



Genetic diversity and differentiation in *Dalbergia sissoo* (Fabaceae) as revealed by RAPD

B.-Y. Wang, L. Shi, Z.-Y. Ruan and J. Deng

The Research Institute of Resource Insects,
Chinese Academy of Forestry, Yunnan, China

Corresponding author: L. Shi
E-mail: leishi@139.com

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ABSTRACT: *Dalbergia sissoo*, a wind-dispersed tropical tree, is one of the most preferred timber tree species of South Asia. Genetic diversity and differentiation among natural populations of *D. sissoo* were examined for the first time. We found a relatively high level of genetic diversity in *D. sissoo*, both at the species level (percentage of polymorphic bands = 89.11%; $H = 0.2730$; $I = 0.4180$) and the population level (percentage of polymorphic bands = 68.7%; $H = 0.239$; $I = 0.358$), along with a relatively low degree of differentiation among populations ($GST = 0.1311$; $AMOVA = 14.69\%$). Strong gene flow among populations was estimated, $N_m = 3.3125$. The Mantel test suggested that genetic distances between populations were weakly correlated with geographic distances ($R = 0.3702$, $P = 0.1236$). The high level of genetic diversity, low degree of differentiation, strong gene flow, and weak correlation between genetic and geographic distances can be explained by its biological character and wide-spread planting. This information will be useful for the introduction, conservation and further studies of *D. sissoo* and related species.

Key words: *Dalbergia sissoo* Roxb.; RAPD; Genetic diversity; Genetic differentiation

INTRODUCTION

Dalbergia sissoo Roxb. (Sissoo) is a wind-dispersed tropical tree, and its natural distribution zone (latitude 24° 42' N to 32° 36' N, longitude 74° 30' E to 94° 36' E, altitude 76 to 460 m asl) is in the foothills of the Himalayas from eastern Afghanistan through Pakistan to India and Nepal (Sagta and Nautiyal, 2001; Ashraf et al., 2010). It is not only valued for timber but also for a range of other natural products such as fibers, alkaloids, tannins, and resins. Due to its economic value, the species is not only widely planted throughout its natural distribution, but also planted worldwide as an exotic species, such as in China, Cuba, Brazil, Honduras, etc. Studies have been conducted on morphological variation (Dhillon et al., 1995; Sagta and Nautiyal, 2001; Devagiri et al., 2007), genetic diversity of plantations (Devagiri et al., 2007), and DNA extraction methods in *D. sissoo* (Pandeya et al., 2007; Ribeiro and Lovato, 2007; Ginwal and Maurya, 2010). However, there have not yet been any studies on *D. sissoo* with regard to genetic diversity and its pattern of natural populations. Genetic diversity and natural distribution pattern are very important for the introduction and conservation of *D. sissoo*.

Among the methods employed to assess genetic diversity and distribution pattern, those based on molecular markers are widely used with forest tree species (Newton et al., 1999). Random amplified polymorphism DNA (RAPD) is one of the most popular DNA-based approaches (Bekessy et al., 2002). It is the least technically demanding and offers a fast method of providing information from a large number of loci, particularly in species where no study has been undertaken. Moreover, the diversity assessed by RAPD is comparable to that obtained with allozymes or restriction fragment length polymorphism (RFLP) (Esselman et al., 2000). Lack of reproducibility is considered to be its limitation, but studies show that the results of RAPD can be reproduced in a stable polymerase chain reaction (PCR) system (Wang et al., 2003). Arif et al. (2009) compared inter-simple sequence repeat (ISSR) and RAPD markers in the study of genetic diversity in *D. sissoo*, and the results show that RAPD markers were relatively more efficient than the ISSR assay.

In this study, we used RAPD to investigate the genetic diversity and its pattern in the natural range of *D. sissoo*. The aims of this study were then i) to estimate the genetic diversity both at the species and population level and ii) to analyze the genetic differentiation in *D. sissoo*.

MATERIAL AND METHODS

Five populations including 120 individuals were collected from their natural distribution in India and Nepal (Table 1 and Figure 1). Their fresh leaves were collected and dried with silica in a zip-lock plastic bag for 2 weeks, and genomic DNA was extracted and isolated from dry leaves according to the modified CTAB procedure (Wang et al., 2003).

Sixteen polymorphic primers were selected of 200 random primers, which were synthesized by the Shanghai Biotechnology Company. PCR amplification was carried out in a 20- μ L solution containing: 10 ng DNA, 5 nmol dNTPs, 45 nmol MgCl₂, 20 pmol primer, 2 U DNA Tag polymerase, 2 μ L buffer (Promega), and sterile water to volume. Optimal amplification conditions for RAPDs were 1 cycle of 5 min at 94°C (initial denaturation), followed by 40 cycles of 1 min at 94°C (denaturation), 1 min at 36°C (annealing) and 2 min at 72°C (extension). A final step of 10 min at 72°C ensured full extension of all amplified products. PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide and visualized by UV transillumination.

Only RAPD bands that could be unequivocally scored were counted in the analysis. As a dominant marker, amplified DNA marker bands were scored in a binary manner as either present (1) or absent (0) and entered into a binary data matrix. Genetic diversity was measured by the percentage of polymorphic bands (PPB), Nei's genetic diversity (Nei, 1973) and Shannon diversity index. These were calculated using POPGENE1.32 (Yeh et al., 2000). The non-parametric analysis of molecular variance (AMOVA) program v1.55 (Excoffier et al., 1992) was used as an approach to describe genetic structure among the populations. A UPGMA (unweighted pair-group method with arithmetic means) dendrogram was constructed by MEGA3 based on the matrix of genetic distance from POPGENE1.32. The Mantel test was performed using NTSYSpc 2.1e in order to test for a correlation between genetic distance and geographic distance between populations.

Table 1. Information on the *Dalbergia sissoo* populations studied.

Population name	Location	Latitude (N)	Longitude (E)
Latitpar	Latitpar, Uttar Pradesh, India	24.7	78.5
Sibsagar	Sibsagar, Assam, India	27.0	94.6
RaptiRiver	Rapti River, Hitaunda, Nepal	27.3	85.2
Kankai	Kankai, Gaire Domukha, Jhapa, Nepal	26.4	87.5
Pathankot	Pathankot, Punjab, India	32.3	75.7

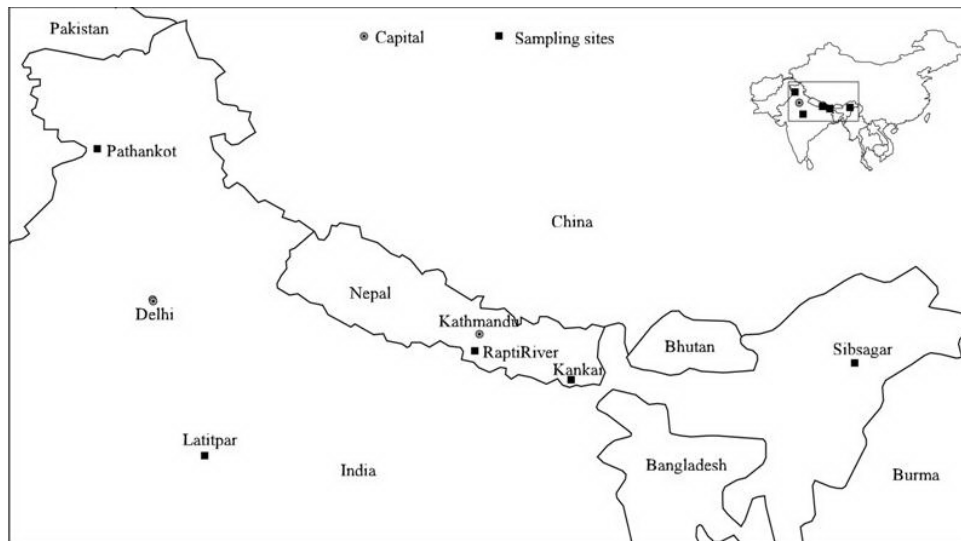


Figure 1. Locations of the *Dalbergia sissoo* populations studied.

RESULTS

Genetic diversity

Screening of primers resulted in 16 decamer primers that showed polymorphisms within the five populations of *D. sissoo* used, and that generated 101 bands ranging in molecular size from 200 to 1700 bp. On average, 6.3 loci were scored per primer. At the species level,

the PPB, Nei's genetic diversity and Shannon diversity index were 89.11%, 0.418 and 0.273, respectively. Within the populations, the PPB ranged from 60.40 to 77.23%; Nei's genetic diversity between the populations ranged from 0.1964 to 0.2618, and the Shannon diversity index between the populations ranged from 0.2991 to 0.3948 (Table 2).

Table 2. Genetic diversity of *Dalbergia sissoo*.

Population	N	H	I	PPB (%)
Kankai	24	0.2439 (0.1899)	0.3668 (0.2707)	72.28
RaptiRiver	24	0.2618 (0.1815)	0.3948 (0.2566)	77.23
Latitpar	24	0.2580 (0.2017)	0.3819 (0.2824)	70.30
Pathankot	24	0.2349 (0.2137)	0.3451 (0.2992)	63.37
Sibsagar	24	0.1964 (0.1865)	0.2991 (0.2725)	60.40
Total	120	0.2730 (0.1696)	0.4180 (0.2281)	89.11

H = Nei's genetic diversity; I = Shannon diversity index; PPB = percentage of polymorphic bands.

Differentiation

Coefficient of overall differentiation (G_{ST}) among populations was 0.1311 (Table 3). AMOVA showed a highly significant (0.001) genetic differentiation among the five populations of *D. sissoo*. Of the total genetic diversity, 14.69% resided among populations and the rest (85.31%) resided among individuals within populations (Table 4). Indirect estimate of the level of gene flow between populations was calculated, and the gene flow (N_m) value was 3.3125 individuals per generation.

Table 3. Coefficient of differentiation for *Dalbergia sissoo*.

Parameter	H_t	H_s	G_{ST}	N_m
Mean	0.2751	0.2390	0.1311	3.3125
SD	0.0289	0.0231		

SD = standard deviation.

Table 4. Analysis of molecular variance (AMOVA) for 5 populations of *Dalbergia sissoo*.

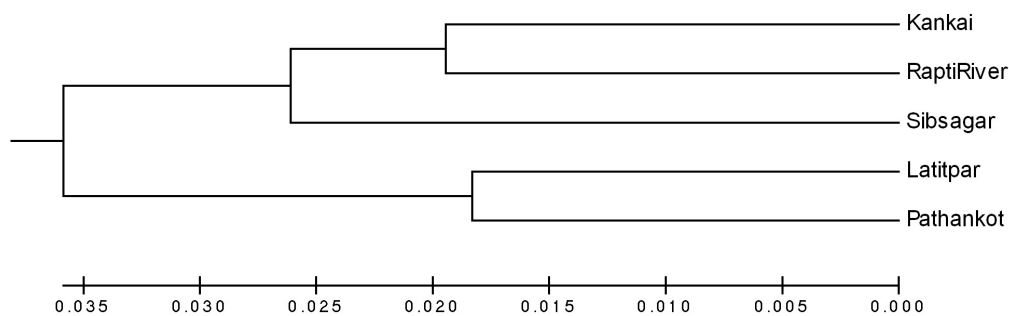
Source of variation	d.f.	Sum of squares	Mean square	Variance component	% of total variance	P
Between populations	4	181.688	45.422	1.5	14.69	<0.001
Within populations	115	1022.12	8.888	8.9	85.31	<0.001

d.f. = degrees of freedom.

Genetic distance between populations ranged from 0.0389 to 0.0883, and geographic distance between populations ranged from 250 to 1930 km (Table 5). The Mantel test showing a coefficient of correlation between genetic and geographic distances was neither high ($R = 0.3702$) nor significant ($P = 0.1236$). A dendrogram based on Nei's genetic distance showed that five populations were divided into two clusters (Figure 2).

Table 5. Genetic distance (below diagonal) and geographic distance (above diagonal) between populations of *Dalbergia sissoo*.

	Kankai	RaptiRiver	Latitpar	Pathankot	Sibsagar
Kankai	-	250	927	1325	712
RaptiRiver	0.0389	-	733	1082	936
Latitpar	0.0938	0.0636	-	895	1639
Pathankot	0.0777	0.0569	0.0366	-	1930
Sibsagar	0.0500	0.0544	0.0883	0.0498	-

**Figure 2.** UPGMA dendrogram based on Nei's genetic distance.

DISCUSSION

Although the PPB, Nei's genetic diversity and the Shannon diversity index are all important parameters to assess the variation within population or species, their emphasis differs. PPB is calculated for the proportion of polymorphic bands, and only polymorphism is considered; in Nei's genetic diversity, both polymorphism and gene heterozygosity are considered; the Shannon diversity index is calculated for each locus and averaged over loci to provide the degree of variation within populations or species, and both polymorphism and its distribution are considered.

With polymorphic and monomorphic loci used for calculation, genetic diversity was higher in *D. sissoo* than estimated for other plants. The mean PPB of plant species is 62% with RAPD markers (Zou et al., 2001). At the species level, the mean PPB in gymnosperms, dicotyledons, and monocotyledons are 70.9, 44.8 and 59.2%, respectively, and at the population level, the mean PPB in gymnosperms, dicotyledons, and monocotyledons are 57.7, 29 and 40.3%, respectively (Hamrick and Godt, 1990). In *D. sissoo*, PPB was 89.11% at the species level and the average PPB was 68.7% at the population level. A high level of genetic diversity may be related to the mating system and geographic distribution. Species with cross-fertilization and a wide geographic range have higher levels of genetic diversity than do selfing and endemic species (Casiva et al., 2002). Although the natural distribution is not very wide, a long planting history allowed its distribution to cover South Asia and even another continent.

Compared to the other species in *Dalbergia*, genetic diversity in *D. sissoo* was also higher at both the species level and population level. In *D. monticola*, PPB, Shannon diversity index and Nei's genetic diversity are 83%, 0.30 and 0.19, respectively, at the species level, and at the population level, the average PPB, Shannon diversity index and Nei's genetic diver-

sity are 51%, 0.223 and 0.15, respectively (Andrianoelina et al., 2006). In *D. odorifera*, PPB, Shannon diversity index and Nei's genetic diversity are 54.55%, 0.3137 and 0.2137, respectively, at the species level, and at the population level, the average PPB, Shannon diversity index and Nei's genetic diversity are 40.9%, 0.2048 and 0.1353, respectively (Yang et al., 2007). In the present study, PPB, Shannon diversity index and Nei's genetic diversity were 89.11%, 0.4180 and 0.2730, respectively, at the species level, and at the population level, the average PPB, Shannon diversity index and Nei's genetic diversity were 68.7%, 0.358 and 0.239, respectively. This may be explained by the special mechanism in seed selection. It is found that seed pairs developing in a given pod are genetically more similar than pairs of seeds chosen randomly from a tree, but of four to five ovules in the flower, generally one and occasionally two or three develop to maturity (Mohana et al., 2001). As a result, a high ratio of pods with one seed improved the level of genetic diversity.

The genetic structure of a species is affected by a number of evolutionary factors including mating system, gene flow and seed dispersal, and mode of reproduction, as well as natural selection (Hamrick et al., 1992). Although there is a large distance between the populations in the present study, varying from 250 to 1930 km, no obvious differentiation was found among the populations in *Dalbergia*. G_{ST} between populations in *Dalbergia* is 0.1311 and much smaller than the mean G_{ST} for dicotyledons, 0.32 (Nybom and Bartish, 2000). The similarity result was obtained by AMOVA: 14.69% between populations and 85.31% within populations. This can be explained by some biological patterns such as long-lived woody perennials and outcrossed insect-pollinated species, according to Nybom and Bartish (2000). *Dalbergia sissoo* is a long-lived woody plant and is insect pollinated, its seeds have wings and are dispersed by wind, and all these characters are helpful for gene flow between populations. Another possible explanation is that *D. sissoo* is widely planted in the natural distribution. Plantations fill the gap between the natural populations, and gene flow can easily occur from one natural population to another. Long-distance planting material transfer during plantation establishment of *D. sissoo* in Nepal was proved by Pandey et al. (2004), and this transfer not only enhances long-distance gene flow, but also breaks down the relationship between genetic and geographic distances. As a result, strong gene flow was detected in *Dalbergia*, 3.3125 individuals per generation ($N_m = 3.3125$), and the correlation between genetic and geographic distances was neither strongly positive ($R = 0.3702$) nor significant ($P = 0.1236$).

This study provides data on the genetic diversity and its distribution of *D. sissoo* in natural populations, and contributes to an overall understanding of living conditions of *D. sissoo*. The study is helpful in resource conservation, introduction, and further studies of *D. sissoo* and related species.

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