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ASSESSMENT OF GENETIC STRUCTURE OF THE ENDANGERED FOREST SPECIES *BOSWELLIA SERRATA* POPULATION IN CENTRAL INDIA

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Boswellia serrata, a commercially important species for its pulp and pharmaceutical properties was sampled from three locations representing its natural distribution in central India for genetic characterisation through 56 RAPD + 42 ISSR loci. The wood fibre dimensions measured for morphometric characterisation confirmed 11.36% variation in length and 8.75% variation in width, indicating its fitness for local adaptation. Bayesian and non-Bayesian approach based diversity measures resulted moderately within population gene diversity (0.26 ± 0.17), Shannon's information index (0.40 ± 0.22) and panmictic heterozygosity (0.28 ± 0.01). A high estimate for genetic differentiation measures i.e. G_{ST} (0.31), G_{ST}-B (0.33 ± 0.02) and θ-II (0.45) led to distinct clusters of sampled genotypes, representing their regional variability due to limited gene flow and total absence of natural regeneration. This study reports the first investigation of the species for its molecular characterisation emphasising the urgent need for a genetic improvement program for in-situ/ex-situ conservation and sustainable commercialisation.

Keywords: Burseraceae, θ-statistics, Shannon's information index, genetic improvement

INTRODUCTION

Boswellia serrata (Burseraceae), commonly known as salai guggul or Indian frankincense (*olibanum indicum*) is a commercially important deciduous tree of India (Shah et al. 2008). It is found in regions having rainfall between 500 mm–2000 mm and temperature up to 45 °C. The species can thrive in the poorest and shallowest soils (Bhat et al. 1952). In India, it is distributed in the states of Rajasthan, Maharashtra, Madhya Pradesh, Karnataka and Chhattisgarh (Pawar et al. 2012). The oleo-gum-resin of the tree contains boswellic acid, which is effective for the treatment of inflammatory disorders, arthritis, cardiovascular diseases, diarrhea, dysentery and other skin diseases (Khare 2004, Ammon 2006). Apart from these medicinal values, the resin of the species was found to be a more effective sizing agent in papermaking than resin obtained from *Pinus* species (Sharma et al. 1985). This wood quality for pulp making led to the establishment of the first paper mill in Nepanagar of Madhya Pradesh, India, as the region was naturally occupied by a wide range of the species (Khan 1972).

The species is highly out-crossing, supported by self-incompatibility to selfing (Sunnichan et al. 2005). However, its poor fruit setting (2.6%–10%) under open pollination condition, inadequate production of viable seeds and scanty seed germination (10%–20%) limit the distribution of the species in nature (Ghorpade et al. 2010). The species population has been harvested for frankincence (Sharma 1983). The scarcity of protocols to regenerate the species through seeds and clones makes mass multiplication difficult (Purohit et al. 1995). This situation resulted in declined abundance of the species, and listed as endangered by the International Union for Conservation of Nature (IUCN). Therefore, the available natural patches of the species require keen attention for both in-situ and ex-situ conservation with actual knowledge of available genetic resources of the species, for management and breeding objectives.

Information on actual genetic variability and the cryptic number of the differentiated genetic resource are important aids for its conservation and genetic improvement. For such purposes,

DNA based markers are of great value; unlike morphometric traits, the molecular markers are independent of environmental influence and assessable at any growth stage. Among the various molecular markers employed to assess diversity studies, PCR-based dominant markers such as random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) have become popular due to their polymorphism and discrimination power, as their application does not need any prior sequence information (Zietkiewiez et al. 1994). These primer systems have been successfully applied for genetic characterisation of tropical tree species populations (Ansari et al. 2012, Abuduli et al. 2016, Vaishnav & Ansari 2018). Bayesian statistics has been extended to dominant markers for precise estimates of population genetic hierarchies equivalent to those obtained with codominant markers, circumventing inbreeding estimate within the population (Holsinger et al. 2002). Therefore, the present investigation was conducted to differentiate the morphometric and genetic variability exhibited by the natural population of *B. serrata* in three agro-climatic regions of central India, applying dominant marker system.

MATERIAL AND METHODS

Population sampling

The distribution of *B. serrata* was obtained from the old forest working plans of the forest department of Chhattisgarh, classified in three agro-climatic zones viz. Northern Hills, Chhattisgarh plain and Bastar plateau (Table 1). The forests of these agro-climatic regions were visited during July to September 2014 to collect samples from 20 trees of each region within the natural distribution of *B. serrata,* with inter-tree distance of 100 m along the latitude (Figure 1). The girth at breast height (gbh) was measured using a measuring tape. A wood radial core sample was extracted from each tree at breast height (1.34 m) and stored in a tube with 40% formaldehyde. A leaf sample was also collected from each tree. The samples were transported to the laboratory in a cryo-box.

Measurement of wood fibre dimensions

In the laboratory, the wood samples were macerated as described by Mahesh et al. (2015). Five slides were prepared for each tree; the wood fibre length (WFL) and width (WFW) were measured using a compatible program integrated with compound microscope.

DNA isolation and amplification

The DNA from the leaf samples was isolated following modified cetyl trimethylammonium bromide (CTAB) method (Deshmukh et al. 2007). To select the reproducible markers, 10 RAPD and 10 ISSR primers were cross-amplified for 12 genotypes of the species (four from each region) and finally, five from each RAPD and ISSR markers were selected based on their polymorphism and reproducibility for the final amplification of all 60 genotypes (Table 3). For RAPD/ISSR amplification, the final PCR reaction mixture, 15 µL/10 µL, contained 50 ng/40 ng genomic DNA, 0.66 µM/0.8 µM of primers, 0.2 mM/0.1mM of dNTP mix, 1.5 mM/2.5 mM $MgCl_2$, 1X buffer with KCl

Table 1 Geo-climatic conditions of the three regions of natural distribution of *B. serrata* populations sampled for the investigation

Regions	Agro-climatic zones	GPS coordinate ranges		Altitude (m)	Mean annual temperature (°C)		Annual precipitation (mm)
		Latitude (N)	Longitude (E)		Min	Max	
Dhamtari (DT)	Chhattisgarh Plain	20.37861-20.40511	81.96636-81.98122	445.80 ± 5.44	21.60 ± 5.22	33.02 ± 3.56	1488 ± 0
Narayanpur (NP)	Bastar Plateau	19.71365-19.71586	81.19447-81.19836	595.60 ± 21.39	20.80 ± 5.22	32.40 ± 3.36	1628 ± 0
Sarguja (SG)	Northern Hills	23.39300-23.39446	83.46277-83.46783	619.75 ± 7.15	19.02 ± 6.35	31.02 ± 4.07	1670 ± 0

± = standard deviation (SD)

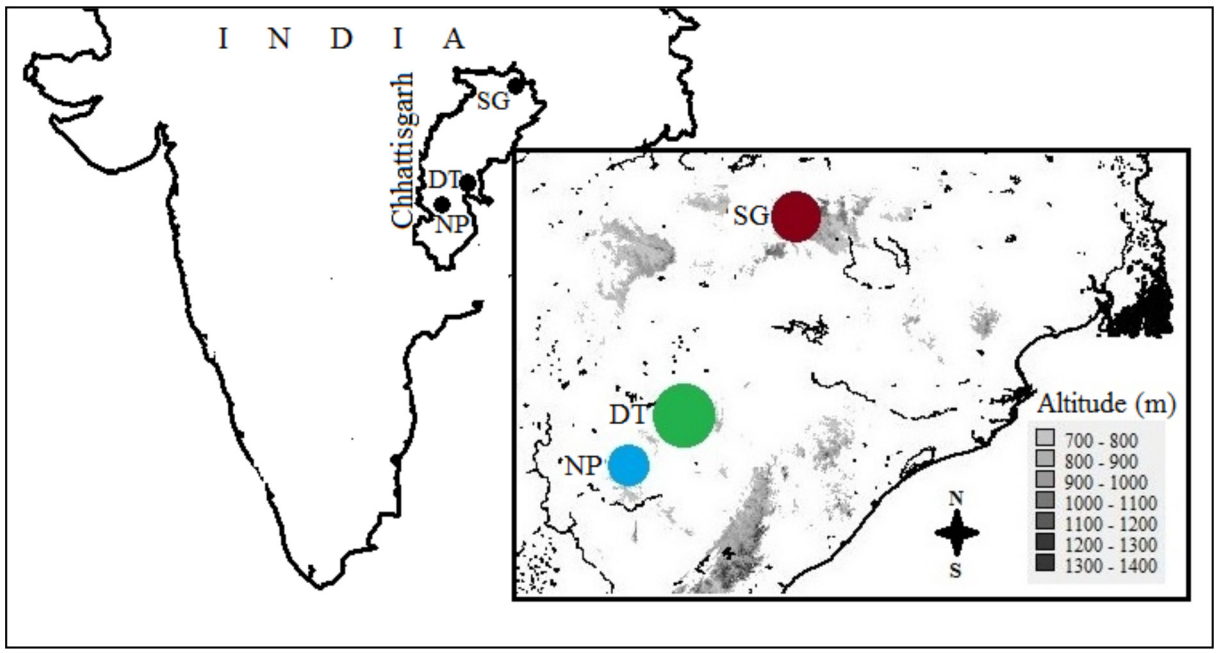

Figure 1 Three regions i.e. Sarguja (SG), Dhamatri (DT) and Narayanpur (NP) of natural distribution of *B. serrata* from central Indian region (Chhattisgarh) were sampled for investigation; every location represents distinct agro-climatic zone namely Northern hills (SG), Chhattisgarh plain (DT) and Bastar plateau (NP); an inset view of altitudinal variation is given in the box, size differences among the location's legend dot depicts differences in gene diversity of the species estimated by employing dominant markers

and 1 unit of Taq polymerase. The PCR assay included an initial 4 minutes denaturation step at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at the annealing temperature of 35 °C/50 °C, 45 s extension at 72 °C, followed by a final extension of 5 minutes at 72 °C. The amplified products were electrophoresed on 1.5% agarose gel containing 0.5µg/ml ethidium bromide (EtBr) in 0.5 X TBE (pH 8.0). The separation was carried out by applying constant 100 V for 3 hours. The fractionated amplified products on agarose gel were visualised on gel documentation system under UV light. To avoid homology, the amplification profile of all sixty genotypes was evaluated through the molecular weight of the bands for each primer and the bands were scored in a Microsoft Excel sheet in binary data format.

Data analysis

The measures of central tendency and the coefficient of variation (CV) were calculated for gbh, WFL and WFW. A genetic profile of 60 genotypes on 10 markers was generated and analysed following both band-based and allele frequency-based approaches as described by Bonin et al. (2007). The information from the markers was evaluated, estimating mean allelic frequency (AF), gene diversity (GD) and polymorphic information content (PIC) using POWERMARKER v 3.1 program (Liu & Muse 2005). Resolving power (RP) of the markers was calculated as described by Prevost and Wilkinson (1999).

The genetic variability and differentiation of the sampled population were evaluated with both non-Bayesian and Bayesian approaches. The program POPGENE v 1.32 (Yeh et al. 1999) was applied for the non-Bayesian estimates of genetic diversity measures i.e. Nei's gene diversity (GD), Shannon's information index (I) and the percentage of polymorphism (P%) for each location, and G_{ST} was calculated to evaluate the level of genetic differentiation. The genetic relationship among the genotypes was calculated based on Jaccard's genetic similarity coefficient using the program DARWIN v 5.0 (Perrier & Jacquemoud-Collet 2006). The principal coordinate analysis (PCoA) was performed to cluster the genotypes in different axes using the program GENALEX v 6.5 (Peakall & Smouse 2012). Analysis of molecular variance

(AMOVA) was performed using the program ARLEQUIN v 3.11 to estimate the hierarchical variation (Excoffier & Lischer 2010). The isolation by distance (IBD) was calculated from the correlation coefficient between two pairwise matrices of genetic and geographical distances among genotypes, through Mantel's test performed using the program ALLELES IN SPACE in independent runs (with and without logarithmic transformation) on 10,000 permutations (Miller 2005).

For Bayesian approach based genetic estimates, the θ-statistics (viz. θ-I, θ-II, θ-III and G_{ST}-B) implemented by using the program HICKORY v 1.1) were preferred (Holsinger & Lewis 2007). It allowed direct estimation of genetic differentiation measure (F_{ST}) from dominant markers without assumption of prior knowledge of the extent of inbreeding and Hardy Weinberg Equilibrium (HWE), even within populations of small size and number. Region-wise panmictic heterozygosity (Hs) and total panmictic heterozygosity (Ht) were also estimated. All these estimations were performed with 50,000 steps of burn-in, 500,000 replicates and 20 thinnings, using the best-suited model for the data amongst all four models (viz. full, f = 0, θ = 0 and f-free models), implemented based on minimum deviance information criterion (DIC) value as described by Spiegelhalter et al. (2002). To determine the most suitable number of cryptic populations (K) for the data, the program STRUCTURE v 2.3.1 was used (Pritchard et al. 2000). The model applied combinations of admixture/no-admixture with correlated/independent allele frequencies among the samples, on 100000 burn-in and 1000000 MCMC repeats, with three-runs from K = 1 to K = 9, for number of K $2 \leq K \leq 8$. The most suitable model and the best-suited K was determined based on the highest delta-K value resulted by using the online program STRUCTURE HARVESTER (Earl and Von Holdt 2012).

RESULTS

Variation in wood fibre dimension

The WFL of the species ranged between 0.803 mm to 1.397 mm with an average of 0.968 ± 0.11 mm (11.36% CV), and the WFW of the species ranged between 0.019 mm to 0.030 mm with average of 0.025 ± 0.002 mm (8.75% CV). Both traits followed normal distribution and no significant correlation was observed between them. Both the WFL and WFW were evenly distributed on standard deviation (σ) based classes, and no significant variation was observed among the locations based on WFL and WFW (Table 2).

Genetic informativeness of markers

The five RAPD primers amplified 826 bands and 56 loci (14.75 bands/locus). The diversity measures, AF and GD, were 0.81 ± 0.065 and 0.27 ± 0.075 respectively. The PIC and RP were 0.22 ± 0.054 and 5.51 ± 3.17 subsequently (Table 3). For RAPD markers, the diversity measures and discriminatory power measures were found insignificant ($p < 0.05$) in linear correlation. The five ISSR primers amplified 1004 bands for 42 loci (23.90 bands/locus). In a significant linear correlation ($p < 0.01$) to each other; AF was 0.76 ± 0.068 and the GD was 0.33 ± 0.073. The PIC and the RP were 0.27 ± 0.052 and 6.69 ± 1.69 subsequently (Table 3).

Table 2 Variability in wood trait parameters of three sub-populations of *B. serrata*

Regions	GBH (m)	WFL (mm)	WFW (mm)
Dhamtari (DT)	0.99 ± 0.25	0.919 ± 0.097	0.025 ± 0.001
Narayanpur (NP)	1.07 ± 0.22	0.975 ± 0.095	0.026 ± 0.002
Sarguja (SG)	1.40 ± 0.25	1.009 ± 0.120	0.024 ± 0.003
Average	1.15 ± 0.30	0.968 ± 0.11	0.025 ± 0.002
CV (%)	26.01	11.36	8.75

GBH = girth at breast height, WFL = wood fibre length, WFW = wood fibre width, CV = coefficient of variation, ± = standard deviation (SD)

Table 3 Genetic informativeness of RAPD and ISSR markers

Marker system	Primers	Sequence (5'–3')	N	AF	GD	PIC	RP
RAPD	OPA1	CAGGCCCTTC	12.0	0.77	0.32	0.26	6.10
	OPA2	TGCCGAGCTG	13.0	0.72	0.37	0.29	10.80
	OPY1	GTGGCATCTC	10.0	0.80	0.27	0.22	3.97
	OPY2	CATCGCCGCA	12.0	0.87	0.19	0.16	3.70
	OPP3	CTGATACGCC	9.0	0.87	0.21	0.18	2.97
	Average		11.20	0.81	0.27	0.22	5.51
ISSR	UBC840	GAGAGAGAGAGAGAGAAT	9.0	0.67	0.42	0.33	9.47
	UBC830	TGTGTGTGTGTGTGTGG	6.0	0.74	0.36	0.29	6.50
	UBC822	TCTCTCTCTCTCTCTCA	8.0	0.74	0.37	0.30	6.50
	UBC845	CTCTCTCTCTCTCTCTGG	8.0	0.84	0.24	0.20	6.17
	UBC836	AGAGAGAGAGAGAGAGTA	11.0	0.80	0.28	0.23	4.83
	Average		8.40	0.76	0.33	0.27	6.69

N = number of amplified loci, AF = allele frequency, GD = gene diversity, PIC = polymorphic information content, RP = resolving power

Genetic diversity and differentiation

Among the non-Bayesian genetic diversity estimates of species population, 100% polymorphism, 0.26 ± 0.17 GD and 0.40 ± 0.22 I were observed among the regional locations (Table 4). For all measures, no significant difference was found among the three regions. Dhamtari was found slightly more diverse and polymorphic than the other two regions (Table 4); G_{ST} was 0.31. The Bayesian model implemented in Hickory estimated the lowest DIC value (896.96) for the full model, and panmictic heterozygosity was 0.28 ± 0.01. No significant variation in panmictic heterozygosity was found among the three regional sub-populations (Table 4). The θ-I, θ-II, and θ-III values were 0.50 ± 0.04, 0.45 ± 0.04 and 0.25 ± 0.01 subsequently, and G_{ST}-B was 0.33 ± 0.02.

The dendrograph based on Jaccard's genetic similarity coefficient (average 0.67) grouped the genotypes according to their regions (Figure 2). The genotypes sampled from Sarguja further bifurcated into two different but conjoint clusters supported by bootstrap values > 60 (Figure 2). The PCoA accounting for 68.97% cumulative separation based on similar genetic distance separated the genotypes of Narayanpur from the rest of the sampled genotypes, and equal proportion of genotypes from Dhamatari and Sarguja was grouped separately, making an overall of four small clusters (Figure 3). The Bayesian STRUCTURE program assigned K = 2 as the most appropriate number of cryptic population for the sampled genotypes based on the highest delta-K value in STRUCTURE HARVESTER. The bar plot generated shows admixing of genotypes assigned to the two cryptic populations (K = 2) with > 80% of ancestry coefficient (Figure 4). The genotypes from Dhamtari were found admixed with both cryptic populations, and the genotypes from Narayanpur and Dhamatri were assigned in the same cluster. On the other hand, genotypes from Sarguja clustered distinctly (Figure 4). The AMOVA showed 46.03 ± 0.65% variation among groupings, given by dendrograph, PCoA and STRUTURE (K = 2), with 53.38 ± 0.15% variation within these groups. Mantel's test found significant ($p < 0.001$) correlation between pair-wise genetic and geographical distances among the genotypes.

DISCUSSION

The *B. serrata*, a member of the family Burseraceae, bifurcated from Terebinthaceae and Anacardiaceae during the Miocene epoch due to lesser intrinsic ability to adopt the changing conditions across the climatic niches (Weeks et al. 2014). Therefore, the distribution of genus *Boswellia* is limited within the mediterranian and tropical regions. *Boswellia serrata* is found only in central India and the species is renowned for its olio-gum-resin and pulp content. Due to

Table 4 Population genetics parameters for 60 genotypes sampled from three sub-populations of *B. serrata*

Regions	P%	GD	I	Hs
Dhamtari (DT)	63.27	0.20 ± 0.20	0.30 ± 0.28	0.20 ± 0.01
Narayanpur (NP)	57.14	0.19 ± 0.19	0.28 ± 0.28	0.19 ± 0.01
Sarguja (SG)	55.10	0.14 ± 0.16	0.22 ± 0.24	0.17 ± 0.01
Overall*	100	0.26 ± 0.17	0.40 ± 0.22	0.28 ± 0.01

± = SD, P% = polymorphism%, GD = Nei (1973) gene diversity, I = Shannon's information index, Hs = panmictic heterozygosity resulted by Hickory, *considering all 60 genotypes as a group

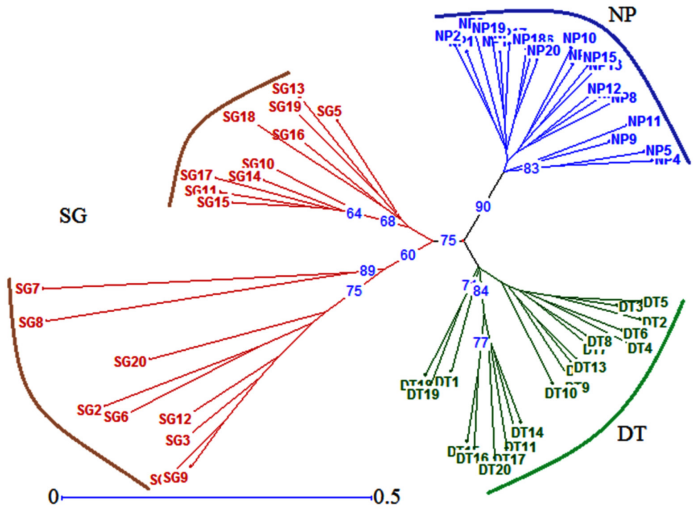

Figure 2 A Jaccard's similarity coefficient based radial dendrograph of 60 accessions from three natural populations i.e. Sarguja (SG), Dhamtari (DT) and Narayanpur (NP) of *B. serrata;* bootstrap values (> 60) are shown on nodes of accessions

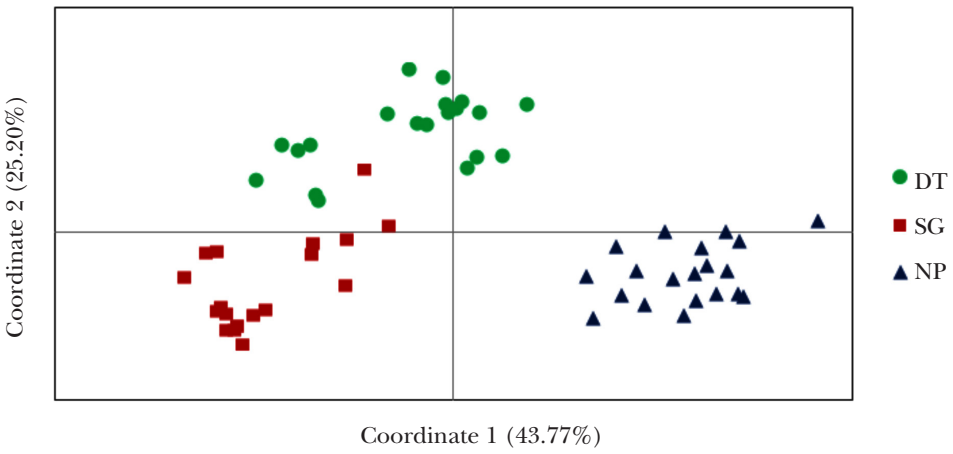

Figure 3 The principal coordinate analysis, showing closeness of genotypes of *B. serrata* sampled from Dhamtari (DT), and Sarguja (SG), and the genotypes from Narayanpur (NP) cluster distinctly, in two coordinates covering 68.97% of variation

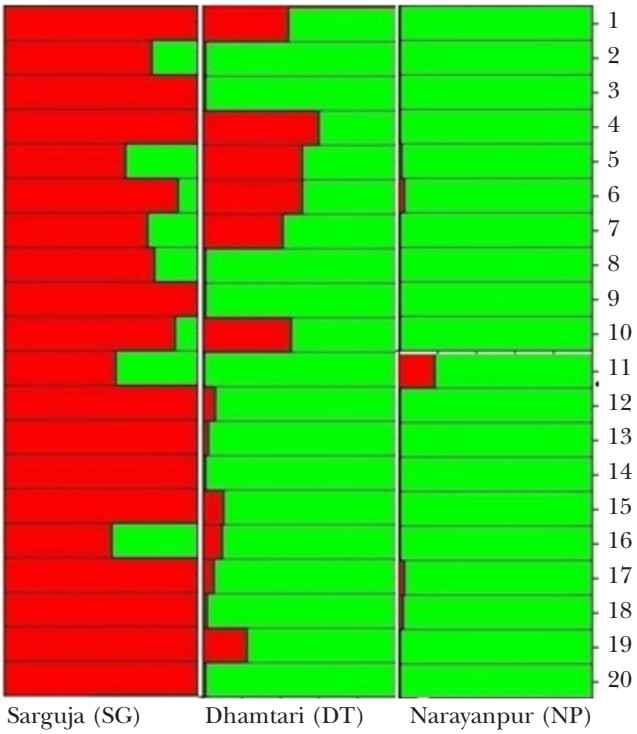

Figure 4 The Bayesian clustering resulted by program STRUCTURE depicted two cryptic populations (K) in 60 genotypes of *B. serrata* sampled from three locations of its natural distribution; each genotype is presented by a separate colored bar with corresponding number; green and red color represents two different cryptic populations.

uncontrolled harvesting it has become scarce in nature, and literature regarding its distribution is inadequate. During the investigation, the species was found in the temperature range of 19.02 ± 6.35 °C to 33.02 ± 4.07 °C with annual rainfall of 1595.33 ± 95.30 mm, confirming its suitability for moist conditions along arid regions. For molecular characterisation of the species, RAPD and ISSR markers were preferred due to the absence of background genomic information of the species, to design microsatellites and low-cost applicability. The problems with dominant markers such as low reproducibility and uncertain homology of alleles were handled during amplification and data profiling. Bayesian model-based θ-statistics was applied based on successful estimation of the genetic variability and differentiation of population, applying dominant markers system in forest species (Holsinger & Wallace 2004, Ci et al. 2008, Vaishnaw et al. 2015).

Variation in wood fibre dimension

The wood fibre length and thickness were considered to be the most important characteristics for mechanical strength of wood for the making of high-quality groundwood in paper industries (Zobel & Jett 1995, Migneault et al. 2008). Comparison with other tropical trees employed in pulp making process established that wood fibre dimensions of *B. serrata* is much higher than *Ailanthus altissima* (WFL= 0.747 ± 0.035 mm and WFW = 0.023 ± 0.0004 mm) and *Eucalyptus globulus* (WFL = 0.785 ± 0.012 mm and WFW = 0.018 ± 0.0005) (Baptista et al. 2014). The WFL is lower than *Eucalyptus grandis* (1.01 ± 0.14 mm) (Bhat et al. 1990). The coefficient of variation of wood fibre dimension in *B. serrata* is higher than *A. altissima* (WFL = 4.76% and WFW = 1.66%) and *E. globulus* (WFL = 1.5% and WFW = 2.72%) and lower than *E. grandis* (WFL= 14.45%) (Bhat et al. 1990, Baptista et al. 2014). In terms of adaptability, such high variation confirms the adaptive fitness of the population to local ecological conditions (Mac-Pherson et al. 2015).

Genetic informativeness of the primers

With an approach to handle the limitation of non-reproducibility, the dominant markers (RAPD/ ISSR) have exhibited moderate values for AF

and GD. It indicates that the approach avoiding null alleles have controlled the informativeness of the primers, helping genetic differentiation (Lynch & Milligan 1994). The AF exhibited by RAPD/ISSR markers are slightly higher to the information resulted in populations of *Tectona grandis,* sampled from India on ISSR markers (0.73 ± 0.02) (Vaishnav & Ansari 2018). On the other hand, negligible differences in AF among the markers of RAPD and ISSR systems ensure the qualitative support for diversity assessment (Lynch & Milligan 1994, Nybom & Bartish 2000). The discriminatory power exhibit a strong and linear relationship between the ability of a primer to distinguish genotypes (Prevost & Wilkinson 1999). The values of PIC and RP are comparable to those obtained with dominant markers in other species, e.g. *Podophyllum hexandrum*, *Jatropha curcas* and *Pongamia pinnata* (Naik et al. 2010, Grativol et al. 2011, Sharma et al. 2014).

Genetic diversity and differentiation

In order to estimate the genetic diversity of *B. serrata* population, both band-based and allele frequency-based approaches were applied with non-Bayesian and Bayesian statistics so that the under-estimation or over-estimation of the diversity measures, caused by the dominant marker systems, could be avoided. A significant correlation between diversity measures i.e. P%, GD, I and Hs, with slight differences among them for different regions, confirmed almost equal heterogeneity of the species population. The gene diversity of the sampled population has been found slightly higher than that reported by Nybom (2004) for out-crossing species (0.18). Nevertheless, Holsinger & Wallace (2007) suggested avoiding comparison among diversity measures resulted by neutral markers (AFLP/RAPD), as these markers only show the differences in rates of mutations at their loci rather than reflecting different migration rates. However, there are few studies based on tree species of central Indian regions. Wang et al. (2011) investigated the genetic diversity of *D. sissoo* sampled from Indian regions and found 89.11% polymorphism, 0.27 ± 0.16 GD and 0.41 ± 0.23 I. Ansari et al. (2012) sampled *Tectona grandis* from central and peninsular Indian regions and found 80.30% polymorphism, 0.32 GD and 0.45 I. Comparison of results indicated that its natural distribution and level of genetic diversity were equally influenced by geographical conditions and landscapes. The significant correlation found between genetic and geographical distances of the genotypes in the study also supports this fact.

The genetic structure of a population reflects long-term evolutionary history of the species along with its mating system, reproductive biology and gene flow influenced by the adaptive selection and fragmentation (Slatkin 1987, Nybom & Bartish 2000, Duminil et al. 2016). In this investigation, the results of Bayesian model-based analysis through HICKORY found full-model as the most suitable for the data, determining the influence of inbreeding within the populations. The low level of panmictic heterozygosity (Hs) for different regions and overall population supports the finding. Like other out-crossing species, the *B. serrata* exhibited higher within-population variability than among populations, but the genetic variation within the population resulted by AMOVA was found comparatively lower than those reported in other out-crossing forest species (Hamrick & Godt 1989, Nybom 2004, Wang et al. 2011, Ansari et al. 2012). The limited gene flow of *B. serrata* is one of the important factors that have led to the high differentiation among sampled populations and genotypes. The species has an entomophilous mode of pollination and self-incompatible flowers, supporting only cross-pollinated pollen to grow the pollen tubes. Moreover, only up to 10% fruit set has been observed in open-pollination condition (Sunnichan et al. 2005). In the field observation, earlier abscission of unripe fruits and ripen fruit with empty seeds led to total absence of natural regeneration in all the sampling sites. These bottlenecks contributed to genetic drift and signatory inbreeding depression to the species population, reflected through different estimates.

The genetic differentiation measures, G_{ST} and G_{ST}-B, have resulted in comparatively equal values indicating the cautions taken to employ dominant markers for investigation, equivalent to Bayesian model correction. The θ-statistic measures exhibit differences in their values due to low number of populations sampled for investigation (Holsinger & Wallace 2004). The θ-I is equivalent to Wright's $F_{ST,}$ and higher value (0.50 ± 0.04) is expected because of the scaled allele frequency measured across evolutionary time (Wright 1969). The θ-II is a more appropriate measure for a small number of admixed population and its higher

value (0.45 ± 0.004) reflects the problematic gene exchange among genotypes, leading to high differentiation.

The Bayesian clustering resulted by STRUCTURE, K = 2, was found most suitable for the data on the basis of delta-K value. Nevertheless, the presence of inbreeding in sampled population and deviation from HWE suggest preference of dendrograph and PCoA over the two admixed cryptic populations resulted by the program STRUCTURE, due to its prior assumption of HWE. In PCoA, it was found that Sarguja population closely related to Dhamtari and Narayanpur population, representing itself distinctly even in geographical closeness to Dhamatri. This could be due to gene flow among populations following geographical limits and biological constraints. On the other hand, the Narayanpur population represented a different source of genetic origin of southern Indian region that could not be sampled. An almost equal polymorphism, genetic diversity and heterozygosity were found, but with highly differentiable genetic resource resulting to distinctly different clusters. This indicates that the sampled populations may belong to a large metapopulation of the species that has been fragmented with due course of time, due to its own intrinsic inability to cope with natural and anthropogenic pressures, later leading to geographical isolation.

The *B. serrata* is a highly out-crossing species with self-incompatibility and therefore, its own constraint to exchange the genes. The fragmentation of the population due to geographical isolation and commercial harvesting could be the reason for detected inbreeding pressure. The estimates of genetic diversity measures, genetic structure and level of differentiation of a species population depend on the molecular marker system employed for the investigation. In this investigation, the dominant markers could be a reason behind low to moderate estimates of gene diversity, panmictic heterozygosity and within-population variability. On the other hand, the Bayesian model-based analysis gave a higher value of θ-II because the θ-statistics has been developed specifically for the dominant markers and has been found more appropriate than G_{ST} and G_{ST}-B to estimate inbreeding and population differentiation (Holsinger et al. 2002, Spiegelhalter et al. 2002, Holsinger & Wallace 2004). The comparatively higher variation in morphometric traits also indicate the existence of higher variability in population. The northernmost region, Sarguja and the southernmost region, Narayanpur represent different centers of genetic origin of a large metapopulation of the species. The genes from Sarguja have been immigrating to the central region, Dhamtari. For a better understanding of the influence of landscape and other geographical features on distribution and gene diversity of *B. serrata,* futher investigation should be conducted with a larger number of locations, representing its entire range of natural distribution, applying co-dominant multiallelic marker system to cross verify the results.

CONCLUSION

The *B. serrata* has been over-exploited due to its significant quality of wood for pulp and paper industries. The paper industries use the species as a raw material, and the oleo-gum-resin obtained from its wood has significant medicinal value to heal arthritis and asthma. The species is endemic to India and unfortunately, there is not a single source of sustainable supply for the raw material required in paper and pharmaceutical industries. Apart from all, the species is understudied and has not received much scientific attention on population genetic. The present investigation found the available genetic resource of the species in central Indian region maintaining high variation in wood fibre dimension and high genetic variability within and among sub-populations. In order to maintain the valuable forest genetic resource, the species need attention for conservation. The locations of its natural distribution can be conserved as nature reserves. A genetic improvement program should be initiated for selection and multiplication of elite genotypes. There is also a need to develop protocols for artificial generation of the species through clonal and tissue culture techniques.

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