreya jackii* (66.11%) (Li et al., 2007), *Antirrhinum microphyllum* (67.65%) (Torres et al., 2003), *Oroxylem indium* (49.6%) (Jayaram and Prasad, 2010). The reason for this might be that the parent plants of this species probably grew for ages in area in which mountainous systems were not a barrier for dispersion. These geographical regions are capable of harbouring genotypes with similar life strategies and adaptations; however, their populations that become isolated by geographical barriers tend to be diverse in their genotype as well (Rzedowski, 1988).

### Conservation implications of *N. Jatamansi*

Analysis of genetic diversity of rare and endangered species might be efficacious measurement and strategy for conservation of these species. Based on our results and the information available for *N. Jatamansi*, two alternative conservation strategies may be proposed. The *in situ* conservation strategies need area free from significant disruption for establishing at least the genetically more different accession (Munsiyari). This would assure the maintenance of species genetic variation. However, because the observed genetic differentiation among accessions of *N. jatamansi* is high and little gene flow appears to exist among the accessions, management for conservation of genetic variability in this species not only try to preserve large accessions collected from MS but also many of the small accessions collected from

HKD and TN. *Ex situ* conservation of the accessions accordingly based on the data of RAPD markers would fulfil the objective of capturing most of the detected genetic variability.

RAPD based diversity analysis revealed that *N. jatamansi* contain high level of variation among their population. However, this study could be more informative if number of population increased. The distinct accession identified in the present study may be further characterized based on chemotyping and functional attributes for subsequent conservation and propagation of elite planting materials for biomedical applications. The present investigation is an initial step to evaluate the molecular diversity of this critically rare and endangered herb. This study will be a boon to conserve this herb *in vitro* and *ex-situ* as well and also to fulfil the growing demand at national and international market.

**REFERENCES**


Airi S, Rawal RS, Dhar U, Purohit AN (2000). Assessment of availability and habitat prefernce of Jatamansi-a critically endangered medicinal plant of west Himalaya. Curr. Sci. 79(10): 1467-1470.

Anon (1970). Medicinal Plants of Nepal. H.M.G. Department of Medicinal Plants Publication, Thapathali, Kathmandu, Nepal.

Anon (2001). Draft conservation assessment and management prioritisation report, Pokhara, Nepal.

Bantawa P, Das A, Ghosh PD, Mondal TK (2011). Detection of natural genetic diversity of *Gaultheria fragrantissima* landraces by RAPDs: An endangered woody oil bearing plant of Indo-China Himalayas. Indian J. Biotechnol. 10: 294-300.

Chauhan RS, Nautiyal MC (2005). Commercial viability of cultivation of an endangered medicinal herb *Nardostachys jatamansi* at three different agroclimatic zones. Curr. Sci. 89(9):1481-1488

Doyle JJ, Doyle J L (1987). A rapid DNA isolation procedure for small quantities of fresh leaftissue. Phytoch. Bull. 19:11-15.

Ghimire SK, Mckey D, Thomas YA (2005). Conservation of Himalayan medicinal plants: harvesting patterns and ecology of two threatened species, *Nardostachys grandiflora* DC. and *Neopicrorhiza scrophulariiflora* (Pennell) Hong. Biol. Conserv. 124(4):463-475.

Hnia C, Yosr Z, Mohamed B (2013). Genetic diversity of natural *Tunisian Lavandula* multifida L. (Lamiaceae) populations assessed by allozymes and random amplification of polymorphic DNA (RAPD). Afr. J. Biotechnol. 12(7):648-657.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bul. Soc. Vaudoise. Sci. Nat. 44:223-270.

Jayaram K, Prasad MNV (2010). Genetic diversity in *Oroxylem indicum* (L.) Vent. (Bignoniaceae), a vulnerable medicinal plant by random amplified polymorphic DNA marker. Afr. J. Biotechnol. 7(3):254-262.

Laribi B, Zoghlami N, Lamine M, Kouki K, Ghorbel A, Mougou A (2011). RAPD based assessment of genetic diversity among annual caraway (*Carum carvi*) populations. Eurasia J. Biosci. 5: 37-47

Li JH, Jin ZX, Li JM (2007). Genetic diversity of endangered plant *Torreya jackii*: a study with RAPD markers. J. Appl. Ecol. 18(12): 2661-67.

Mulliken T, Crofton P (2008). Review of the status, harvest, trade and management of seven Asian CITES-listed Medicinal and Aromatic Plant Species. Bundesamt für Naturschutz, Bonn.

Nei M, Li W H (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 76:5269-5273.

Rahman H, Muralidharan P (2011). *Nardostacys Jatamansi* DC Protects from the loss of memory and cognition deficits in sleep deprived Alzheimer's disease (Ad) mice model. Int. J. Pharm. Sci. Rev. Res. 5(3):160-167.

Rzedowski J (1988). Analissis de la distibucion geographica del compleyo prosopis (Leguminosae, Mimosoideae) en Norte America. Acta Bot. Mex. 3:7-19.

Sharma PC, Yelne NB, Dennis TJ (2001). Data base on Medicinal Plants used in Ayuveda , Vol-1,CCRAS, New Delhi.

Singh UM, Gupta V, Rao VP, Sengar RS, Yadav MK (2013). A review on biological activities and conservation of endangered medicinal herb *Nardostachys jatamansi*. Int. J. Med. Aromat. Plant. 3(1):113-124.

Tonk FA, Gıachıno RRA, Sönmez C, Yüce S, Bayram E, Telci I, Furan MA (2011). Characterization of various *Hypericum perforatum* clones by hypericin and RAPD analyses. Int. J. Agric. Biol. 13(1): 31-37.

Torres E, Iriondo JM, Pérez C (2003). Genetic structure of an endangered plant, *Antirrhinum microphyllum* (Scrophulariaceae): allozyme and RAPD analysis. Am. J. Bot. 90(1):85-92.

Vijay N, Sairkar P, Silawat N, Garg RK, Mehrotra NN (2009). Genetic variability in *Asparagus racemosus* (Willd.) from Madhya Pradesh, India by random amplified polymorphic DNA. Afr. J. Biot. 8(14):3135-3140.

Weberling F (1975). On the systematic of Nardostachys (Valerianaceae). Taxon. 24:443-452.

Weberling F (1978). Monographie der Gattung *Nardostachys grandiflora* DC. (Valerianaceae). Bot. Jahrb. Syst. 99:188-221.

Yadav SK, Gupta SK, Prabha S (2011). Hair growth activity of *Nardostachys jatamansi* and *Cyperus rotundus* rhizomes extract on chemotherapy induced alopecia. Int.J. Drug.Discov..Herb.Res. 1(2):52-54.