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RESEARCH ARTICLE

ASSESSMENT OF EST-SSR AND RAPD MARKERS FOR GENETIC ANALYSIS ON *ARTEMISIA* SPECIES

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ABSTRACT

Artemisia species has various medicinal properties, constituting the main source artemisinin, an antimalarial drug which has widespread importance to mankind. The production of artemisinin either in cell/tissue/the whole plant of *Artemisia* species is therefore highly desirable. This artemisinin varies in different species of *Artemisia* based on their genetic diversity. Thus in the present study an attempt has been made successfully to assess the genetic diversity among *Artemisia* species using EST-derived SSR marker as well as RAPD marker. The five species of *Artemisia* were collected from different regions of Karnataka, India and used for the DNA isolation using CTAB method and DNA quantified using 260/280 in UV-Vis spectrophotometer. The Isolated DNA was amplified using five EST derived Simple Sequence Repeat (SSR) markers and 10 RAPD marker. Further the amplified products were separated by Gel Electrophoresis. The bands were scored for the presence or absence of bands. SSR produced a total of 25 alleles with an average of 5 alleles per locus. The polymorphism information content (PIC) reflections of allele diversity, frequency among the varieties ranged from 0.03 to 0.33, with an average of 0.18. The 10 decamer-RAPD primers generated 352 RAPD fragments. Most of the RAPD markers studied showed different level of genetic polymorphism. Pairwise Nei and Li's similarity coefficient value ranged from 0.62-0.83 for 5 species of medicinal plants. A dendrogram was constructed using Unweight Pair Group Method with Arithmetic Mean (UPGMA) revealing 5 genotype with 4 clusters (SSR marker) and 3 clusters (RAPD marker), wide range of dissimilarity values which showed a high degree of diversity among the cultivars. The *Artemisia* species showed rich allelic diversity, indicating that there is great potential for identification of associations with present genotypes. Thus this method of analysis can be a helpful tool in selecting diverse parents and give broadness to the germ plasm base of medicinal plant breeding programs for the future, which can be utilized to develop new varieties with traits of interest.

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INTRODUCTION

Artemisia species are an aromatic, herbaceous plant belongs to Asteraceae family. It grows mainly in the middle, eastern and southern parts of Europe, northern, middle and eastern parts of Asia and in North Africa, it grow naturally in cold arid desert region of trans-Himalaya in India (Simon and Cebert, 1994; Ferreiraand Janick, 1996).The plant species of the genus *Artemisia* possess a plethora of bioactivities, such as antihelminthic, antiseptic and anti-inflammatory properties (Tigno *et al*., 2000; Govindaraj *et al*., 2008). *Artemisi*a was originally collected by the Chinese as herbal medicine and is currently processed by pharmaceutical firms for the production of artemisinin derivatives for its effective treatment to malaria. So, *Artemisia* species still remain as one of major source of drug in the traditional and modern system of medicine throughout the world. Therefore the enhanced production of artemisinin in the whole plant of *Artemisia* species is highly desirable (Jitendra Kumar *et al*., 2014). For the purpose of efficient conservation and successful breeding programme of different species of *Artemisia*, it will be prudent to study the populations of *Artemisia* species at genetic and molecular levels. Studying the variations at molecular level provides devising strategies to protect the genetic diversity of the plant. Moreover this study of genetic diversity also helps us to compare the genotypes of various species of *Artemisia* for an effective taxonomic and evolutionary studies thereby helping in providing a supportive data for taxonomical conformation of the genotypes. The genetic variability also can be exploited to select useful genotypes that could be utilized as cultivars to avoid batch to batch variation in extraction of standard drugs

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(Jitendra Kumar *et al*., 2014). It also helps in developing DNA based molecular markers for identification of genotypes with better traits. However, so far reports are not available regarding genetic characterization of species in *Artemisia* plants, hence detailed investigation is required. Therefore, the present investigation of SSR and RAPD molecular marker were selected for molecular characterization. The SSR markers are extremely valuable and are increasingly becoming popular in comparative genomics where SSR markers developed from one species could be utilized in a related or heterologous species towards genetic mapping, characterization, gene cloning, diversity, evolutionary studies of genetic variation, linkage mapping, gene tagging, establishment of genetic maps, integration of physical and genetic maps, determination of evolutionary relationships and comparative genome analysis (Karlin *et al*., 1998; Katti *et al*., 2001). Further, DNA fingerprinting distinguishes different varieties according to their DNA variations at a set of genetic loci. A Powerful and convenient molecular marker system such as randomly amplified polymorphism of DNA (RAPD) analysis is also used for the genetic mapping, taxonomic and polygenic studies of many plants. Their environmental stability and nearly unlimited availability have made RAPD markers an ideal tool for the plants (Gebhardt, *et al*., 1989; Slocum *et al*., 1990). Therefore a detailed karyological features of SSR and RAPD profiling have been applied to assess the genetic diversity among populations of five species of *Artemisia*. Hence for the first time a detailed comparative investigation of molecular characterization of *Artemisia* species has been undertaken for the study.

MATERIALS AND METHODS

Plant Materials

The five species of *Artemisia* genus were selected for the present research work. *Artemisia annua*, *Artemisia japonica* and *Artemisia nilagirica* were collected from Indian Institute of Horticultural Research, Bangalore. *Artemisia pellan* wall was collected from Hulimavu Horticulture Research Centre, Bangalore and *Artemisia abrotanum* was collected from Lalbagh, Bangalore. Which are grown in green house of Visveswarapura College of Science, Bangalore.

Isolation of genomic DNA

Total genomic DNA from all the 5 genotypes of *Artemisia* was extracted using the fresh leaves by CTAB method as described by Sambrook *et al*., (2001). CTAB (Cetyl trimethyl ammonium bromide) is a detergent and is used along with other reagents to liberate nucleic acids from the cell. To remove RNA contamination, RNase A (10 mg/ml, Sigma, USA) was added. The DNA concentration and purity was determined (A260/A280) by UV spectrophotometric analysis (Sambrook *et al*., 1993; 2001).

Amplification conditions for SSR Primers PCR Program

Five selected SSR makers (Table 1) were used for amplification and carried out in a Thermal cycler (Eppendorf make). The amplification profile was as follows:Initial Denaturation – 94^0C for 4 min, Denaturation – 94^0 C for 45 sec, Annealing – 59^0 C for 30 sec (touch down every cycle by 0.30^0C for 10cycles), Extension – 72^0 C for 1 min, Denaturation – 94^0 C for 45sec. Annealing – 56^0 C for 30 sec, Extension – 72^0 C for 1 min (25 cycles). Final Extension – 72^0 C for 5 min. The genetic diversity of the samples as a whole was estimated based on the number of alleles per locus (total number of loci analysed) and polymorphism was determined according to the presence or absence of the SSR locus. The value of PIC was calculated.

RAPD Marker Analysis and PCR Program

Ten decamer Oligonucleotide primers (Table2) (Operon Technologies, Alameda, USA) were used for polymerase chain reaction (PCR) for screening of amplification and detection of polymorphism. The RAPD-PCR reactions were performed in 10 µl volumes in 100 µl PCR tubes (Tarson Pvt., Ltd., India). The reaction mixture contained 25-30 ng of template DNA, 1× amplification buffer (10 mM of Tris–HCl – pH 8, 50 mM of KCl, 1.8 mM of $MgCl_2$ and 0.01 mg/ml gelatine), 2.0 mM each of dCTP, dGTP, dATP, and dTTP, 5 pM primers and 1 U Taq DNA polymerase (Bangalore Genei, Pvt., Ltd., India). The reactions were performed in a Master Cycler Gradient 5331 (Eppendorf version 2.30. 31-09, Germany) with an initial denaturation step at 94 °C for 4 minutes, followed by 35 cycles of 94 °C for 1 minute, 37 °C for 1 minute, 72 °C for 2 minutes. The final extension step was at 72 °C for 10 minutes. The reactions were then cooled and hold at 4 °C.

GEL Electrophoresis

The PCR products were separated on 1.5% (w/v) agarose (Sigma–Aldrich, USA) gel at 5 V/cm in 1 × TBE (89 mM Tris–HCl, 89 mM boric acid and 2 mM EDTA, pH 8.0) buffer. Agarose gel was prepared using electrophoresis grade agarose in 1×TBE buffer. Ethidium bromide was added at concentration of 0.5 µg/ml of gel. The gel was allowed to set fully before removing the comb and loading the sample. 5 µl of loading dye was added to 20 µl of PCR products and mixed well before loading into the wells. A voltage of 5v/cm was given for a time period of three hours for separation of PCR fragments. The DNA profile image was captured using the (BioRad) gel documentation unit and stored.

Data Analysis

DNA fragments amplified by SSRand RAPDprimer was scored for each band as present (1) or absent (0) and from band scores a binary data matrix was constructed. Pair-wise genetic similarity coefficients were calculated according to Jaccard (1908). The similarity coefficient was used to construct a Dendrogram by the un-weighted pair group method with arithmetic average (UPGMA) and Sequential, Agglomerative, Hierarchical and Nested (SHAN) features of NTSYS-pc version 2.02 as per (Sneath and Sokal, 1973). In addition, the discrimination power of each markers was evaluated by the polymorphism information content (PIC) according to Powell *et al*. (1996) as follows. PIC = $\Sigma 1–pi^2$, where pi is the frequency of the i^{th} band or percentage of varieties in which the fragment is present.

RESULTS

Data analysis of SSR marker

Overall allelic diversity: Using DNA sample isolated from 5 genotypes of genus *Artemisia* varieties as templates, polymorphic DNA fragments were amplified from 5 out of 5 SSR primer pairs selected in the study. The size of these fragments ranged from 150 to 300bp. A total 25 alleles with the average alleles per locus of 5 were detected. Each single allele detected identified as polymorphic (Fig.1).

PICVALUE: PIC refers to the value of a marker for detecting polymorphism within a population or set of genotypes by taking in to account not only the number of expressed alleles but also the relative frequencies of alleles.

$PIC = 1 - \Sigma fi^2$
$i = 1 – n$

Where, fi is the frequency of the i^{th} amplicon. The number of amplicons refers to the number of scored bands. The frequency of an amplicon was obtained by dividing the number species, where it was found by the total number of species. The PIC value (Polymorphism information content) provides an estimate of the discriminating power of a marker. Marker index was calculated for each primer as the product of PIC and the number of polymorphic bands. Primer 5 gives 96% of polymorphism with 0.96 PIC value and primer 4 gives 88% polymorphism with 0.88 PIC value (Table 3).

Genetic distance-based analysis: A significant high genetic variation was found among a genotypes with Jaccards similarity coefficient ranged from 0.03 to 0.33. All 5 genotypes could be discriminated successfully by SSR marker (Fig.2). The genotype collected were distributed among 4 genetic clusters, group 1 contained *Artemisia japonica* and *Artemisia nilagirica*, group 2 having *Artemisia abortanum*, group 3 having *Artemisia pellan* wall, and group 4 is a *Artemisia annua*, which all are single cluster genotype(cluster2 to 4).

Pairwise genetic dissimilarity: A dissimilarity matrix was used to determine the level of relatedness, among the Cultivars studied. The pairwise genetic dissimilarity indices (Table 4) indicated that the highest genetic dissimilarity was between *Artemisia annua* to *Artemisiajaponica* (100%), *Artemisia pellan wall* to *Artemisia japonica* (100%), *Artemisia abortanum* to *Artemisia japonica* and *Artemisia nilagirica* (100%), the lowest genetic dissimilarity is between *Artemisia nilagirica* to *Artemisia jap*onica (69%), *Artemisia pellan* wall to *Artemisia nilagirica* (90%). Thus the SSR marker provides adequate power of resolution to discriminate between *Artemisia* species and it could serve as a potential tool in the identification and characterization of genetically distant cultivars from sources.

Data analysis of RAPD marker

The RAPD banding patterns of the 5 *Artemisia* species are illustrated in Figure 2. The ten primers used for RAPD-PCR were able to amplify the DNA from all the plant species studied. A total of 352 bands were observed for 5 plant species using ten primers. For 5 plant species, the maximum number of well-defined or major bands was observed with primer OPA 15 (47 bands) and the minimum number with primer OPAC 2(18 bands) (Figure 1 to 5). Primers OPAC 5, OPAC 1, OPAC 15, OPA 08, OPA 05, OPA 11, OPD 08 and OPAC4 produced distinct banding patterns for all the plant species tested.

RAPD Analysis of Medicinal Plants using Primer OPA-8

The genomic DNA of 5 *Artemisia* plants was amplified with decamer Oligonucleotide primers such as OPA-08 and as shown in Figure 2. The distinct and abundant RAPD fragments were recorded. The total numbers of bands generated were 40 RAPD gel profiles. The primer produced monomorphic banding pattern in all the five medicinal plant species, the number of RAPD bands per plant were 8.

RAPD Analysis of Medicinal Plants using Primer OPA–05

The data obtained in the present investigation revealed a total number of 34 RAPD bands. The genomic DNA of 5 *Artemisia* plants amplified with OPA-05 revealed polymorphic RAPD bands. The distinct and abundant RAPD fragments were recorded. The primer produced medium low and high resolution of RAPD bands. The number of bands per plant was recorded at a maximum of 7 bands to 4 bands. The no of polymorphic bands are 22 and 65% polymorphism. The plant *Artemisia abortanum* and *Artemisia pellan* wall give the same banding pattern, as shown in Figure 2.

RAPD Analysis of Medicinal Plants using Primer OPA–11

The data obtained in the present investigation revealed a total number of 39 RAPD bands. The genomic DNA of 5 *Artemisia* plants amplified by OPA-11, 27 Polymorphic RAPD bands and 71% polymorphism. The distinct and abundant RAPD fragments were recorded. The primer produced medium low and high resolution of RAPD bands. The number of bands per plant was recorded at a maximum of 9 bands to 7 bands.

RAPD Analysis of Medicinal Plants using Primer OPA–15

The data obtained in the present investigation revealed a total number of 47 RAPD bands. The genomic DNA of 5 *Artemisia* plants amplified by OPA-15, 20 Polymorphic RAPD bands and 43% polymorphism. The distinct and abundant RAPD fragments were recorded. The primer produced medium low and high resolution of RAPD bands. The number of bands per plant was recorded at a maximum of 10 bands to 5 bands. Plant *Artemisia annua*, *Artemisia nilagirica* and *Artemisia abortanum* gives slightly similar banding pattern.

RAPD Analysis of Medicinal Plants using Primer OPD-8

The data obtained in the present investigation revealed a total number of 34 RAPD bands. The genomic DNA of 5 *Artemisia* plants amplified by OPD-8,17 Polymorphic RAPD bands and 51% polymorphism. The distinct and abundant RAPD fragments were recorded. The primer produced medium low and high resolution of RAPD bands. The number of bands per plant was recorded at a maximum of 7 bands to 6 bands.

Table 1. List of primers used for SSR amplification. GC content, total number of loci, the level of polymorphism and PIC value

Primer	Primer sequence(5'-3')G	GC (%)	Tm (c)	Allele number	Product size	PIC
1F	GCATGCATTTATGTTGGATCAC	40.9	56.5	2	100-400	0.565
1R	CAGCAGCAACAACAACAACAG	47.6	57.9			
2F	GTAAGTTATACCTGGTTTCCAGC	43.5	58.9	4	300-600	0.595
2R	ACCACTACACCTTGCATTCTA	42.9	55.9			
3F	CTCTCTTCCTCTTTGTGTGTCT	45.5	58.4	4	150-500	0.503
3R	CAAGATGGTACGAATACTGTTG	40.9	56.5			
4F	CGAGCAATCGGAGAGTTAGC	55.0	59.4	7	250-550	0.802
4R	ATGCATCTCGCGAATCTTCT	45.0	55.3			
5F	GTGTGAGGCCTCTGCTCTG	63.2	61.0	5	120-480	0.716
5R	ACCGCCATGTCTTCTCCATA	50.0	57.3			

Table 2.Sequence information of RAPD oligo nucleotide primers used for amplification and polymorphism study in 5 medicinal plants

S.No.	Oligo Primer	Sequence
1	OPA-05	AGGGGTCTTG
2	OPA-08	GTGACGTAGG
3	OPA-11	CAATCGCCGT
4	OPA-15	TTCCGAACCC
5	OPD-08	GTGTGCCCCA
6	OPAC-1	TCCCAGCAGA
7	OPAC-02	GTCGTCGTCT
8	OPAC-04	ACGGGACCTG
9	OPAC-05	GTTAGTGCGG
10	OPAC-15	TGCCGTGAGA

Table 3. PIC values (SSR) for *Artemisia* species

S.No	Primer	PIC	% Polymorphism
1	Primer 1	0.93	93
2	Primer 2	0.87	87
3	Primer 3	0.93	93
4	Primer 4	0.88	88
5	Primer 5	0.96	96

Table 4. Pairwise genetic distance (Nei's genetic distance) indices among 5 varieties of *Artemisia* varieties obtained from SSR marker analysis

	A.annua	*A.japonica*	*A.nilagirica*	*A.abrotanum*	*A.pellan wall*
A.annua	0				
A.japonica	1	0			
A.nilagirica	0	0.69	0		
A. abrotanum	0	1	0.9	0	
A. pellan wall	0	1	1	0	0

Table 5. RAPD analysis (Total bands, number of polymorphic bands and percent of polymorphism per primer

S.No	Marker	Total bands/primer	No of polymorphic bands	%polymorphism
1	OPA11	39	27	71
2	OPA15	47	20	43
3	OPA08	40	0.00	0
4	OPA 05	34	22	65
5	OPD08	34	17	51
6	OPAC 1	29	22	76
7	OPAC 2	18	11	64
8	OPAC 4	34	9	27
9	OPAC 5	37	11	31
10	OPAC 15	40	18	47

Table 6. Pairwise genetic distance (Nei's genetic distance) indices among 5 varieties of *Artemisia* varieties obtained from RAPD marker analysis

	A.annua	*A.japonica*	*A.nilagirica*	*A.abrotanum*	*A.pellan wall*
A.annua	0				
A.japonica	2.6085	0			
A.nilagirica	2..7794	1.6314	0		
A. abrotanum	1.3246	2.3041	1.7784	0	
A. pellan wall	3.4830	3.4089	3.3606	2.7556	0

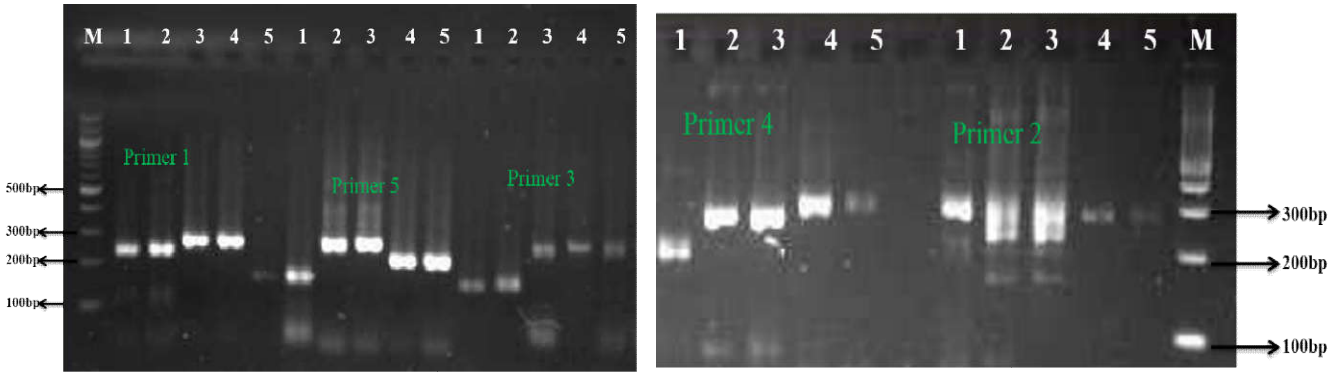

Figure 1. M= ladder, lane 1= *Artemisia annua*, lane 2= *Artemisia japonica* , lane 3= *Artemisia nilagirica,* lane 4= *Artemisia abortanum,* lane 5= *Artemisia pellan wall*

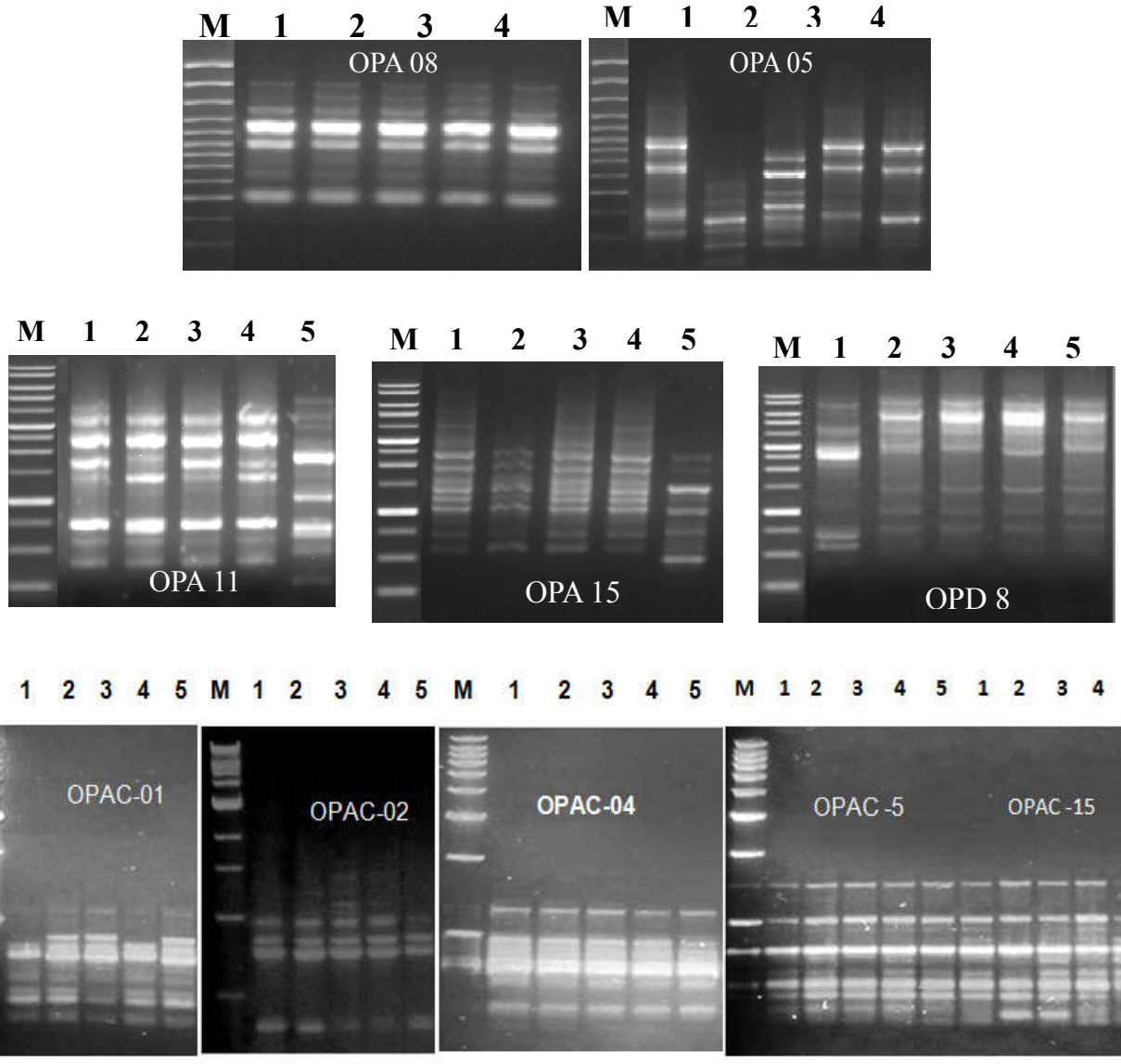

Figure 2. M= 100bp ladder, lane 1= *Artemisia annua*, lane 2= *Artemisia japonica,* lane 3= *Artemisia nilagirica* , lane 4= *Artemisia abrotanum,* lane 5= *Artemisia pellan wall*

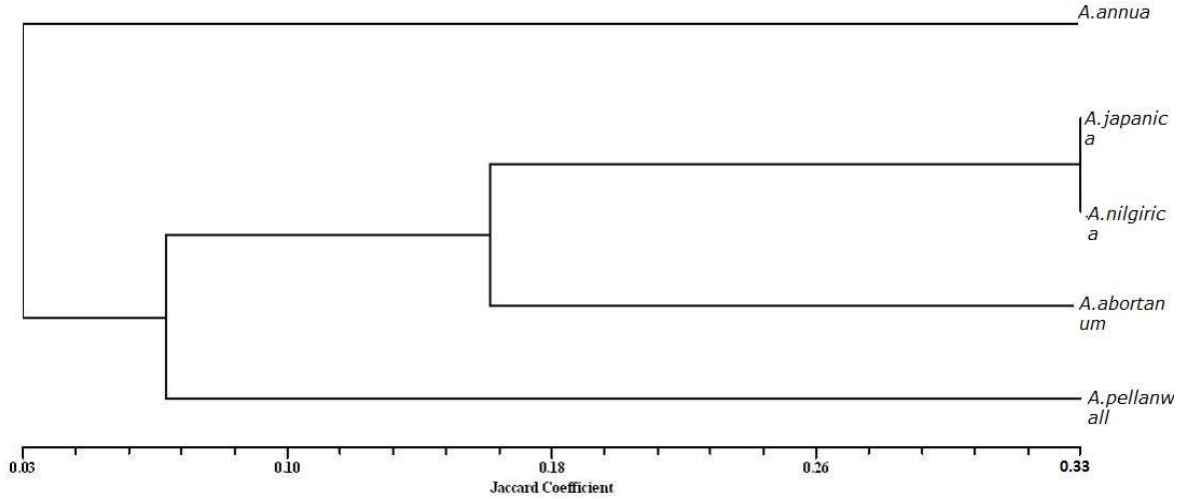

Figure 3.An UPGMA cluster Dendrogram showing the genetic relationships among 5 *Artemisia* verities based on 5 SSR markers

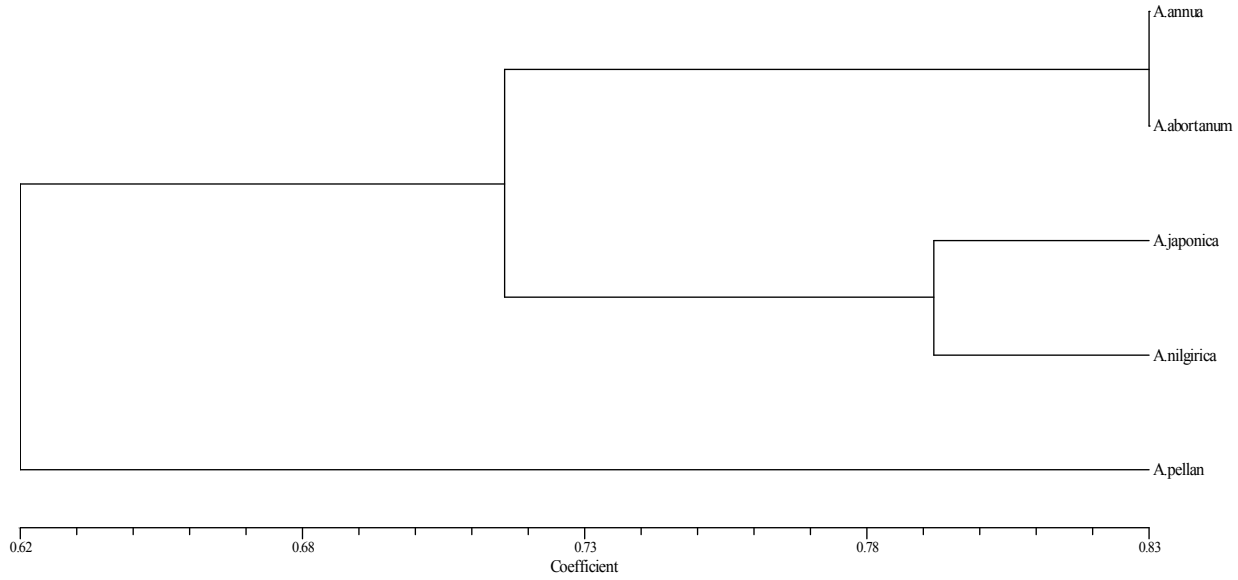

Figure 4. Dendrogram depicting the clustering among *Artemisia* genotypes

RAPD Analysis of Medicinal Plants using Primer OPAC 05

The genomic DNA of 5 *Artemisia* plants was amplified with decamer Oligonucleotide primers such as OPAC-05 and as shown in Figure 2. The distinct and abundant RAPD fragments were recorded. The total numbers of bands generated were 37RAPD gel profiles. The primer produced polymorphic banding pattern in all the five medicinal plant species, the number of RAPD bands per plant were 9 to 7. The no of polymorphic bands are 11 and 31% polymorphism. The plant *Artemisia annua, Artemisia japonica* and *Artemisia nilagirica* give the same banding pattern.

RAPD Analysis of Medicinal Plants using Primer OPAC–15

The data obtained in the present investigation revealed a total number of 40 RAPD bands. The genomic DNA of 5 *Artemisia* plants amplified with OPAC-15 revealed polymorphic RAPD bands. The distinct and abundant RAPD fragments were recorded. The primer produced medium low and high resolution of RAPD bands. The number of bands per plant was recorded at a maximum of 10 bands to 6 bands. The no of polymorphic bands are 18 and 47% polymorphism.

RAPD Analysis of Medicinal Plants using Primer OPAC–01

The data obtained in the present investigation revealed a total number of 29 RAPD bands. The genomic DNA of 5 *Artemisia* plants amplified by OPAC-01, 22 Polymorphic RAPD bands and 76% polymorphism. The distinct and abundant RAPD fragments were recorded. The primer produced medium low and high resolution of RAPD bands. The number of bands per plant was recorded at a maximum of 7 bands to 5 bands.

RAPD Analysis of Medicinal Plants using Primer OPAC–02

The data obtained in the present investigation revealed a total number of 18 RAPD bands. The genomic DNA of 5 *Artemisia* plants amplified by OPAC-02, 11 Polymorphic RAPD bands and 64% polymorphism. The distinct and abundant RAPD fragments were recorded. The primer produced medium low and high resolution of RAPD bands. The number of bands per plant was recorded at a maximum of 5 bands to 2 bands. Plant *Artemisia annua* and *Artemisia japonica* gives slightly similar banding pattern.

RAPD Analysis of Medicinal Plants using Primer OPAC-04

The data obtained in the present investigation revealed a total number of 34 RAPD bands. The genomic DNA of 5 *Artemisia* plants amplified by OPAC-04, 9 Polymorphic RAPD bands and 27% polymorphism. The distinct and abundant RAPD fragments were recorded. The primer produced medium low and high resolution of RAPD bands. The number of bands per plant was recorded at a maximum of 8 bands to 6 bands.

Genetic distance-based analysis

A relatively high genetic variation was found among a genotypes with Jaccards similarity coefficient ranged from 0.62 to 0.83. All 5 genotypes could be discriminated successfully by RAPD marker (Fig 4). The genotype collected were distributed among 3 genetic clusters, group 1 contained *Artemisia* annuaand *Artemisia abortanum*, group 2 having *Artemisiajapanica* and *Artemisia nilagirica and* group 3 having *Artemisia pellan wall*.

Pairwise genetic dissimilarity

Overall, pairwise similarity for all ten primers ranged from 0 to 3.4. The highest pairwise similarity (3.4) was observed between *Artemisia annua* and *Artemisia pellan wall* when ten primers were combined. The lowest pairwise similarity (1.3) was observed between *Artemisia annua* and *Artemisia abortanum*, the *Artemisia annua* to *Artemisia japonica* is 2.6, *Artemisia annua* to *Artemisia nilagirica is 2.7*(Table 6).

DISCUSSION

Molecular markers take its own importance for genome identification and selection with the help of PCR technology since 1980s. The above mentioned genomic study was possible mainly by using three widely used PCR-based markers viz., Random Amplified Polymorphic DNA (RAPD) (Williams *et al*., 1990), Simple Sequence Repeats or microsatellites (Tautz, 1989), and Amplified Fragment Length Polymorphism (Vos *et al*., 1995). Moreover, each marker technique has its own advantages and disadvantages. Comparatively, RAPD markers are very quick and easy to develop (because of the arbitrary sequence of the primers) but lack reproducibility (Karp *et al*., 1997; Hansen *et al*., 1998; Virk *et al*., 2000).While, AFLP has medium reproducibility but is labour intensive and has high operational and development costs (Karp *et al*., 1997). Further, Microsatellites are specific and highly polymorphous (Karp *et al*., 1997; Jones *et al*., 1997), but they require knowledge of the genomic sequence to design specific primers and thus, are limited primarily to economically important species.However, SSRs are co-dominant molecular markers that distinguish homozygotic and hetrozygotic individuals and also possess a large number of alleles. In fact, the use of single SSR marker may not provide authentic information hence in the present investigation we have used five different EST derived SSR markers for reliable and accurate differentiation of *Artemisia* plants, as recommended earlier (Jitedra kumar *et al*., 2014). Further, the present study was conducted on 5 genotypes of *Artemisia species* with 5 SSR primers used for assessment of genetic diversity. All the 5 primer were polymorphic in nature. The sizes of these fragments ranged from 150 to 300 bp, total 25 alleles, average allele per locus is 5 at 5 loci. Comparatively, the reports of the other study revealed that 20 genotypes of *Artemisia annua* with 16 primers produced 13 SSR fragments in which the size ranged from 90 to 600 bp, total 38 allelesand average allele per locus is 2.92 at 13 loci (Jitendra Kumar *et al*., 2014). Further, the polymorphic markers ratio ranged between 96% to 87% with 5 genotypes of *Artemisia* species when compared to the work of Jitendra kumar (2014) with the polymorphic markers ratio of 92% to 23% genetic relationships among the 20 genotypes of *Artemisia annua*. The similarity level in *Artemisia* inbred lines based on ANOVA and Neis similarity coefficient, although was sufficient for general identification and in testing programs, it may not be sufficient in studies that require higher resolution, such as fine mapping of quantization trait loci. Further, the genetic relationship were found to be very close between *Artemisia japonica* and *Artemisia nilagirica* but *Artemisia annua* was found to be highly genetically different from the other four species. Additionally, *Artemisia pellans and Artemisia abortanum* are different from each other and lies in between *Artemisia annua* and *Artemisia japonica* as per the findings of the present investigation.

Moreover, the study on RAPD has frequently been used for the detection of genetic variability in plants. The advantage of this method is its rapidity, simplicity and lack of need for any prior genetic information about the plant. RAPD patterns are consistent irrespective of the plant source or age. Understanding of the genetic variation within and between populations is essential for the establishment of effective and efficient conservation of plants (Shafie *et al*., 2009). RAPD molecular marker technique indicated its potential for studying the genetic variability within *Artemisia* species. Higher similarity indices suggested that the tested individuals have closer genetic relation. In relation to this the present study of RAPD molecular work indicated 76% of polymorphism, the lowest polymorphism being 27%, the average percentage of polymorphism in the 5 species of *Artemisia* plants is 49%. On the other hand the studies reported in *Artemisia judaica* reflects the genetic difference between the *A.judica* populations. (Ibrahim Mohammed Al Rawshdeh, 2011) while in *Artemisia cappillari* 95% polymorphic bands showed the percentage of polymorphism being high. (Mohammed Shafie, *et al*., 2011). Further, *Artemisia pellans* and *Artemisia vulgaris* showed high variance in bands by using different RAPD marker (Robin Chandra *et al*., 2014), and high correlation between cluster formed by the analysis of genetic diversity and chemotypes has also been observed in aromatic medicinal plant like *Artemisia annua* (Sangwan *et al*., 1999). According

to the present investigation the *Artemisia annua* and *Artemisia abortanum* are genetically very close to each other and also *Artemisia nilagirica* and *Artemisia japonica* are also found to be very close to each other, but *Artemisia pellan* wall is found to be genetically highly different from other four species. The 5 genotypic molecular markers study of *Artemisia* species provided a highly informative set of genetic distance and clusters, indicating the abundant and useful genetic diversity of 5 *Artemisia* species of India.

From the above details, our present investigation focuses on the fact that both SSR and RAPD molecular marker studies implies that *Artemisia japonica* and *Artemisia nilagirica* are genetically very close to each other but other three plants shows much genetical variation. Thus, the present investigation in comparison with the earlier work of Yasmin Taj and Narasegowda (2016) though goes hand in hand with respect to RAPD profiling to know the genetic variability at molecular level for taxonomic and evolutionary study, the present investigation brings forward an illustrative explanation in specificity with the comparative study of SSR and RAPD molecular markers.

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