*Research Article*

# Genetic and Biochemical Diversity among *Valeriana jatamansi* Populations from Himachal Pradesh

**Sunil Kumar Singh,[1,2] Rajan Katoch,[1] and Rakesh Kumar Kapila[1]**

[1]*Department of Biotechnology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur 176062, India*
[2]*National Research Centre on Plant Biotechnology, Lal Bahadur Shastri Building, Pusa Campus, New Delhi 110012, India*

Correspondence should be addressed to Sunil Kumar Singh; agrilsunshine@gmail.com

*Valeriana jatamansi* Jones is an important medicinal plant that grows wild in Himachal Pradesh, India. Molecular and biochemical diversity among 13 natural populations from Himachal Pradesh was assessed using RAPD and GC-MS to know the extent of existing variation. A total of seven genetically diverse groups have been identified based on RAPD analysis which corroborated well with the analysis based on chemical constituents. The essential oil yield ranged from 0.6% to 1.66% (v/w). A negative correlation between patchouli alcohol and viridiflorol, the two major valued constituents, limits the scope of their simultaneous improvement. However, other few populations like Chamba-II and Kandi-I were found promising for viridiflorol and patchouli alcohol, respectively. The analysis of chemical constitution of oil of the populations from a specific region revealed predominance of specific constituents indicating possibility of their collection/selection for specific end uses like phytomedicines. The prevalence of genetically diverse groups along with sufficient chemical diversity in a defined region clearly indicates the role of ecology in the maintenance of evolution of this species. Sufficient molecular and biochemical diversity detected among natural populations of this species will form basis for the future improvement.

## 1. Introduction

*Valeriana jatamansi* Jones. (*Valeriana wallichii* DC), also known as Indian valerian, is an erect pubescent herb, having horizontal, thick rootstock/rhizomes, with thick descending fibrous roots [1]. The species is found growing on moist slopes in the Himalayas and Khasi hills in shrubberies and open slopes between 1500 and 4000 m elevation from Pakistan to Southwest China, Burma, and South-East Asia. The plant also grows well in different agroclimatic regions of India. In Himachal Pradesh, it grows profusely in Bharmour division of Chamba, Kanda area of Karsog, and Chansil of Rohru forest division [2].

*Valeriana jatamansi has* long been in use in the Ayurvedic and Unani system of medicine [3]. Herbal medicine remains one of the most common forms of the therapy available for much of the world's population. In traditional medicines, the roots of the plant are used for various ailments like ulcers, convulsions, jaundice, cardiac debility, dry cough, asthma, seminal weakness, chronic and intermittent fevers, skin diseases, falling hairs, nephropathy, leprosy, general debility, and sleep enhancement [4–6]. Besides, *Valeriana jatamansi* is also known to possess fungicidal activity against *Fusarium oxysporum* and *Macrophomina phaseolina* [7].

The medicinal property of the plant is attributed to various chemical components present in its essential oil. The composition of oil has been reported to vary with geographical location and altitude [8–12]. The assessment of the genetic composition of species collected from different phytogeographical regions helps to assess the available diversity, whereas biochemical analysis of economical parts is important to know quantitative as well as qualitative aspects and to choose the economically superior genotypes for the active ingredients.

Among different PCR based markers, random amplified polymorphic DNA (RAPD) is an easy method for discovering random polymorphism in the genome [13]. In contrast to other molecular markers, RAPD is a very simple technique for taxonomic and systemic analysis and phylogenetic studies
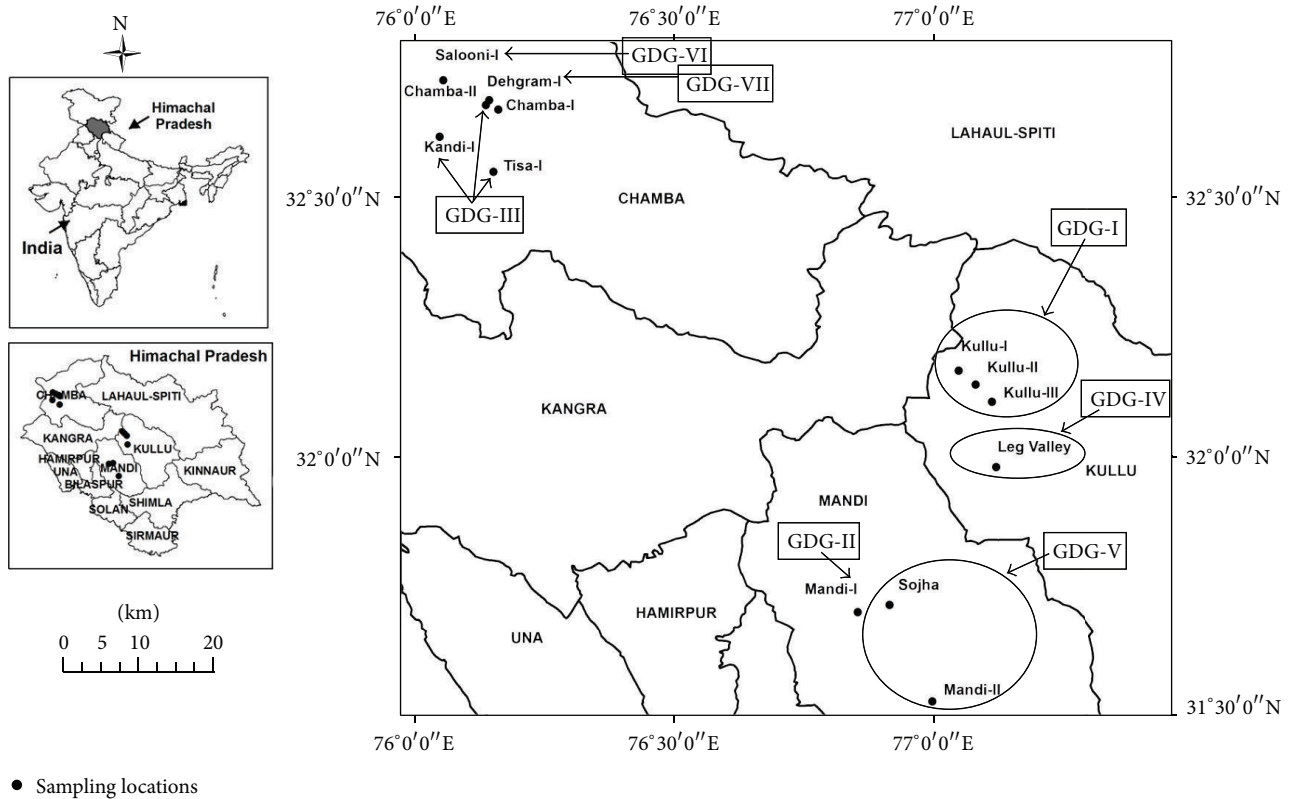
● Sampling locations

FIGURE 1: Sampling locations of thirteen populations of *Valeriana jatamansi* from three districts of Himachal Pradesh. The seven genetically diverse groups (GDG) have been indicated by arrow.

of plants [14–18]. The usefulness of RAPDs in diversity analysis has been demonstrated at the species level [19], subspecies level [20], population level [21], and cultivar level [22]. The present study was conducted to elucidate the biochemical and molecular diversity among different representative population samples of *Valeriana jatamansi* collected from different geographical locations of Himachal Pradesh, India.

## 2. Materials and Methods

*2.1. Plant Material.* A total of 64 samples from thirteen natural populations (five from each population except Mandi-I, having four samples) of *Valeriana jatamansi* Jones (Table 1 and Figure 1) were collected from different location/regions of Himachal Pradesh (HP), India, for the present study. Since the collections were made from the available natural populations at different locations, representative samples collected were limited in number ranging from 4 to 5.

*2.2. DNA Extraction and PCR Amplification.* Genomic DNA was isolated from young leaves using CTAB method given by Murray and Thompson [23]. The isolated genomic DNA was stored at −20°C until being used. Decamer primers of arbitrary sequence from operon (A, C, D, E, F, J, P, Q, and X series) were used to amplify genomic DNA of twenty-six samples (2 per collections) and based on polymorphism obtained forty-five primers were selected (Table 2) for final amplification of all the collections. DNA amplification

TABLE 1: Geographical descriptors of collections of *Valeriana jatamansi* from Himachal Pradesh, India, used in the present study.

| Population | Sample size | Altitude (m) | Latitude | Longitude |
|---|---|---|---|---|
| Kullu-I | 5 | 3647 | 32°09′56″N | 77°02′41″E |
| Kullu-II | 5 | 2734 | 32°08′19″N | 77°04′37″E |
| Kandi-I | 5 | 854 | 32°36′58″N | 76°02′42″E |
| Tisa-I | 5 | 1220 | 32°32′54″N | 76°08′55″E |
| Chamba-II | 5 | 2104 | 32°40′36″N | 76°08′03″E |
| Salooni-I | 5 | 1730 | 32°43′29″N | 76°03′08″E |
| Mandi-I | 4 | 764 | 31°42′00″N | 76°51′00″E |
| Kullu-III | 5 | 2541 | 32°06′20″N | 77°06′30″E |
| Dehgram-I | 5 | 2165 | 32°41′11″N | 76°08′25″E |
| Leg Valley | 5 | 1720 | 31°58′47″N | 77°06′59″E |
| Chamba-I | 5 | 2368 | 32°40′06″N | 76°09′29″E |
| Mandi-II | 5 | 945 | 31°31′41″N | 76°59′41″E |
| Sojha | 5 | 2692 | 31°42′47″N | 76°54′47″E |

was carried out by making final reaction volume of 20 $\mu$L containing 1.6 $\mu$L of dNTP mix (0.2 mM each of dATP, dGTP, dCTP, and dTTP), 0.16 $\mu$L of *Taq* DNA polymerase (5 U/$\mu$L), 2.0 $\mu$L DNA template (25 ng/$\mu$L), 1.0 $\mu$L of 5 $\mu$M primer, 2.0 $\mu$L of 10X PCR buffer, 1.2 $\mu$L of MgCl$_2$ (25 mM), and 12.04 $\mu$L of sterilized distilled water.

Table 2: Level of polymorphism detected using 45 RAPD primers in 13 populations of *Valeriana jatamansi*.

| Marker | Scored bands | Polymorphic bands | PIC value (%) |
| --- | --- | --- | --- |
| OPA-01 | 9 | 8 | 85.40 |
| OPA-02 | 9 | 9 | 83.29 |
| OPA-03 | 9 | 9 | 87.88 |
| OPA-04 | 10 | 9 | 83.82 |
| OPA-09 | 7 | 6 | 81.93 |
| OPA-11 | 11 | 10 | 88.58 |
| OPA-13 | 10 | 4 | 87.75 |
| OPA-14 | 10 | 7 | 85.70 |
| OPA-15 | 8 | 6 | 84.19 |
| OPA-19 | 9 | 8 | 86.78 |
| OPA-20 | 10 | 7 | 82.40 |
| OPC-06 | 11 | 11 | 89.23 |
| OPC-20 | 6 | 5 | 67.19 |
| OPD-07 | 7 | 5 | 84.03 |
| OPD-12 | 9 | 9 | 82.70 |
| OPD-13 | 9 | 9 | 85.32 |
| OPD-16 | 6 | 5 | 79.51 |
| OPD-18 | 5 | 5 | 76.81 |
| OPD-19 | 7 | 7 | 77.39 |
| OPE-07 | 7 | 6 | 79.18 |
| OPF-01 | 3 | 2 | 63.95 |
| OPF-02 | 6 | 5 | 79.31 |
| OPF-10 | 7 | 7 | 84.44 |
| OPF-12 | 7 | 5 | 66.44 |
| OPF-13 | 12 | 12 | 88.06 |
| OPJ-01 | 3 | 2 | 55.40 |
| OPJ-04 | 11 | 10 | 85.73 |
| OPJ-10 | 10 | 10 | 85.62 |
| OPJ-11 | 5 | 4 | 75.72 |
| OPJ-14 | 15 | 14 | 92.25 |
| OPJ-18 | 7 | 5 | 81.57 |
| OPP-08 | 10 | 8 | 86.76 |
| OPP-10 | 7 | 2 | 84.93 |
| OPP-11 | 8 | 4 | 86.01 |
| OPQ-01 | 6 | 5 | 86.08 |
| OPQ-04 | 12 | 7 | 88.57 |
| OPQ-06 | 8 | 7 | 82.25 |
| OPQ-09 | 8 | 1 | 80.22 |
| OPQ-12 | 9 | 8 | 83.28 |
| OPQ-13 | 8 | 8 | 82.25 |
| OPQ-14 | 4 | 1 | 70.09 |
| OPQ-15 | 6 | 3 | 77.15 |
| OPQ-16 | 12 | 6 | 89.59 |
| OPX-02 | 8 | 6 | 81.86 |
| OPX-19 | 7 | 6 | 84.20 |
| Total | 368 | 293 | 81.79 (mean) |

The DNA amplification was carried out in a thermal cycler (Applied Biosystems). The PCR program was set at initial cycle of 94°C for 5 minutes, 37°C for 1 min, and 72°C for 2 min. Further amplification was repeated 40 times consisting of denaturation at 94°C for 1 minute, annealing at 37°C for 1 min and extension at 72°C for 2 min. Final extension of 5 min at 72°C was carried out before rapid cooling to 4°C. Amplification products were separated by agarose gel electrophoresis in 1 X TAE on 1.4% agarose containing 0.5 ng/$\mu$L ethidium bromide. Images were photographed and captured by Gel Doc (Bio-Rad). Molecular weights were estimated using a 1000 bp DNA ladder.

*2.3. RAPD Data Scoring and Analysis.* The RAPD profiles generated by different primers were compared to determine relatedness within and among different populations. The presence and absence of each RAPD band of a particular molecular weight in all genotypes were scored manually. A binary data matrix with "1" indicating presence of a particular molecular weight band and "0" indicating its absence was generated separately for each primer. The binary data were used for principal coordinate analysis (PCA), analysis of molecular variance (AMOVA), and mantel test [24] of geographic and genetic distance using GenAlEx software [25]. PCA was done based on genetic distance measure calculated from binary data for multiple samples with multiple populations using GenAlEx software. The neighbor-joining tree and bootstrap analysis were executed using DARWIN version 6.0 [26]. The statistical analysis was done by using StatSoft Inc. [27], STATISTICA (data analysis software system), version 7. Fst and Nm values were calculated using GenAlEx software following formula given by Nei [28, 29].

*2.4. GC-MS Analysis of Essential Oil.* Roots and rhizomes of *Valeriana jatamansi* dried for 20 days under ambient room conditions in shade [30, 31] were used for essential oil extraction by hydrodistillation in Clevenger apparatus. The essential oil was dried over anhydrous $Na_2SO_4$. The purified fraction was used for recording GC-MS data. Two $\mu$L of essential oil fraction was used for injection. GC-MS (70 eV) data were measured in MS-QP-2010 series Shimadzu, Tokyo, Japan, equipped with MS, AOC-20i autosampler, and BP-20 capillary column (SGC International, Ringwood, Australia) 30 m length, 0.25 mm I.D., and film thickness 0.25 $\mu$m (poly ethylene glycol), with helium as a carrier gas. The injector temperature was 220°C with split ratio of 1 : 50. The GC oven temperature was programmed to hold at 70°C for 4 min and then to increase up to 220°C at increments of 4°C/min and finally it holds at 220°C for 5 min. Column flow rate was set at 1.10 mL/min. Ion source temperature was 200°C and interface temperature was set at 220°C. The MS was scanned at 70 eV over 40–600 a.m.u. The individual components of the essential oils were identified by comparing their mass spectra with a computerized MS-database using WILEY7, NIST 147, NIST 27, and SZTERP libraries.

## 3. Results and Discussion

*3.1. RAPD Polymorphism.* A total of 150 primers were initially screened for amplification of DNA of a subset of 26 samples (two plants randomly selected from each population). Based on the polymorphic information content (PIC) [32],
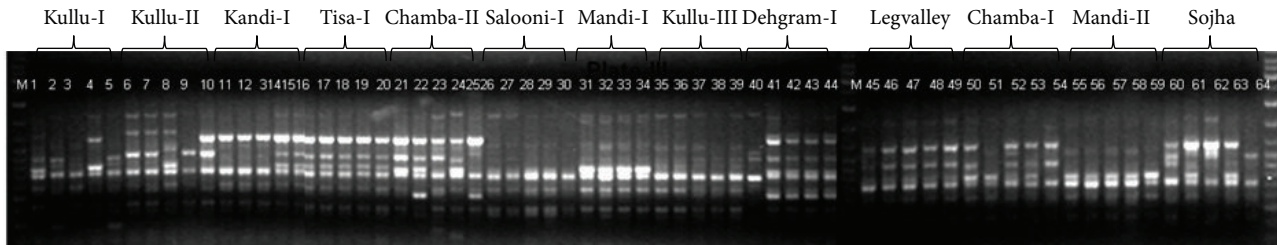
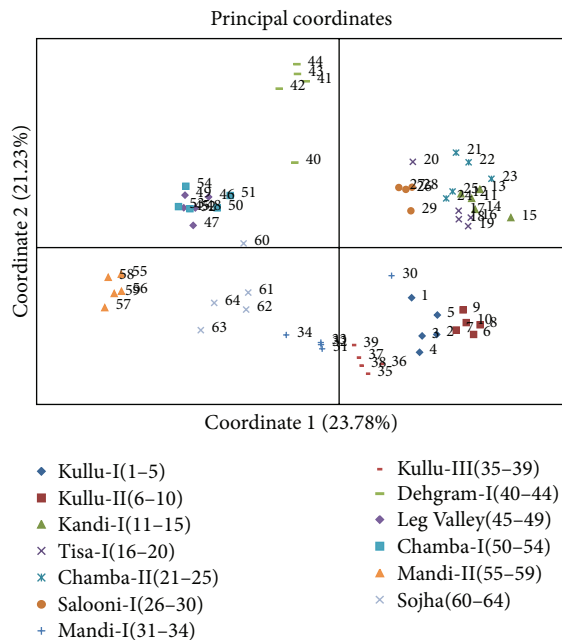FIGURE 2: RAPD profile of thirteen populations of *Valeriana jatamansi*.



FIGURE 3: Principal coordinate analysis of genetic differences among 13 populations of *Valeriana jatamansi*. Values in parenthesis show level of variation explained by the coordinate.

the signal intensity, and number of bands, 45 primers were selected for final analysis (Table 2). The representative RAPD profile generated by OPA-3 primer is shown in Figure 2. All forty-five primers, generated a total of 368 bands with a mean of 8 bands per primer ranging from 3 to 15 per primer. Of 368 bands, only 75 (20.39%) amplified fragments were present in all the 64 plants, whereas 293 (79.61%) were polymorphic. It indicated considerable variation among the 64 samples of 13 populations. Kumar [33] had reported 90.18%, while Rajkumar et al. [34] reported a range of 65–81% polymorphism in the sampled populations of the species in their studies. The difference in the level of polymorphism among these reports might be due to less number of primers used by Kumar [33] and lower number of polymorphic loci (241) obtained by Rajkumar et al. [34], as compared to the present investigation. Besides, it can also be due to inherent differences in the samples collected from different geographical regions in our case.

*3.2. Genetic Diversity Analysis and Population Structure.* The binary data used for principal coordinates analysis (PCA) distributed the samples in two coordinates; coordinate one accounted for 23.78%, whereas coordinate two accounted for

21.23% of the total variation among populations (Figure 3). Distribution pattern of all the samples from different populations revealed consistency with their geographical origin. It clearly revealed lesser intrapopulation variation as compared to interpopulation variation. The same was evident from the analysis of molecular variance (AMOVA) wherein 48% ($P$ = 0.001) variation was recorded within population as compared to 52% ($P$ = 0.001) among populations (Table 3). The pairwise differences (Fst) between populations (calculated based on allele sharing) varied from 0.25 to 0.74 (Table 4). The estimated extent of gene flow (Nm) among populations is 0.253, ranging from 0.08 (between populations from Salooni-I and Leg Valley-II) to 0.72 (between populations from Kullu-I and Kullu-II). Based on Nei's genetic similarity index (Table 4), 13 populations clustered in seven different groups are designated hereafter as genetically diverse groups (GDGs) at 85% genetic similarity level (Figure 4, Table 4). This clustering into seven GDGs was further corroborated based on biochemical profiling of their essential oil. As evident from Figures 1, 3, and 4, most of the samples from a particular area/region (population) were grouped separately, deciphering the level and

TABLE 3: Analysis of molecular variance (AMOVA) of 64 samples of 13 populations of *Valeriana jatamansi*.

| Source | df | Sum of squares | Mean squared deviation | Estimated variance | % Total variance | Probability |
|---|---|---|---|---|---|---|
| Among populations | 12 | 1778.137 | 148.178 | 25.337% | 52% | <0.001 |
| Within populations | 51 | 1197.050 | 23.472 | 23.472% | 48% | <0.001 |

df = degree of freedom, SS = sum of squares, and MS = mean square.

TABLE 4: Pairwise Fst and Nm (in parenthesis) value among populations of *Valeriana jatamansi* (below diagonal) and and Nei's genetic similarity (above diagonal) index.

| | Kullu-I | Kullu-II | Kandi-I | Tisa-I | Chamba-II | Salooni-I | Mandi-I | Kullu-III | Dehgram-I | Leg Valley | Chamba-I | Mandi-II | Sojha |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Kullu-I | | 0.903 | 0.853 | 0.828 | 0.838 | 0.805 | 0.846 | 0.858 | 0.784 | 0.812 | 0.812 | 0.801 | 0.842 |
| Kullu-II | 0.256 (0.727) | | 0.869 | 0.822 | 0.839 | 0.780 | 0.825 | 0.853 | 0.770 | 0.798 | 0.806 | 0.778 | 0.822 |
| Kandi-I | 0.407 (0.364) | 0.394 (0.384) | | 0.873 | 0.872 | 0.823 | 0.789 | 0.807 | 0.812 | 0.793 | 0.837 | 0.773 | 0.831 |
| Tisa-I | 0.467 (0.285) | 0.501 (0.249) | 0.421 (0.344) | | 0.875 | 0.790 | 0.799 | 0.805 | 0.782 | 0.799 | 0.820 | 0.784 | 0.806 |
| Chamba-II | 0.374 (0.418) | 0.393 (0.385) | 0.342 (0.481) | 0.347 (0.471) | | 0.847 | 0.808 | 0.821 | 0.829 | 0.799 | 0.835 | 0.778 | 0.806 |
| Salooni-I | 0.528 (0.193) | 0.586 (0.152) | 0.549 (0.173) | 0.609 (0.137) | 0.448 (0.308) | | 0.811 | 0.801 | 0.770 | 0.743 | 0.825 | 0.787 | 0.785 |
| Mandi-I | 0.453 (0.419) | 0.521 (0.314) | 0.603 (0.244) | 0.605 (0.230) | 0.419 (0.347) | 0.529 (0.223) | | 0.851 | 0.750 | 0.812 | 0.812 | 0.837 | 0.801 |
| Kullu-III | 0.451 (0.304) | 0.488 (0.262) | 0.593 (0.172) | 0.609 (0.160) | 0.498 (0.252) | 0.698 (0.108) | 0.468 (0.284) | | 0.773 | 0.785 | 0.812 | 0.813 | 0.837 |
| Dehgram-I | 0.525 (0.226) | 0.565 (0.193) | 0.529 (0.223) | 0.583 (0.179) | 0.433 (0.327) | 0.648 (0.136) | 0.575 (0.185) | 0.636 (0.143) | | 0.820 | 0.830 | 0.776 | 0.777 |
| Leg Valley | 0.533 (0.219) | 0.579 (0.182) | 0.609 (0.161) | 0.615 (0.157) | 0.530 (0.222) | 0.740 (0.088) | 0.562 (0.195) | 0.680 (0.118) | 0.572 (0.187) | | 0.855 | 0.829 | 0.813 |
| Chamba-I | 0.468 (0.284) | 0.499 (0.251) | 0.469 (0.283) | 0.510 (0.240) | 0.407 (0.364) | 0.562 (0.195) | 0.463 (0.289) | 0.567 (0.191) | 0.483 (0.268) | 0.490 (0.261) | | 0.881 | 0.881 |
| Mandi-II | 0.550 (0.205) | 0.604 (0.164) | 0.633 (0.145) | 0.633 (0.145) | 0.558 (0.198) | 0.706 (0.104) | 0.501 (0.249) | 0.644 (0.138) | 0.630 (0.147) | 0.619 (0.154) | 0.427 (0.335) | | 0.872 |
| Sojha | 0.390 (0.391) | 0.450 (0.306) | 0.454 (0.301) | 0.507 (0.243) | 0.434 (0.327) | 0.596 (0.170) | 0.458 (0.296) | 0.498 (0.252) | 0.538 (0.215) | 0.537 (0.215) | 0.329 (0.510) | 0.422 (0.342) | |

robustness of diversity analysis using large number of RAPD markers with high PIC value encompassing larger genome coverage. The populations from specific regions such as Kullu, Chamba, and Mandi are grouped together except some of the samples that exhibited variation and were grouped in different clusters. This possibly can be due to their natural habitat and geographic confinement. The clustering pattern further indicates that the populations are not much differentiated during the evolution and the slight genetic variation present within population(s) might have evolved under the influence of environmental factors. The mantel test did not exhibit significant correlation ($r = 0.002$; $P = 0.05$) between genetic distance and geographic distance of populations (Figure 5).

### 3.3. Essential Oil Composition and Chemical Diversity. The essential oil yield from roots was found to vary from 0.6% to 1.66% (Table 5) with a mean oil yield of 1.090 ± 0.052 among

13 populations studied. The DMRT analysis revealed that the populations differ in oil content significantly from each other with Tisa-I and Dehgram-I having highest oil content as compared to others, while populations Leg valley, Chamba-I, Mandi-II, and Kullu-I have the lowest oil content. Based on the GC and GCMS analysis, ten major chemical constituents were identified in all the populations, namely, endobornyl acetate (0 to 4.56%), calarene (0.58 to 24.21%), *alpha*-guaiene (0.51 to 4.16%), seychellene (0.89 to 5.29%), azulene (0.24 to 6.73%), selinene (0.38 to 2.21%), viridiflorol (1.82 to 48.8%), epiglobulol (0 to 3.16%), patchouli alcohol (0.98 to 65.04%), and pogostol (0 to 2.39%). The correlation analysis indicated a negative trend of major chemical constituents and essential oil with altitude, though it was not significant (Table 6), while a significant positive and negative correlation exists among the chemical constituents of essential oil, for example, calarene has a significant negative trend in relation
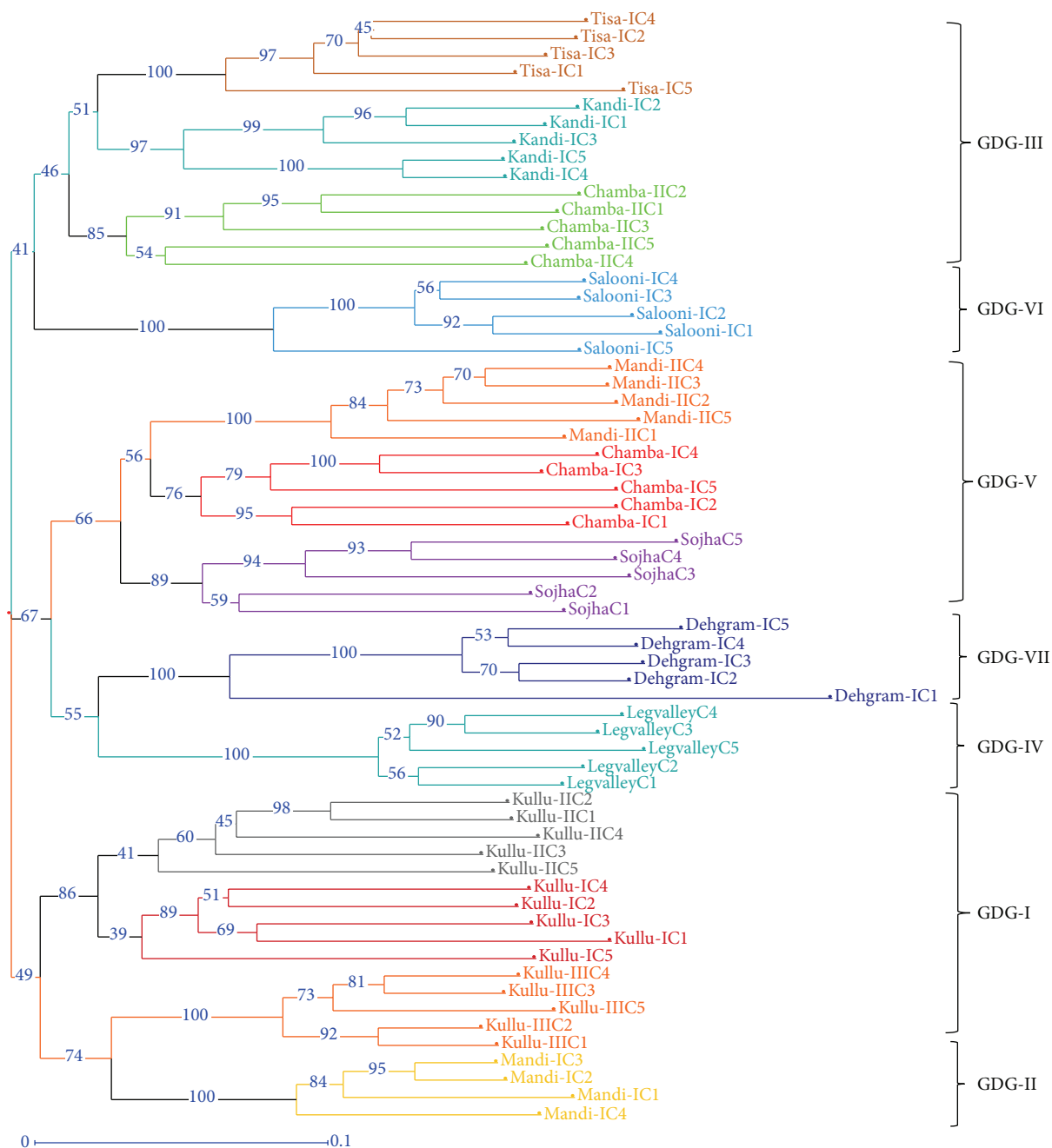
FIGURE 4: Dendrogram of 64 samples from 13 populations of *Valeriana jatamansi* representing clustering of samples in a separate group showing the population specific grouping. Cluster analysis was performed using the neighbor-joining method. Bootstrap values obtained from 500 replicate analyses higher than 40% are indicated on nodes.

to patchouli alcohol, seychellene, and pogostol, whereas with epiglobulol it is positive. This indicates that the accumulation of one type of constituent hinders the accumulation of other forms. The variation in chemical composition of essential oil with change of altitude has already been documented in the literature [9, 35]. Amongst constituents, patchouli alcohol content was found to have significantly high positive correlation with *alpha*-guaiene (0.816), seychellene (0.884), and

azulene (0.602), while it has significant negative association with calarene (−0.953), viridiflorol (−0.820), and epiglobulol (−0.954). As the patchouli alcohol and viridiflorol are the major economic constituents of *Valeriana jatamansi*, these correlation analyses will provide a yardstick for the selection of plants from the population having high contents of desired constituent in the essential oil. It is also evident from the analysis that the two major chemical constituents are negatively

TABLE 5: Essential oil contents and their major constituents in 13 populations of *Valeriana jatamansi*.

| | Endobornyl acetate (%) | Calarene (%) | *alpha*-Guaiene (%) | Seychellene (%) | Azulene (%) | Selinene (%) | Viridiflorol (%) | Epiglobulol (%) | Patchouli alcohol (%) | Pogostol (%) | Oil (DW) (Vol/Wt) % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kullu-I | 2.1 | 2.04 | 2.15 | 2.97 | 2.49 | 1.17 | 16.62 | 0.25 | 56.29 | 2.1 | 0.67[h] |
| Kullu-II | 4.56 | 15.43 | 0.55 | 2.27 | 1.33 | 1.22 | 29.1 | 1.67 | 14.73 | 0 | 1.17[e] |
| Kandi-I | 0.84 | 0.58 | 3.54 | 4.39 | 6.23 | 2.12 | 1.82 | 0.0 | 65.04 | 2.36 | 1.37[cd] |
| Tisa-I | 0.0 | 24.21 | 0.73 | 0.89 | 0.51 | 1.99 | 26.72 | 3.16 | 0.98 | 0.0 | 1.66[a] |
| Chamba-II | 2.72 | 10.41 | 0.51 | 1.39 | 0.4 | 0.38 | 48.8 | 1.49 | 15.7 | 0.75 | 1.12[ef] |
| Salooni-I | 0.76 | 2.35 | 1.23 | 3.77 | 1.53 | 0.85 | 14.92 | 0.26 | 59.29 | 2.29 | 1.5[bc] |
| Mandi-I | 0.27 | 3.09 | 2.6 | 3.46 | 3.75 | 1.43 | 19.58 | 0.34 | 50.96 | 2.15 | 0.93[g] |
| Kullu-III | 0.5 | 2.39 | 2.63 | 3.56 | 5.15 | 1.57 | 15.53 | 0.21 | 48.47 | 2.12 | 1.25[de] |
| Dehgram-I | 1.99 | 13.46 | 1.19 | 2.43 | 1.58 | 0.93 | 22.71 | 1.7 | 30.16 | 1.32 | 1.56[ab] |
| Leg Valley | 0.0 | 0.72 | 4.16 | 5.29 | 6.73 | 2.12 | 3.15 | 0.0 | 60.17 | 2.27 | 0.64[h] |
| Chamba-I | 0.26 | 1.55 | 3.04 | 3.12 | 0.24 | 1.95 | 6.42 | 0.0 | 59.93 | 2.39 | 0.69[h] |
| Mandi-II | 0.76 | 7.06 | 2.42 | 3.13 | 3.89 | 1.67 | 24.47 | 0.76 | 38.52 | 1.66 | 0.6[h] |
| Sojha | 1.01 | 1.07 | 3.18 | 3.94 | 5.84 | 2.21 | 8.07 | 0.0 | 59.11 | 2.11 | 1.0[fg] |

Superscripts on oil content value denote significant homogeneous grouping at $P \leq 0.05$ using DMRT.
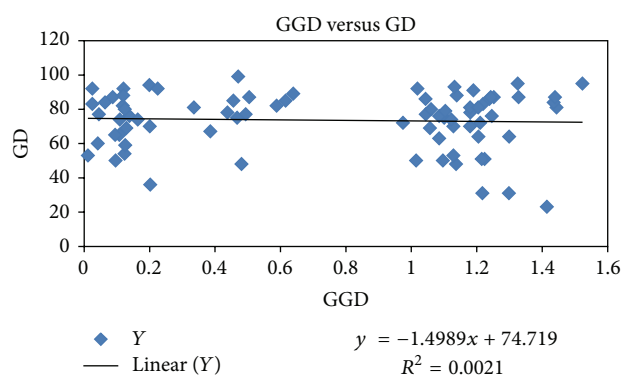


FIGURE 5: Test of correlation between genetic and geographic distances among 13 populations of *Valeriana jatamansi*.

correlated and selection for one will hinder the selection of the other. Hence, for a particular type of chemical constituent and end use, one has to choose specific population. In present study, Chamba-II and Kandi-I were observed as best populations for viridiflorol and patchouli alcohol, respectively.

Singh et al. [36] reported prevalence of chemotypes of *Valeriana jatamansi* in Himachal Pradesh according to the area of their natural habitat. Table 7 documents the area wise diversity of genetic groups present and the chemical diversity documented for each group in a particular area. The table clearly indicates that there is prevalence of microclimate on the chemical composition of the essential oil from plants inhabiting the particular area. For example, the samples from Kullu region have been divided into two distinct groups on the basis of genetic identity and the chemical constituents also corroborating molecular analysis. Based upon information generated under the present investigation, specific population of a region can be selected and targeted for a particular chemical constituent; for example, the samples

collected from Chamba region will in general be having highest level of oil content (1.48%) with highest chemical constituents such as viridiflorol (22.31%), longifolenaldehyde (2.02%), calarene (9.18%), and *alpha*-patchoulene (4.98%). Similarly samples from Mandi could be targeted for highest level of pogostol (2.10%), (−)-*alpha*-selinene (2.33%), and spathulenol (0.95%).

## 4. Conclusion

RAPD profiling of 13 populations of *Valeriana jatamansi* with 45 oligo primers generated a total of 368 amplicons and showed 79.61% polymorphism as a whole. The PCA and AMOVA revealed that variation among populations was slightly higher than that within population(s). It might be due to local environmental effect during adaptation of plant to given environment. The chemical analysis of essential oil, which ranged from 0.6% to 1.66% (v/w), led to identification of ten major chemical constituents, namely, endobornyl acetate (0 to 4.56%), calarene (0.58 to 24.21%), *alpha*-guaiene (0.51 to 4.16%), seychellene (0.89 to 5.29%), azulene (0.24 to 6.73%), selinene (0.38 to 2.21%), viridiflorol (1.82 to 48.8%), epiglobulol (0 to 3.16%), patchouli alcohol (0.98 to 65.04%), and pogostol (0 to 2.39%). Two major components of oils, namely, patchouli alcohol and viridiflorol, exhibited negative association, thus limiting scope of simultaneous gains from selection for both constituents. Chamba-II and Kandi-I populations were found to be best populations for viridiflorol and patchouli alcohol, respectively. Since variable contents of patchouli alcohol and viridiflorol can potentially affect therapeutic efficacy of the plant, it will be important to understand and consider negative association between two major constituents while making any attempts of genetic enhancement in order to breed new cultivars with more desirable chemical constituents. This study also provides indicative guideline for collection of samples from a suitable

Table 6: Correlation among essential oil contents, its constituents, and altitude of growth habitat in 13 *Valeriana jatamansi* populations.

| | Endobornyl acetate | Calarene | *alpha*-Guaiene | Seychellene | Azulene | Selinene | Viridiflorol | Epiglobulol | Patchouli alcohol | Pogostol | Oil (DW) | Altitude |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Endobornyl acetate | 1 | | | | | | | | | | | |
| Calarene | 0.334 | 1 | | | | | | | | | | |
| *alpha*-Guaiene | −0.598* | −0.774** | 1 | | | | | | | | | |
| Seychellene | −0.407 | −0.844** | 0.862** | 1 | | | | | | | | |
| Azulene | −0.37 | −0.600* | 0.804** | 0.816** | 1 | | | | | | | |
| Selinene | −0.584* | −0.224 | 0.728** | 0.469 | 0.584* | 1 | | | | | | |
| Viridiflorol | 0.554* | 0.649* | −0.837** | −0.819** | −0.625* | −0.734** | 1 | | | | | |
| Epiglobulol | 0.301 | 0.993** | −0.785** | −0.861** | −0.606* | −0.265 | 0.680* | 1 | | | | |
| Patchouli alcohol | −0.445 | −0.953** | 0.816** | 0.884** | 0.602* | 0.362 | −0.820** | −0.954** | 1 | | | |
| pogostol | −0.558* | −0.940** | 0.796** | 0.824** | 0.561* | 0.295 | −0.742** | −0.924** | 0.969** | 1 | | |
| Oil (DW) | 0.091 | 0.535 | −0.358 | −0.366 | −0.271 | −0.229 | 0.289 | 0.432 | −0.387 | −0.417 | 1 | |
| Altitude | 0.502 | −0.102 | −0.148 | −0.105 | −0.194 | −0.244 | 0.042 | −0.118 | 0.045 | −0.03 | −0.186 | 1 |

* Significant at 0.05 level. ** Significant at 0.01 level.

TABLE 7: Highly diverse biochemical profile revealed by oil constituents analysis of population from different ecological niches which also possess high level of genetic diversity.

| GDG | Kullu | | | Mandi | | | Chamba | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | I | IV | Avg. | V | II | Avg. | III | VI | VII | Avg. |
| Viridiflorol (%) | 21.13 | 3.15 | **12.14** | 13.24 | 19.92 | **16.58** | 27.33 | 15.18 | 24.41 | **22.31** |
| Isopatchoulane (%) | 0.62 | 0.67 | **0.64** | 0.60 | 0.55 | **0.57** | 0.81 | 0.87 | 0.45 | **0.71** |
| Longifolenaldehyde (%) | 0.91 | 1.53 | **1.22** | 1.86 | 0.73 | **1.30** | 3.80 | 0.38 | 1.89 | **2.02** |
| Patchouli alcohol (%) | 39.83 | 60.17 | **50.00** | 52.52 | 50.96 | **51.74** | 27.24 | 59.29 | 30.16 | **38.90** |
| Pogostol (%) | 1.41 | 2.27 | **1.84** | 2.05 | 2.15 | **2.10** | 1.04 | 2.29 | 1.32 | **1.55** |
| Juniper camphor (%) | 1.55 | 1.55 | **1.55** | 1.49 | 1.66 | **1.58** | 2.10 | 1.14 | 0.81 | **1.35** |
| DL-Limonene (%) | 0.42 | 0.34 | **0.38** | 0.33 | 0.24 | **0.28** | 0.29 | 0.00 | 0.00 | **0.10** |
| Camphene (%) | 0.90 | 0.50 | **0.70** | 0.41 | 0.22 | **0.32** | 0.25 | 0.00 | 0.00 | **0.08** |
| p-Cymene (%) | 0.93 | 0.00 | **0.47** | 0.17 | 0.00 | **0.08** | 0.42 | 0.00 | 0.00 | **0.14** |
| beta-Patchoulene (%) | 1.37 | 1.32 | **1.35** | 1.03 | 0.64 | **0.84** | 0.11 | 0.00 | 0.00 | **0.04** |
| Endobornyl Acetate (%) | 2.39 | 0.00 | **1.19** | 0.68 | 0.27 | **0.47** | 1.19 | 0.76 | 1.99 | **1.31** |
| Calarene (%) | 6.62 | 0.72 | **3.67** | 3.23 | 3.09 | **3.16** | 11.73 | 2.35 | 13.46 | **9.18** |
| alpha-Guaiene (%) | 1.78 | 4.16 | **2.97** | 2.88 | 2.60 | **2.74** | 1.59 | 1.23 | 1.19 | **1.34** |
| cis-Farnesol (%) | 0.13 | 0.00 | **0.06** | 0.26 | 0.46 | **0.36** | 0.20 | 2.43 | 0.34 | **0.99** |
| alpha-Patchoulene (%) | 0.89 | 0.58 | **0.74** | 2.12 | 0.00 | **1.06** | 5.65 | 1.07 | 8.22 | **4.98** |
| Seychellene (%) | 2.93 | 5.29 | **4.11** | 3.40 | 3.46 | **3.43** | 2.22 | 3.77 | 2.43 | **2.81** |
| alpha-Humulene (%) | 0.99 | 1.86 | **1.43** | 1.52 | 1.14 | **1.33** | 1.06 | 0.70 | 0.83 | **0.86** |
| Azulene (%) | 2.99 | 6.73 | **4.86** | 3.32 | 3.75 | **3.54** | 2.38 | 1.53 | 1.58 | **1.83** |
| (−)-alpha-Selinene (%) | 1.34 | 3.07 | **2.20** | 2.60 | 2.06 | **2.33** | 2.74 | 0.85 | 0.93 | **1.51** |
| ar-Curcumene (%) | 0.44 | 0.00 | **0.22** | 0.29 | 0.00 | **0.15** | 0.54 | 0.00 | 0.24 | **0.26** |
| Isoaromadendrene epoxide (%) | 0.15 | 0.37 | **0.26** | 0.06 | 0.42 | **0.24** | 0.27 | 0.78 | 0.51 | **0.52** |
| Valerenal (%) | 0.53 | 0.47 | **0.50** | 0.14 | 0.80 | **0.47** | 0.74 | 0.26 | 0.00 | **0.33** |
| Spathulenol (%) | 0.60 | 0.94 | **0.77** | 0.44 | 1.45 | **0.95** | 0.14 | 0.24 | 0.00 | **0.13** |
| Oil content (%) | 1.03 | 0.64 | **0.84** | 0.76 | 0.93 | **0.85** | 1.38 | 1.50 | 1.56 | **1.48** |

region for a particular end use based on the predominance of particular chemical constituents in its oil.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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## References

[1] R. Sharma, *Medicinal Plants of India—An Encyclopedia*, Daya Publishing House, 2003.

[2] S. S. Purohit and S. P. Vyas, *Medicinal Plant Cultivation—A Scientific Approach*, Agrobios, Jodhpur, India, 2005.

[3] L. K. Gupta and S. C. Shah, "Cultivation and importance of *Valeriana wallichii* in the hills of Uttar Pradesh," *Indian Drugs*, vol. 18, no. 11, pp. 393–395, 1981.

[4] N. Prajapati and U. Kumar, *Agro's Dictionary of Medicinal Plants*, Agrobios, 2003.

[5] S. Bent, A. Padula, D. Moore, M. Patterson, and W. Mehling, "Valerian for sleep: a systematic review and meta-analysis," *The American Journal of Medicine*, vol. 119, no. 12, pp. 1005–1012, 2006.

[6] T. Komori, T. Matsumoto, E. Motomura, and T. Shiroyama, "The sleep-enhancing effect of valerian inhalation and sleep-shortening effect of lemon inhalation," *Chemical Senses*, vol. 31, no. 8, pp. 731–737, 2006.

[7] C. Arora and R. D. Kaushik, "Fungicidal activity of plants extracts from Uttaranchal hills against soybean fungal pathogens," *Allelopathy Journal*, vol. 11, no. 2, pp. 217–227, 2003.

[8] R. Bos, H. J. Woerdenbag, F. M. S. van Putten, H. Hendriks, and J. J. C. Scheffer, "Seasonal variation of the essential oil, valerenic acid and derivatives, and valepotriates in *Valeriana officinalis* roots and rhizomes, and the selection of plants suitable for phytomedicines," *Planta Medica*, vol. 64, no. 2, pp. 143–147, 1998.

[9] J. Ibañez and A. Usubillaga, "Analysis of the essential oil of two different altitudinal populations of *Coespeletia moritziana* (Sch. Bip.ex Wedd) cuatrec," *Flavour and Fragrance Journal*, vol. 21, no. 5, pp. 760–763, 2006.

[10] P. C. Santos-Gomes and M. Fernandes-Ferreira, "Organ- and season-dependent variation in the essential oil composition of *Salvia officinalis* L. cultivated at two different sites," *Journal of Agricultural and Food Chemistry*, vol. 49, no. 6, pp. 2908–2916, 2001.

[11] S. Echeverrigaray, F. Fracaro, A. C. A. dos Santos, N. Paroul, R. Wasum, and L. A. Serafini, "Essential oil composition of

south Brazilian populations of *Cunila galioides* and its relation with the geographic distribution," *Biochemical Systematics and Ecology*, vol. 31, no. 5, pp. 467–475, 2003.

[12] M. J. Oliveira, I. F. P. Campos, C. B. A. Oliveira et al., "Influence of growth phase on the essential oil composition of *Hyptis suaveolens*," *Biochemical Systematics and Ecology*, vol. 33, no. 3, pp. 275–285, 2005.

[13] J. G. K. Williams, A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey, "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers," *Nucleic Acids Research*, vol. 18, no. 22, pp. 6531–6535, 1990.

[14] P. Rath, G. Rajaseger, C. J. Goh, and P. P. Kumar, "Phylogenetic analysis of *Dipterocarps* using random amplified polymorphic DNA markers," *Annals of Botany*, vol. 82, no. 1, pp. 61–65, 1998.

[15] I. V. Bartish, L. P. Garkava, K. Rumpunen, and H. Nybom, "Phylogenetic relationships and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae) estimated with RAPDs and isozymes," *Theoretical and Applied Genetics*, vol. 101, no. 4, pp. 554–563, 2000.

[16] A. Chaveerach, H. Kunitave, S. Nuchadomrong, N. Sattayasai, and H. Kamulsu, "RAPD patterns as a useful tool to differentiate Thai *Piper* from morphologically alike Japanese *Piper*," *Science Asia*, vol. 28, pp. 221–226, 2002.

[17] L. A. Hug and A. J. Roger, "The impact of fossils and taxon sampling on ancient molecular dating analyses," *Molecular Biology and Evolution*, vol. 24, no. 8, pp. 1889–1897, 2007.

[18] P. Mokkamul, A. Chaveerach, R. Sudmoon, and T. Tanee, "Species identification and sex determination of the genus *Nepenthes* (Nepenthaceae)," *Pakistan Journal of Biological Sciences*, vol. 10, no. 4, pp. 561–567, 2007.

[19] K. Isoda, S. Shiraishi, S. Watanabe, and K. Kitamura, "Molecular evidence of natural hybridization between *Abies veitchii* and *A. homolepis* (Pinaceae) revealed by chloroplast, mitochondrial and nuclear DNA markers," *Molecular Ecology*, vol. 9, no. 12, pp. 1965–1974, 2000.

[20] J. B. dos Santos, J. Nienhuis, P. Skroch, J. Tivang, and M. K. Slocum, "Comparison of RAPD and RFLP genetic markers in determining genetic similarity among *Brassica oleracea* L. genotypes," *Theoretical and Applied Genetics*, vol. 87, no. 8, pp. 909–915, 1994.

[21] J. Herbert, P. M. Hollingsworth, M. F. Gardner, R. R. Mill, P. I. Thomas, and T. Jaffré, "Conservation genetics and phylogenetics of new Caledonian *Retrophyllum* (Podocarpaceae) species," *New Zealand Journal of Botany*, vol. 40, no. 2, pp. 175–188, 2002.

[22] K. Wolff and R. J. Peters-Van, "Rapid detection of genetic variability in chrysanthemum (*Dendranthema grandiflora* Tzvelev) using random primers," *Heredity*, vol. 71, pp. 335–341, 1993.

[23] M. G. Murray and W. F. Thompson, "Rapid isolation of high molecular weight plant DNA," *Nucleic Acids Research*, vol. 8, no. 19, pp. 4321–4326, 1980.

[24] N. Mantel, "The detection of disease clustering and a generalized regression approach," *Cancer Research*, vol. 27, no. 2, pp. 209–220, 1967.

[25] R. Peakall and P. E. Smouse, "GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research," *Molecular Ecology Notes*, vol. 6, no. 1, pp. 288–295, 2006.

[26] X. Perrier, A. Flori, and F. Bonnot, "Data analysis methods," in *Genetic Diversity of Cultivated Tropical Plants*, P. Hamon, M. Seguin, X. Perrier, and J. C. Glaszmann, Eds., pp. 43–76, Enfield, Science Publishers, Montpellier, France, 2003.

[27] StatSoft, *STATISTICA (Data Analysis Software System)*, version 7, StatSoft, 2004, http://www.statsoft.com/.

[28] M. Nei, "Genetic distance between populations," *The American Naturalist*, vol. 106, pp. 283–392, 1972.

[29] M. Nei, "Estimation of average heterozygosity and genetic distance from a small number of individuals," *Genetics*, vol. 89, no. 3, pp. 583–590, 1978.

[30] K. Javidnia, R. Miri, M. Kamalinejad, and H. Khazraii, "Chemical composition of the volatile oil of aerial parts of *Valeriana sisymbriifolia* Vahl. grown in Iran," *Flavour and Fragrance Journal*, vol. 21, no. 3, pp. 516–518, 2006.

[31] R. Bos, H. J. Woerdenbag, H. Hendriks, H. F. Smit, H. V. Wikström, and J. J. C. Scheffer, "Composition of the essential oil from roots and rhizomes of *Valeriana wallichii* DC," *Flavour and Fragrance Journal*, vol. 12, no. 2, pp. 123–131, 1997.

[32] S. K. Singh, Gopichand, P. S. Ahuja, and S. Rajkumar, "Estimation of genetic diversity in *Ginkgo biloba* trees from northwestern India using AFLP and microsatellite markers," *Journal of Plant Genetics and Transgenics*, vol. 1, no. 1, pp. 16–20, 2010.

[33] A. Kumar, *Molecular characterization of Indian valerian (Valeriana jatamansi) germplasm in Himachal Pradesh using molecular markers [M.S. thesis]*, Dr. Y. S. Parmar University of Horticulture and Forestry, Himachal Pradesh, India, 2003.

[34] S. Rajkumar, S. K. Singh, A. Nag, and P. S. Ahuja, "Genetic structure of Indian Valerian (*Valeriana jatamansi*) populations in western Himalaya revealed by AFLP," *Biochemical Genetics*, vol. 49, no. 9-10, pp. 674–681, 2011.

[35] M. A. Curado, C. B. A. Oliveira, J. G. Jesus, S. C. Santos, J. C. Seraphin, and P. H. Ferri, "Environmental factors influence on chemical polymorphism of the essential oils of *Lychnophora ericoides*," *Phytochemistry*, vol. 67, no. 21, pp. 2363–2369, 2006.

[36] S. K. Singh, R. Katoch, and R. K. Kapila, "Chemotypic variation for essential oils in *Valeriana jatamansi* Jones populations from Himachal Pradesh," *Journal of Essential Oil Research*, vol. 25, no. 2, pp. 154–159, 2013.