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GENETIC VARIABILITY BETWEEN THE POPULATIONS OF BOSWELLIA SERRATA ROXB. BY USING RAPD PRIMERS

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ABSTRACT

Boswellia is the gummy resin plant of the boswellia tree. It has the family *Burseraceae*. It's local name are Guggal, Salai Guggal, Sallaki. Boswellia Gum Resin is the most important component of *Boswellia Serrata* tree. It has the indigenous origin to India and being applied over hundred years since Ayurvedic times by the doctors. In this study, three populations having 57 accessions (Jabalpur, Mandla and Balaghat) were selected to find genetic diversity between and within the individuals. The extracted genomic DNAs obtained the purity from 1.5-1.7. Three highly polymorphic RAPD (Random amplified polymorphic DNA) primers were selected randomly to amplify these genomic DNA samples. Here, the primer OPAW-01 showed the minimum PIC 0.93 and the average of PIC obtain from three primer was 0.83 it means the heir amount of PIC showed that there is a huge genetic difference between groups are individual and the gene diversity which is also maximum in primer OPAW-01 (0.93) showed that there genetic character and it may be within the groups and individual this may be occurs due to the climates change are variation at gene or allele level

KEYWORDS: Boswellia serrata Roxb., RAPD, PIC, phylogeny.

1. INTRODUCTION

Boswellia serrata Roxb. belongs to family Burseraceae. 25 known species of the genus *Boswellia* are known and distributed widely in tropical region, parts of Asia and Africa. In India this species is available naturally in dry hilly forests of Rajasthan, Madhya Pradesh, Gujarat, Bihar, Assam, Orissa as well as central penisular regions of Andhra Pradesh and Assam. Its common name is Indian frankincense/olibanum and usually called salai.

B. serrata is a moderate-sized tree; up to 18m in height and 2.4m in the girth (normally 1.5m) The bark of this plant is thin, greenish grey, yellow or reddish and finally turning to ash colour, peeling off in smooth, exfoliating papery flakes; blaze pinkish and exuding small drops of resin (Saxena & Brahmam, 1994).The leaves of *Boswellia serrata* occurs at the tops of the branch with unequally pinnated ten pairs leaflets with an odd one opposite, oblong, obtuse, serrated, pubescent, sometimes alternate short petioles. *B. serrata* has white or pale rose flowers with short pedicels in single auxillary racemes which are shorter than the leaves. Gum resin containing *B. serrata* is a native tree that grows in the mountainous regions of India. Also known as Indian frankincense, In India the gum is tapped from the incision made on the trunk of the tree which is then stored in basket specially made by bamboo in the form of different flavor, color, shape and size. This fresh gum is obtained in the form of hot dry having pleasant flavor but slightly bitter in taste. Ancient Egyptians, Greeks and Romans used this 'frankincense' for burning purpose to produce sweet smell with fumigant as well as a multipurpose aromatic It is generally used in making incense powder and sticks. In India, the white flower appear in shout racemes at the end of branches from the end of January to March –April; sometime flower may emerge after the emergence of new leaves or before the fall of old leaves. The drupes ripen in May- June. In December before the complete fall out the leaves become yellowish to light brown and after the new leaves

emerge in May-June month. When the trees over covers from flowers and fruits it remain leafless. In year 1991 Welsh and McClelland demonstrated the PCR-based genetic assay technique called randomly amplified

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polymorphic DNA. This procedure detects nucleotide sequences polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence which is done by the annealing of single primer at two different locus of the genomic DNA and all these process is completed on complementary strands of DNA template. RAPDs are DNA fragments amplified by the PCR using short synthetic primers, of random sequence having both forward and reverse oligo nucleotides primer and amplified the fragments from 1-10 genomic sites at the same instant. Jones et al. (1997) demonstrated the procedure for amplified fragments on agarose gels with binding of ethidium bromide and observed under ultraviolet light to check the presence and absence of band. The advantage of RAPDs is that they are quick and easy to assy. This report revealed that the RAPD primers are used to investigate the genetic diversity between and among the populations which will be very helpful to generate the conservation strategy for valuable medicinal plants to maintain its beneficial use for living organisms.

The product size of RAPD is 831-4,268bp (Padmalatha K, et al., 2006). RAPD banding patterns distinguished the different mutilocus genotypes even in species exhibiting no allozyme diversity. (White *et al.* 1980). They showed the RAPD band diversities ranged from 0.003 to 0.022 within species; >90% of total diversity was among species and <10% within them It is found that allozyme and RAPD showed the similar diversity particularly those have highest and lowest diversities with no significant correlation. RAPD also shows the 75.2 % polymorphism across the genotypes 12.5 bands per primer. Jaccard's coefficient of similarity varied from 0.00 to 1.00 indicative of high levels of genetic variation among the genotypes studied. This type of study provides valid guidelines for the collection, conservation and characterization of forest trees. The objective of this work was to generate a comparative study among the four selected population of different area by using three primers (Subramanyamna et al. 2010).

2. MATERIAL AND METHOD

The leaf samples for the extraction of total genomic DNA were collected from the three different geographical regions having Jabalpur, Mandla and Balaghat with total 57 accessions (20 from Jabalpur, 17 from Mandla and 20 from Balaghat region)..

DNA Extraction

500mg leaf sprouts from each ramjet were taken for genomic DNA extraction following the method of Doyle and Doyle (1990). The collected leaves were ground in liquid nitrogen in chilled mortar and pestle. The leaf powder obtained so was transferred to sterile polypropylene tubes containing 20 ml of pre-warmed (65^0C) CTAB extraction buffer (pH 8.0) and mixed gently and thoroughly till no clump was visible. The content was incubated in water bath for 40 min at 65°C and stirred regularly after every 5 min. Subsequently, the equal volume of chloroform: isoamyl alcohol (24:1) was added, mixed thoroughly and centrifuged for 20 min at 8000 rpm at 20^0C. The resultant upper aqueous phase was transferred to a new sterile polypropylene tube to which the adjusted volume making final a concentration of 100 µg ml^{-1} RNAse A was added and incubated at 37^0C for 30 min. The sample was re-extracted with equal volume of chloroform: isoamyl alcohol (24:1) and centrifuged for 20 min at 6000 rpm at 20^0C. The aqueous upper layer was again collected in a new sterile polypropylene tube followed by addition of ice cold isopropanol in equal volume and incubation for 30 min at –20 ^{0}C for precipitation of genomic DNA, which was made into a pellet by centrifugation for 30 min at 10,000 rpm at 4^0C. The pellet DNA was washed with 70% ethanol, air dried, dissolved in 200 µl TE buffer and stored at -20^0C.
The integrity and quantity of the extracted DNA were estimated by spectrophotometer and visually verified on 1% agarose gel. Quantification of genomic DNA is done by taking absorbance on uv spectrophotometer. The optical density was measured at 260nm and 280 nm. Optical density and the concentration of the DNA is related to each other and calculated
by the following:

$$\text{Concentration of DNA } (\mu g/ml) = \frac{OD_{260} \times 50 \times \text{Dilution factor}}{1000}$$

The ratio of $OD_{260/280}$ was an indication of the amount of RNA or protein concentration in the preparation. A value of 1.8 is optimum for best DNA preparation. If this ratio is obtained below the 1.8, it means sample has the protein contamination and above the 1.8, indicates the RNA contamination (Table 1).

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Table 1: Purity Index of genomic DNA of selected trees of B.serrata.

REFERENCE TREE ACCESSION	*AVEARGE PURITY INDEX (260/280)*
P5 (Jabalpur)	1.6
P6 (Mandla)	1.7
P7 (Balaghat)	1.5

PCR Parameters*:-*

PCR amplification of genomic DNA of *Boswellia serrata* were conducted to check the genetic variability using RAPD primer (Table 2) and using 20 ng genomic DNA of *Boswellia serrata* trees. DNA amplification (modified Williams *et al.1990*) was carried in 25 µl reaction volume containing 2X Taq polymerase buffer, 2 mM $MgCl_2$, 200µmoles dNTP (dATP, dCTP, dGTP and dTTP) 1 unit Taq DNA polymerase, 20 pm of decamer primer, 20 ng genomic DNA in programmable thermal cycler. Amplification reaction were cycled 35 time for 1 min at 92º C (denaturation), 1 min at 37ºC (annealing), 72º C (extension) with final extension at 72º C for 5 min.

Table 2:-List of RAPD Primer with sequences

S.No.	**Primer**	**Sequence(5'-3')**
1	OPAW-01	ACCTAGGGGA
2	OPA-01	CAGGCCCTTC
3	OPP-03	CTGATACGCC

3. DATA ANALYSIS

The banding profiles generated by RAPD assay were separately compiled into matrix on the basis of presence (1) and absence (0) of bands. The binary matrices were use to estimate DNA polymorphisms and genetic relatedness of Boswellia genotype. Data analyses were performed using the computer software NTSYS-pc (Numerical Taxonomy system for windows). Both monomorphic and polymorphic bands were used to calculate pair-wise genetic similarity among *Boswellia serrata* tree using Jaccard coefficient (Jaccard, 1908).

4. RESULT DISCUSSION

In Molecular Biology the amplification of genomic DNAs is a challenging task scene, if there is a impurity of protein and RNA are other secondary metabolites they inhibit the amplification. In this study the genomic DNA of *B. Serrata* species used in amplification was free from any contaminants and the quantity was obtained an average 1500 µg/gm leaf weight and the ratio of 260 and 280 (observance of DNA and protein) was an average 1.6.

The amplification product were observed and prepared the data sheet (0, 1data). These data were and analyzed by using NT-SYS version 2.1 and formed UPGMA (unweighed paired group method for arithmetic mean) dendrogram. This analysis showed this similarity between within the population. In this analysis to measure group were formed with their two sub group. In these groups some individuals showed these 100% similarity all the groups and individuals showed the similarity more than 80%. Besides, the

polymorphic information content (PIC) and gene diversity also calculated by using power marker software (Version 3.2). The primer OPAW-01 showed the minimum PIC 0.93 and the average of PIC obtain from three primer was 0.83 it means the heir amount of PIC showed that there is a huge genetic difference

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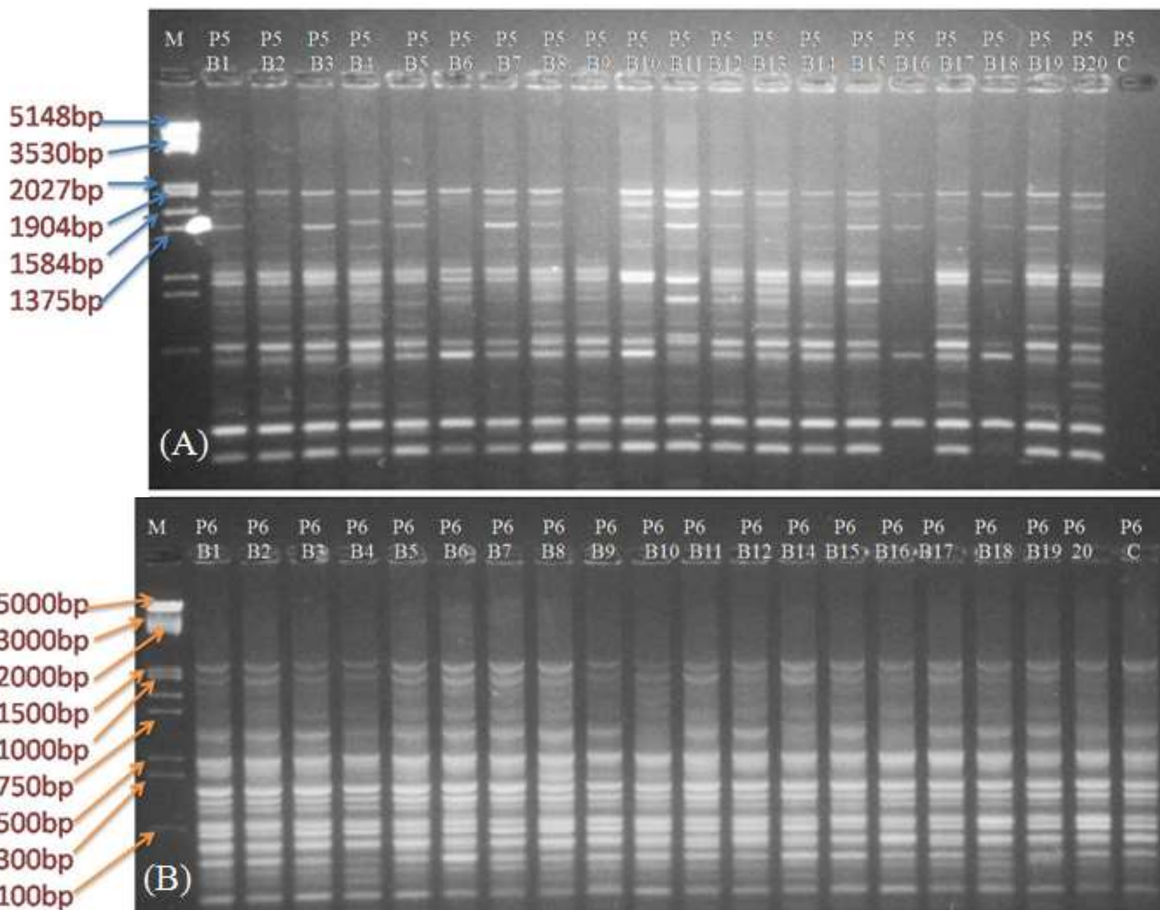
between groups are individual and the gene diversity which is also maximum in primer OPAW-01 (0.93) showed that there genetic character and it may be within the groups and individual this may be occurs due to the climates change are variation at gene or allele level (**Table 3**).

Table 3: Genetic parameters resulted by RAPD primers on B. serrata

Marker	Allele No	Gene Diversity	PIC
loci	80.0000	0.9875	0.9873
OPAW-01	50.0000	0.9288	0.9269
OPA-01	15.0000	0.7384	0.7168
OPP-03	11.0000	0.7075	0.6796
Mean	39.0000	0.8405	0.8276



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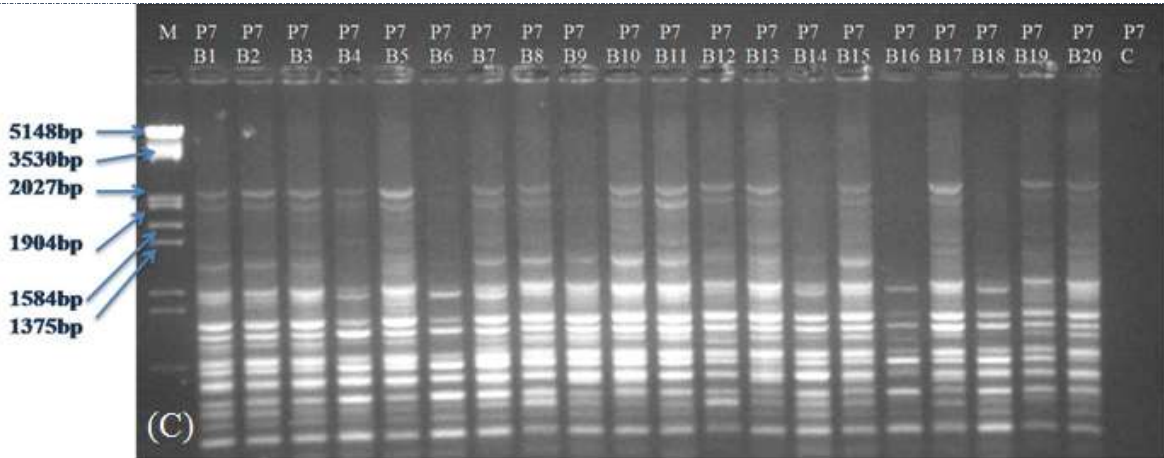

Figure : Showing the amplification of B. serrata genomic DNAs using RAPD primers OPAW-01 (A), OPA-01 (B) and OPP-03 (C).

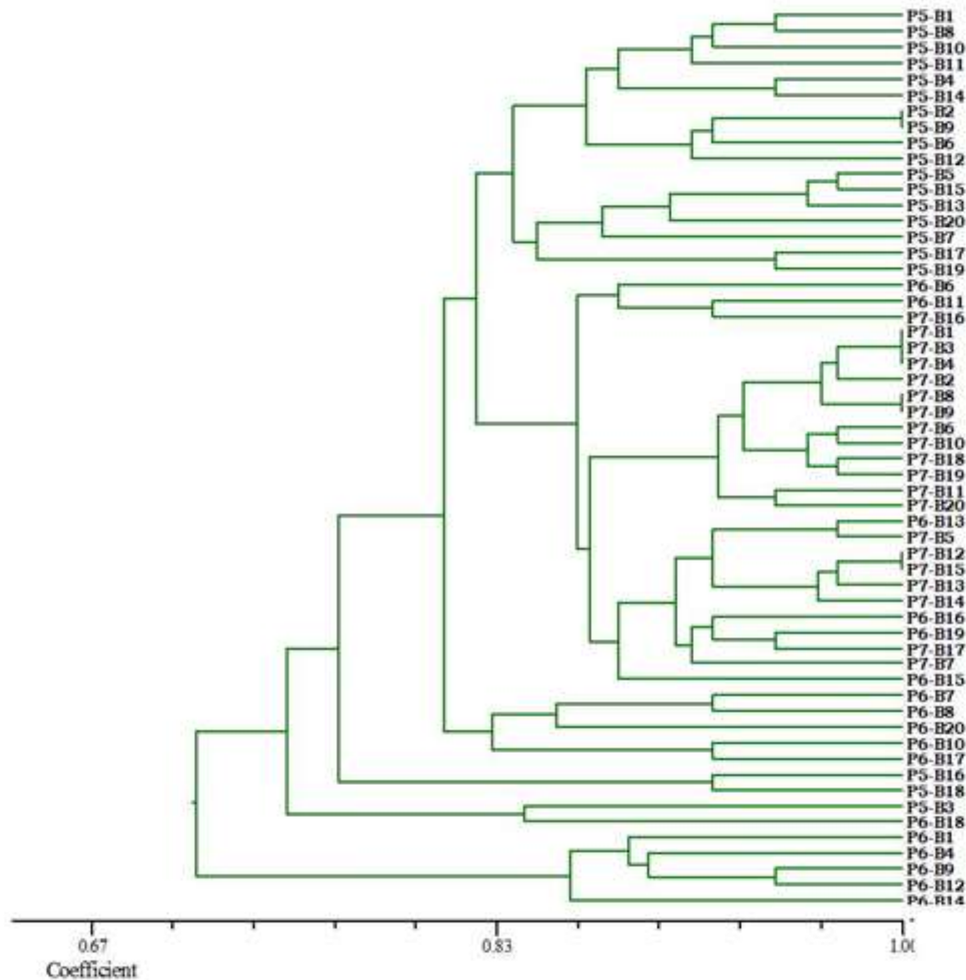

Figure : Phylogenetic analysis of B. serrata populations

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5. CONCLUSION

B.Serrata species is a medicinal plant and recently large numbers of groups are involved to investigate in this species for human well fair. It is also an important species for making a paper purpose but due to the natural poor regeneration there is a limited population are available mainly M.P and C.G. With the help of this study we can investigated the different population with their genetic information contain and preserve the species as per valued genetic information.

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