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Studies on artemisinin, morphotypic and genetic characteristics of seventeen species of Artemisia growing in Indian Himalayan Region

Shilpi Paul[1,2*], K Chandra Sekar[1], Gopal Singh[1], Aseesh Pandey[1,3] and Monisha Bisht[1]

[1]G.B. Pant National Institute of Himalayan Environment (NIHE), Kosi-Katarmal, Almora 263643, Uttarakhand, India

[2]Science and Engineering Research Board,Vasant Square Mall, Vasant Kunj, 110070, New Delhi, India

[3]G.B. Pant National Institute of Himalayan Environment (NIHE), Sikkim Regional Centre, Pangthang, Gangtok 737101, Sikkim, India

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Seventeen species of *Artemisia* L. growing in the Indian Himalayan region were investigated for artemisinin content, morphological and genetic characters. During the investigation, artemisinin content was found to be in the range of trace to 0.12%. The highest was in *A. dracunculus* (0.12%) and *A. roxburghiana* (0.12%). The essential oil content was found in the range of 0.03-1.5%, *A. dracunculus* showed 1.5% oil (w/v) followed by *A. nilagirica* (0.6%) and *A. maritima* (0.6%). The AFLP analysis revealed 25.4% (*Hind*-ACC & *Mse*-CAC) to 67.38% (*Hind*-ACA & *Mse*-CAG) polymorphism while overall it was 51%. The dendrogram generated from AFLP data classified 8 species in one cluster (I) and 9 in another (II). The important pathway genes (HMGR, ADS and CYP71AV1) showed higher expression in *A. dracunculus*, *A. roxburghiana* and *A. sieversiana*. Based on the chemical analysis, the presence of a high amount of essential oil and artemisinin in *A. dracunculus* and *A. roxburghiana* suggested that these could be important medicinal plants for future research.

Keywords: *Artemisia* species, Artemisinin pathway genes, Artemisinin, Genetic diversity, Phenotypic diversity.

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Introduction

The genus *Artemisia* L. (Asteraceae) includes over 400 species which are mainly grown in Asia, Europe and North America[1,2]. About 32 species of *Artemisia* are found in the Indian Himalayan Region[3,4,5]. Most of the species namely *A. capillaris*, *A. dracunculus*, *A. edgeworthii*, *A. gmelinii*, *A. maritima*, *A. myriantha*, *A. nilagirica*, *A. parviflora*, *A. roxburghiana* *A. siversiana*, *A. verlotiorum*, and *A. wallichiana*, grow naturally in the Ladakh and the Indian Trans-Himalayan region at an altitude of 2,700 to 6,100 m a.s.l.[3]. Taxonomically it is a complex genus belongs to family Asteraceae, comprising many individual species with different morphological forms. Thus, individual plants of the different species may resemble each other in appearance which makes correct identification quite difficult, if only morphology is taken into account.

The genus *Artemisia* possess a large number of specialized metabolites like triterpenes, steroids, hydrocarbons, polyacetylenes, flavonoids, coumarins, mono- and sesqui-terpenes. These compounds have potential antibacterial, anti-inflammatory, antifeedant, cytostatic, antidiabetic, hepatoprotective, gastrointestinal and antimalarial activities[6,7,8]. Presence of different compound may be used to identify the different species of their chemotaxonomic characters. The genus *Artemisia* is chemically rich and contains medicinally important compounds such as Artemisinin and essential oil.

Cytology, genomic DNA isolation, and chemical diversity in few species of *Artemisia* have been reported[9,10], but detailed studies in most of the species remain to be done. Further, it is also reported that the genus *Artemisia* is highly heterozygous[11]. High heterozygosity and genetic relatedness in some species of *Artemisia* have been reported, Kumar *et al.*[12] while in terms of their chemical composition along with genetic markers studies remains to be done, hence research in such species requires attention. Terpenoid pathway is a well-studied pathway and most of the key pathway genes are reported, while studies on their expression under different environmental conditions in high valued medicinal plants remain to be carried out. In view of the importance of *Artemisia* species and to estimate their

*Correspondent author
Email: shilpipaul77@yahoo.com, shilpipaul01@gmail.com

artemisinin content in the other species of *Artemisia* growing in Indian Himalayan Region (IHR), the aim of present investigation is to 1) estimate phenotypic and genetic relationship and heterozygosity among the seventeen taxa of *Artemisia* growing in the high altitude; 2) carry out the investigation for artemisinin and essential oil content in the collected species of *Artemisia*; and 3) develop comparative expression profiles of key genes of terpenoid pathway such as HMGR, ADS and CYP71AV1.

Materials and Methods

Plant material

Twigs of seventeen species of *Artemisia* were collected in the month of September from wild (Lahul-Spiti district of Himachal Pradesh) along with the Thandi and Kelong (altitude ranging from 2,800-3,500 m a.s.l.) areas and were used to study the morphological, chemical, and molecular variation. The seeds of these plants were sown in the greenhouse of the Institute (NIHE), Kosi- Katarmal-Almora; altitude 1,150 m a.s.l.; 29°38'15" N and 79°38'10" E) to check survival at low altitude. One of the author (Dr. K C Sekar) has expertise in taxonomical identification, especially in cold desert regions of IHR. The collected species were identified using recent flora[4] and compared with the authentic specimens available in the Herbarium of Botanical Survey of India, Dehradun (BSD). Seeds of all collected species were sown, seedlings of *A. dubia, A. sieversiana, A.roxburghiana, A.dracunculus* and *A. biennis* were grown and showed 70% germination. One voucher specimen of all the species of *Artemisia* collected for the study was made following the standard procedure[13] and preserved as herbarium specimens. The voucher specimens are kept in the herbarium of the Institute.

Morphological characteristics

Quantitative and qualitative characters in the form of plant height (cm), leaf size (length and width), growth forms, shapes, the colour of stem and leaves, base and tip of leaves and colour of flowers were recorded for each species. The harvested shoot biomass was used for oil distillation and simultaneously air shade-dried (appx. 15-20% moisture) plant material was subjected for artemisinin analysis[14,15]. Fresh leaf samples were used for DNA and RNA isolation, AFLP and gene expression analysis. Plants were harvested in the month of September (at the time of seed setting) from the field and seeds of each species were stored at 4ºC.

Extraction of artemisinin and oil

Air shade-dried plant material of each species was powdered and the extraction was carried out following Paul *et al.*[15]. Plant material (10-50g) of each (full flowering stage) were taken for essential oil extraction using hydro-distillation (Clevenger type apparatus). The obtained essential oil was measured in W/V and collected in a vial for further analysis[16].

DNA isolation and AFLP analysis

Fresh leaf tissue was used for DNA isolation and AFLP analysis, following the method of Paul *et al.*[17]. The final character matrix used as input data for the phylogenetic analysis consisted of 340 binary characters representing the presence and absence in the species of *Artemisia*.

Isolation and amplification of RNA

Total RNA isolation and cDNA synthesis were carried out using the protocol mentioned in Paul *et al.*[15]. Primers of three key terpenoid pathway genes i.e. HMGR (HMGR, 3-hydroxy-3-methylglutaryl- CoA reductase; Gene bank No AF142473;[18] of MEP (MEP, 2-C-Methy-D-erythritol 4-phosphate) pathway, ADS (ADS, amorpha-4,11-diene synthase; Gene bank No EF197888;[19], and CYP71AV1 (CYP71AV1**,** amorpha-4,11-diene hydroxylase; Gene bank No DQ268763;[20,21]) were used to amplify the cDNA. The amplification was carried out using 94°C for 3 minutes, 94°C for 30 seconds, 56°C for 30 seconds, 72°C for 5 minutes and final extension was 72°C for 8 minutes for 42 cycles in a thermal cycler (Biometra, Germany). Further quantification of different genes was determined by comparing with the equal amount of cDNA template on formaldehyde gel[15].

Statistic analysis

For each primer pair, the number of polymorphic and monomorphic bands were determined; PI (polymorphic information content), MI (marker index) and β (proportion of polymorphic bands) were calculated following the method given in Paul *et al.*[15]. For the AFLP analysis, each polymorphic fragment was scored as a locus with two allelic classes. The PIC values were then calculated for each locus of every species.

The Nei and Li[22] similarity matrix was calculated with the presence and absence of bands and then subjected to cluster analysis, by an unweighted pair-group method with the arithmetic averages (UPGMA, Statistica version 8.0). Ne's heterozygosity, Gene diversity per locus, per population and Weir &

Cockerham 23 estimation of Fit, Fst and Fis were calculated using Fstat software.

Results

Morphological observations

Morphological data of each species of *Artemisia* were recorded at the time of collection from the field. The species *A. dubia* was tallest (80-90 cm) while *A. macrocephala, A. edgeworthii* and *A. roxburghiana* were small in height (20-30 cm). Varied leaf size and shape were observed and it was also found that all the species were aromatic except *A. biennis* (non-aromatic; Table 1). The leaves of *A. dubia, A. roxburghiana, A. eriocephala, A. dubia* var *subdigitata* and *A. edgeworthii* were bi-pinnatisect whereas *A. biennis, A. nilagirica, A. japonica, A. gmelinii, A. filiformilobulata, A. maritima, A. macrocephala, A. capillaris, A. salsoloides, A. sieversiana* and *A. myriantha* possessed pinnate-sected leaves while simple and undivided leaves were found in *A. dracunculus* which is specific character of this species. Most of the species were perennial except *A. capillaris* and *A. biennis* (biennial), whereas *A. maritima, A. macrocephala, A. edgeworthii* and *A. siversiana* were annual. The species *A. biennis* and *A. eriocephala* have brown coloured stem while other species have a green coloured stem (Table1).

Table 1 — Morphological characteristics of seventeen *Artemisia* species growing in the Indian Himalayan region

S.No	*Artemisia* species (Voucher No.)	GF	NLN	Aroma	LS	LA	CS	CL	LT	BTL	CF
1	*A. dracunculus* (GBP 2759)	P	Simple	*Aromatic*	linear-lanceolate to oblanceolate	Keylong 3350	Green	Green	Simple, undivided	Acute	White
2	*A. biennis* (GBP 2762)	*B*	pinnatisect	Non-aromatic	narrowly elliptic-lanceolate	Keylong 3350	Light brown	Green	pinnatisect/ pinnatifid	Acute	White
3	*A. dubia* (GBP 6035a)	*P*	bi-pinnatisect	Aromatic	oblong-elliptic	Keylong 3350	Green	Green	bi-pinnatisect	Acute	White
4	*A. roxburghiana* (GBP 2764)	*P*	bi-pinnatisect	Aromatic	broadly elliptic	Keylong 3350	Green	Green	bi-pinnatisect	Acute	White
5	*A .nilagirica* (GBP 2384)	*P*	pinnatisect	Aromatic	elliptic - lanceolate	Tandi 2500	Green	Green	pinnatisect/ pinnatifid	Acute	White
6	*A. japonica* (GBP 6025)	*P*	pinnatisect	Aromatic	linear to narrow lanceolate	Keylong 3350	Green	Green	pinnatisect/ pinnatifid	Acute	White
7	*A. eriocephala* (GBP 2336)	*P*	bi-pinnatisect	Aromatic	broadly elliptic	Keylong 3010	Brown	Green	bipinnatisect	Acute	White with purplish tinge
8	*A. gmelinii* (GBP 2378a)	*P*	pinnatisect	Aromatic	linear-lanceolate	Keylong 3200	Green	Green	pinnatisect/ pinnatifid	Acute	Yellowish
9	*A. filiformilobulata* (GBP 2367)	*P*	pinnatisect	Aromatic	linear-lanceolate	Tandi 2800	Green	Green	pinnatisect/ pinnatifid	Acute	White
10	*A. dubia var subdigitata* (GBP 6035b)	*P*	bi-pinnatisect	Aromatic	oblong-elliptic	Keylong 3300	Green	Green	bipinnatisect	Acute	White
11	*A. maritima* (GBP 3523)	*A*	pinnatisect	Aromatic	elliptic-linear	Tandi 2900	Green	Green	pinnatisect/ pinnatifid	Acute	White
12	*A. macrocephala* (GBP 2378b)	*A*	pinnatisect	Aromatic	linear - oblanceolate	Keylong 3300	Green	Green	pinnatisect/ pinnatifid	Acute	White
13	*A. edgeworthii* (GBP 2386)	*A*	bi-pinnatisect	Aromatic	linear - lanceolate	Keylong 3300	Green	Green	bipinnatisect	Acute	White
14	*A. capillaris* (GBP 2379)	*B*	pinnatisect	Aromatic	linear - lanceolate	Tandi 2950	Green	Green	pinnatisect/ pinnatifid	Acute	White
15	*A. salsoloides* (GBP 2383)	*P*	pinnatisect	Aromatic	linear - oblong	Keylong 3320	Green	Green	pinnatisect/ pinnatifid	Acute	White
16	*A. sieversiana* (GBP 2733)	*A*	pinnatisect	Aromatic	linear - oblong	Tandi 2850	Green	Green	pinnatisect/ pinnatifid	Acute	White
17	*A. myriantha* (GBP 2391)	*P*	pinnatisect	Aromatic	Ovate - elliptic	Tandi 2850	Green	Green	pinnatisect/ pinnatifid	Acute	White

Note: GF - Growth form (Annual / Biennial / Perennial), NLN- No of leaves per node; LS Leaf Shape; LA- Locations and Altitude (masl); CS- Color of stem; CL- Color of leaves; LT- Type of leaf; BTL-Base and tip of leaf; CF- Color of flower

Chemical analysis

All the collected species (wild) were analyzed for artemisinin and oil content. Artemisinin content was found in the range of trace to 0.12%, while the highest artemisinin content was found in *A. dracunculus* (0.12%) and *A. roxburghiana* (0.12%). Oil content was in the range of 0.03-1.5% (Fig. 1). The species *A. dracunculus* showed 1.5% oil (w/v) followed by *A. nilagirica* (0.6%) and *A. maritima* (0.6%). The species *A. salsoloides*, *A. myriantha*, *A. edgeworthii* and *A. filiformilobulata* showed zero-trace artemisinin content. Few species like *A. dracunculus* showed highest artemisinin and oil content at full grooming stage in the month of June and July in seed-grown plants (in institute greenhouse).

AFLP analysis

Twelve AFLP primer combinations (*Hind* III and *Mse* I) were used to generate finger printing profiles. A total of 595 AFLP bands were identified in this study, among them 340 were polymorphic with clear and discrete patterns. The number of polymorphic bands ranged from 20 to 47 per gel with an average of 39.47 per primer combination. The percent polymorphism ranged from 25.4% (combination *Hind*-ACC and *Mse*-CAC, H1M1) to 67.38% (combination *Hind*-ACA & *Mse*-CAG, H3M1). The overall polymorphism among the seventeen species was 51%. The marker index (MI) per primer combination varied from 8.90 to 23.00 with an average of 14.17. In addition, the proportion of missing values had no influence on these quality parameters. In respect of the entire collection of plant species, PIC values were found to range from 0.56 to 0.92.

To evaluate the genetic distance and interspecific relationships among the seventeen species of *Artemisia*; similarity matrix was calculated based on 340 AFLP loci. Among the seventeen species, matrix data showed a normal distribution with the range of 0.442-0.816.

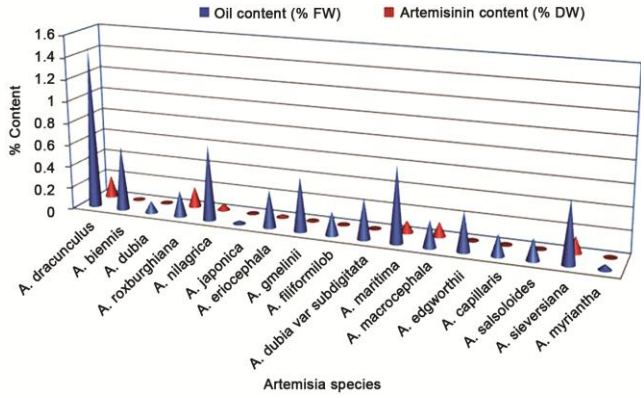

Fig. 1 — Essential Oil and Artemisinin content in seventeen species of *Artemisia*.

Phylogenetic and heterozygosity analysis

Phylogenetic relationships were estimated using quantitative (morphological) and AFLP data. The morphological dendrogram showed two major groups. In Group I *A. maritima*, *A. japonica*, *A. filiformilobulata*, *A. myriantha*, *A. eriocephala*, *A. macrocephala*, *A. edgeworthii*, *A. roxburghiana*, *A. salsoloides*, *A. sieversiana*, *A. capillaris*, *A. gmelinii*, *A. dracunculus*, *A. edgeworthii*, while group (II) comprises *A. nilagirica*, *A. biennis*, *A. dubia*, *A. dubia* var. *subdigitata*. This dendrogram revealed a close relationship among *A. nilagirica*, *A. biennis*, *A. dubia*, *A. dubia* var. *subdigitata* (Fig. 2).

Further AFLP markers were also used to establish phylogenetic relationship among the species. The dendrogram showed two major clusters, 9 species were in the cluster (I) and 8 were in the cluster (II). The Ist cluster was found to have two sub-clusters: one (IIa) consisted only *A. roxburghiana* and another (IIb) comprises seven species. It was found that *A. dracunculus* and *A. biennis* were closely related (Fig. 3). All the alleles generated through AFLP

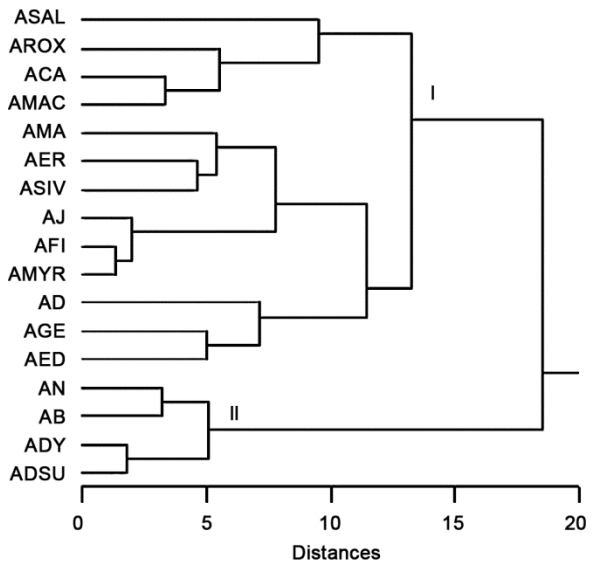

Fig. 2 — Dendrogram illustrating the morphological (quantitative) relationship among the 17 species of *Artemisia*.
Note: *ASAL- A. salsoloides; AROX- A. roxburghiana; ACA- A. capillaris; AMAC- A. macrocephala; AMA- A. maritima; AER- A. eriocephala; ASIV- A. sieversiana; AJ- A. japonica; AFI- A. filiformilobulata; AMYR-A. myriantha; AD-A. dracunculus; AGE- A. gmelinii; AED- A. edgeworthii; AN- A. nilagirica; AB- A. biennis; ADU- A. dubia; ADSU- A. dubia var subdigitata*

profile were used to estimate heterozygosity among the studied species. Average expected heterozygosity was higher than observed heterozygosity. The magnitude of genetic diversity among the investigated *Artemisia* species was 0.377. As *Artemisia* genus falls under highly open-pollinated plant category, therefore, heterozygosity is highly expected and was also observed (Table 2).

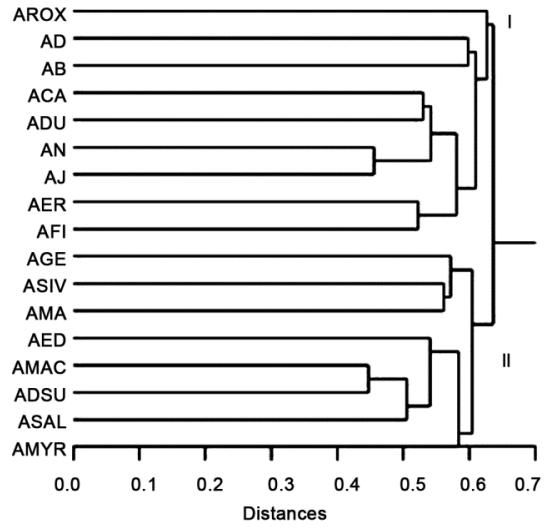

Fig. 3 — Dendrogram illustrating inter-specific relationships (based of AFLP) among 17 analyzed species of *Artemisia*.
Note: *ASAL- A. salsoloides; AROX- A. roxburghiana; ACA- A. capillaris; AMAC- A. macrocephala; AMA- A. maritima; AER- A. eriocephala; ASIV- A. sieversiana; AJ- A. japonica; AFI- A. filiformilobulata; AMYR-A. myriantha; AD-A. dracunculus; AGE- A. gmelinii; AED- A. edgeworthii; AN- A. nilagirica; AB- A. biennis; ADU- A. dubia; ADSU- A. dubia var subdigitata*

Gene expression analysis

Semi-quantitative differential gene expressions were observed among the 17 species of *Artemisia* with three genes i.e. HMGR, ADS and CYP71AV1 of terpenoid pathway were studied to understand artemisinin biosynthesis in the species. These genes showed higher expression in *A. dracunculus*, *A. roxburghiana* and *A. sieversiana* than the rest other species. Some species e.g *A. myriantha, A. dubia*, *A. dubia* var. *subdigitata, A. nilagirica* showed low expression with HMGR and ADS (Fig. 4), although all species showed expression with CYP71AV1 gene suggested that plant cytochrome P450 oxygenase enzyme may regulate the other enzymes of terpenoid pathway.

Discussion

It was found from fossil records that this genus was originated from semi-arid Asia about 20 million years ago during mid-Cenozoic era[23]. Hence from central Asia diverse population of *Artemisia* have been observed[24,25]. Most of the members of this genus belong to herb, shrub and have perennial habitat[25]. While it was also recorded that some species have annual or biannual habitat[26]. Similarly in our investigation 10 out of 17 species were observed to be perennial. Leaf characters i.e. leaf size and shape, tip and base were also found as distinguishing feature in *A. dubia*, *A. dubia* var. *subdigitata*, *A. roxburghiana* and *A. maritima* etc. Species of *Artemisia* e.g. *A. dracunculus* possess simple and undivided leaves while it was also observed that

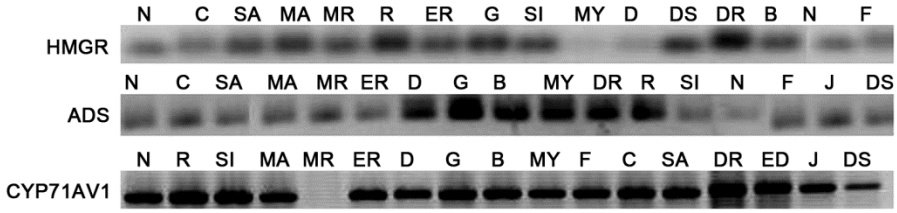

Fig. 4 — Differential expression of key terpenoid pathway genes of *Artemisia* species.
Note. HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase gene; ADS: amorpha-4, 11-diene synthase gene; CYP71AV1: amorpha-4, 11-diene hydroxylase gene
N- *A. nilagirica; C- A. capillaris; SA- A. salsoloides; MA- A. maritima; MR- A. macrocephala; ER- A. eriocephala; D- A. dubia; G- A. gmelinii; B- A. biennis; MY- A. myriantha; F- A. filiformilobulata;R-A. roxburghiana;SI-A. sieversiana;DR-A. dracunculus; ED-A. edgeworthii; J-A. japonica; DS- A. dubia* var *subdigitata*

Table 2 — Heterozygosity and gene diversity in the studied plants of seventeen species of *Artemisia*

	Ho	*Hs*	*Ht*	*Ht'*	*Dst*	*Dst'*	*Gst*	*Gst'*
Seventeen species of *Artemisia*	0.032	0.2722	0.375	0.3174	0.353	0.377	0.1144	0.135

Note: Ho-Observed heterozygosity; Hs- Average expected heterozygosity

Ht-Total gene diversity in population; Ht'-Average gene diversity in the population Dst- Absolute magnitude of gene differentiation among the population Dst'-Average gene diversity among subpopulation; Gst-Relative gene diversity Gst'-Standard measure of gene diversity

A. dubia, A. roxburghiana, A. dubia var. *subdigitata,* and *A. edgeworthii* have bi-pinnatisect leaves.

Level of artemisinin in the leaves of some *Artemisia* species was also investigated by some authors[27,28], where 0.44 to 0.05% artemisinin was reported while in our investigation 0.12%- to trace artemisinin (*A. roxburghiana*-0.12%, *A. dracunculus*-0.12% and *A. siversiana*-0.10%) was observed. Although high artemisinin yielding varieties are from *A. annua* species[14,29] and it is only source of artemisinin production till today. Artemisinin not only has antimalarial properties but also have some anticancer properties which are able to quench the cancer cells causing breast cancer[30]. Some species of *Artemisia* have essential oil along with artemisinin which has huge economic demand, in this category, the species *A. dracunculus* (known as 'tarragon") have high medicinal properties related to its essential oil and artemisinin presence. The essential oil of the species *A. dracunculus* showed antidiabetic property and posses compounds namely tarralin, 2'4'-dihydroxy-4-methoxy dihydrochalcone, 4,5 di-O-caffeoyliquinic acid in the essential oil[31]. Therefore, this species was found in a separate group in the chemical cluster which has been well supported with the report of Mannan *et al.*[27]. It was also reported that some *Artemisia* species (*A. maritima, A. gmelinii, A. roxburghiana* var. *hypoleuca, A. wallichiana, A. myriantha* var. *pleiocephala, A. elegantissima* var. *kumaonensis, A. indica* var. *indica, A. velutina, A. roxburghiana* var. *purpurascens, A. capillaris, A. parviflora, A. dracunculus* and *A. nilagirica)* growing in Western Himalayan exhibited low molecular weight volatile compound[32]. Whereas in the present investigation the high essential oil content was observed in *A. nilagirica, A. maritima* and *A. dracunculus.* Some species also possess strong and aromatic properties which are due to the presence of high concentrations of volatile terpenes, constituents in their essential oils, especially in leaves and flowers. Based on the existing reports the genus *Artemisia* is highly heterogenous therefore diverse inter species populations were observed. The phylogenetic analysis through RAPD[33] and a high level of intra specific diversity in *A. vulgaris* was observed in comparison to other species of *Artemisia*[34]. While in another study, a close association during the evolutionary process was observed between *A. japonica* and *A. persica* of sections *Dracunculus* Besser and *Absinthium* DC. In a separate study[22], Korean *Artemisia* species were investigated through PCR- RFLP using *trn*L–F sequences (chloroplast DNA) and showed specific banding patterns for *A. apiacea, A. Keiskeana* and *A. sieversiana*[35]. The AFLP profiles in the present investigation showed significant polymorphism among the seventeen species and high heterozygosity. The AFLP dendrogram showing close association among *A. dubia* var. *subdigitata, A. salsoloides* and *A. myriantha*. In a report by Mahmood *et al.*[36], RFLP with *rps* 11 gene which revealed the phylogenetic relationship among the species of *Artemisia* and reported 38% cluster diversity among *A. brevifolia, A. japonica, A. vulgaris, A. dubia, A. roxburghiana. A. tangutica* species of *Artemisia*. Based on the molecular phylogenic analysis[37], two main groups i.e the *Artemisia*-group and the *Chrysanthemum*-group (*Dendranthema* group) of subtribe Artemisiinae was suggested. However, different taxonomic studies have divided *Artemisia* into five large groups; *Absinthium* DC., *Artemisia* (*Abrotanum* Besser), *Dracunculus* Besser, *Seriphidium* Besser and *Tridantatae*[38] but, this infrageneric classification is not accepted by all taxonomists, because naturally occurring groups are not properly represented. Therefore, to dissolve this ambiguity, molecular evidence is required. High heterozygosity within the species has been reported in *A. annua* by Graham *et al*[11]. Similarly, in present investigation high heterozygosity was observed among the species of *Artemisia* this is attributed to the open-pollinated nature of the genus.

Biosynthetic pathway of artemisinin in *A. annua* is well investigated and most of the terpenoid pathway genes e.g DXR, DXS, HMGR, FPS, and CYP71AV1 etc. were reported in *A. annua*[39,40,41,42] and also expressed in yeast[43]. The gene amorpha -4,11-diene hydroxylase (CYP71AV1) catalyzes multiple oxidations of the sesquiterpene intermediate amorpha-4, 11-diene to form artemisinic acid[44,19]. It is a multifunctional enzyme, and expression analysis reveals that CYP71AV1 specifically expressed in glandular secretory trichomes (GSTs), hence it is known as the biological factory of artemisinin biosynthesis[21,45]. The other genes such as HMGR and ADS were also well-investigated and over-expressed in *A. annua*, suggested enhanced ability to synthesized artemisinin[46]. Similarly, in the present investigation HMGR and ADS showed differential expression in all species, whereas, higher expression of all the three genes were found in *A. dracunculus, A. roxburghiana* and *A. sieversiana,* suggesting

synthesis of artemisinin in these species. Although the artemisinin content in these species was quite low as compared to *A. annua* the presence of artemisinin opens new avenues for future research for producing more artemisinin and other specialized metabolites.

Conclusion

The observations found in the present investigation suggested more than 40% divergence among the studied species, which may be their phenotypic variations. Presence of artemisinin in the range of trace to 0.12% where high artemisinin was found to be in *A. dracunculus* (0.12%) and *A. roxburghiana* (0.12%), essential oil content was observed in the range of 0.03-1.5%, were high in *A. dracunculus (*1.5% oil; w/v) followed by *A. nilagirica* (0.6%) and *A. maritima* (0.6%) suggested that more attention is required in such species of *Artemisia.* Gene expression profile also supported artemisinin synthesis in these species. Based on the investigation, presence of essential oil and artemisinin content in species like *A. dracunculus, A. roxburghiana* and *A. siversiana* suggested that these could be the potential medicinal plants for further future research.

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Conflict of interest

Authors state that they have no conflict of interest.

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