

Genome size variation and evolution in some species of *Dalbergia* Linn.f. (Fabaceae)

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Abstract — The present study deals with the extent of genome size variation and evolution in some species of *Dalbergia*. The interspecific 4C DNA content variation has been analyzed in 30 accessions belonging to ten species of *Dalbergia*. The 4C DNA content in ten *Dalbergia* species ranges from 5.85 pg in *D. lanceolaria* to 7.88 pg in *D. horrida*, about 1.3 fold variation is apparent at a constant chromosome number $2n=2x=20$. ANOVA test reveals significant variation between species. Like other woody angiospermic taxa, tree species of *Dalbergia* exhibit small genome size ranging from 5.85 pg in *D. lanceolaria* to 7.22 pg in *D. sissooides*. In contrast, the shrubs and climbers of this genus also reveal a narrow range of genome size variation from 7.36 pg in *D. rubiginosa* to 7.88 pg in *D. horrida*. The climber species of this genus exhibits higher DNA amount than the tree species. The present investigation indicates that lianas or climber species of *Dalbergia* have evolved from tree species through evolutionary increase in genome size. The extent of DNA amount differences between the *Dalbergia* species has been discussed in the light of their interrelationships and diversity.

Key words: cytophotometry, *Dalbergia*, DNA content, evolution, genome size.

INTRODUCTION

Study of nuclear DNA amount is crucial for the overall understanding of the genome of an organism. Nuclear DNA C-values differ by approximately 1000 fold among angiosperms, ranging from about 0.13 pg in *Arabidopsis thaliana* to 127.4 pg in *Fritillaria assyriaca*, and tend to be characteristic of a taxon (BENNETT and SMITH 1976, 1991; BENNETT and LEITCH 1995, 1997; BENNETT *et al.* 2000; HANSON *et al.*, 2003). Genome size is a character of fundamental biological importance, thus the knowledge of nuclear DNA amount or genome size is widely used in diverse areas of biology like Cell and molecular biology, Taxonomy, Systematics, Genome evolution and phylogeny, ecology and environment, phytogeography, in predicting radiosensitivity, ozone depletion, global warming, increased atmospheric CO₂ (BENNETT and LEITCH 1995; LEITCH *et al.* 1998), genome evolution, plant breeding, conservation, physiology and development (BENNETT *et al.* 2000). Thus, an understanding of pattern of C-value distribution and evolution is important in several areas of plant biology.

Interspecific DNA content variations are wide spread and has been well documented in numerous genera of angiosperms like *Oxalis* (DE AZKUE and MARTINEZ 1988), *Guizotia* (HIREMATH *et al.* 1992), *Eleusine* (HIREMATH and SALIMATH 1991), *Pisum* (BARANYI and GREILHUBER 1996), *Arachis* (SINGH *et al.* 1996), *Plantago* (PRAMANIK and RAYCHAUDHURI 1997), *Acacia* and *Prosopis* (BUKHARI 1997), *Mammillaria* (MOHANTY *et al.* 1997), *Dendrobium* (JONES *et al.* 1998), *Cistus* (ELLUL *et al.* 2002). These aspects have been reviewed by BENNETT *et al.* (2000).

Dalbergia Linn. F. a fabaceous genus of tribe Dalbergiæ comprising of about 100 odd species of trees, shrubs and woody climbers is widely distributed throughout the tropics of the world. In India about 35 species are reported and about ten species are known in Karnataka from western mountain ranges or commonly known as western ghats (THOTHATHRI 1987). Asian species of *Dalbergia* are placed in four sections viz. Sect. Sissoa, Sect. Dalbergia, Sect. Selenolobia and Sect. Ecastaphylla (THOTHATHRI 1987). The *Dalbergia* species included in the present study belongs to Sect. Sissoa and Sect. Dalbergia. The sect. Sissoa includes *D. latifolia*, *D. sissoo*, *D. sissooides*, *D. malabarica*, *D. rubiginosa*, *D. horrida* and *D. melanoxylon*, whereas Sect. Dalbergia includes *D. lanceolaria*, *D. paniculata* and *D. volubilis*. *Dalbergia* is an exclusively diploid genus with $2n=20$ chromosomes.

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The genus is of considerable economic importance, as many of its species yield valuable timbers of commerce. The Indian rosewood is derived from *D. latifolia*, whose timber is greatly valued for fine furniture and cabinet works. The timber of *D. sissoo* used for boat building, carts, carriages and rail-sleepers. The *D. sissooides* wood, commonly known as Malabar black wood is used for cabinet works. *D. melanoxylon* better known in commerce as African or Chinese black wood or Sudan ebony is another species whose timber is used for musical instruments and handles of surgical instruments. The *D. lanceolaria* wood is used for agricultural implements (THOTHATHRI 1987).

Genome size diversification is an important process during speciation in plants (GREILHUBER 1998; SOLTIS *et al.* 2003). Speciation in flowering plants is normally accompanied by massive changes in nuclear DNA amounts. Both evolutionary increase and decrease in genome size is well documented during the course of speciation in higher plants (PRICE 1976, 1988; HIREMATH and SALIMATH 1991; AHMAD *et al.* 1992; HIREMATH *et al.* 1992). Until now, most of the genome size studies are based on temperate species and crop plants. In contrast, tropi-

cal and subtropical species particularly the hardwoods have not been studied well (BENNETT and SMITH 1976; BENNETT *et al.* 1982). The present study deals with extent of genome size variation and evolution in some species of *Dalbergia*.

MATERIALS AND METHODS

Germplasm Collection - The seeds of ten *Dalbergia* species involving 30 collections were collected from western mountain ranges or western ghats of Karnataka, India and their details are given in Table 1. One set of vouchered herbarium specimens have been deposited in Herbarium, Royal botanic garden, KEW, UK.

Feulgen Cytophotometry - Seeds of *Dalbergia* species were germinated on moist filter paper in a petridish. Actively growing root apices of *Dalbergia* species and *Pisum sativum* (obtained from Dr. M. D. BENNETT, Jodrell Laboratory, KEW) were excised and fixed in pre-cooled 1:3 acetic acid: absolute ethanol for 24 hours at 5°C (DOLEZEL and NOVAK 1984). Then they were washed in distilled water and hydrolyzed in 5N HCl at 20°C ± 0.5°C for 60 min. To de-

Table 1 — Collection number, Habit, place of collection of *Dalbergia* germplasm.

Sl. No.	Taxa	Habit	Coll. No.	Place of collection
1.	<i>D. latifolia</i>	Tree	DL-433	Dharwad, Karnataka, India
2.	<i>D. latifolia</i>	Tree	DL-450	Chikmagalur, Karnataka, India
3.	<i>D. latifolia</i>	Tree	DL-456	Madikeri, Karnataka, India
4.	<i>D. sissoo</i>	Tree	DS-406	Dharwad, Karnataka, India
5.	<i>D. sissoo</i>	Tree	DS-402	Belgaum, Karnataka, India
6.	<i>D. sissoo</i>	Tree	DS-404	Jamboti, Karnataka, India
7.	<i>D. sissooides</i>	Tree	Ds-475	Hassan, Karnataka, India
8.	<i>D. sissooides</i>	Tree	Ds-476	Madikeri, Karnataka, India
9.	<i>D. sissooides</i>	Tree	Ds-477	Thithimathi, Karnataka, India
10.	<i>D. lanceolaria</i>	Tree	DL-407	Dharwad, Karnataka, India
11.	<i>D. lanceolaria</i>	Tree	DL-479	Belgaum, Karnataka, India
12.	<i>D. lanceolaria</i>	Tree	DL-461	Khanapur, Karnataka, India
13.	<i>D. paniculata</i>	Tree	DP-412	Belgaum, Karnataka, India
14.	<i>D. paniculata</i>	Tree	DP-415	Kalghatgi, Karnataka, India
15.	<i>D. paniculata</i>	Tree	DP-417	Dharwad, Karnataka, India
16.	<i>D. melanoxylon</i>	Shrub	DM-416	Dharwad, Karnataka, India
17.	<i>D. melanoxylon</i>	Shrub	DM-410	Bangalore, Karnataka, India
18.	<i>D. melanoxylon</i>	Shrub	DM-485	Dharwad, Karnataka, India
19.	<i>D. volubilis</i>	Climber	DV-441	Shimoga, Karnataka, India
20.	<i>D. volubilis</i>	Climber	DV-436	Belgaum, Karnataka, India
21.	<i>D. volubilis</i>	Climber	DV-435	Jamboti, Karnataka, India
22.	<i>D. horrida</i> var. <i>concanensis</i>	Climber	DH-424	Shimoga, Karnataka, India
23.	<i>D. horrida</i> var. <i>concanensis</i>	Climber	DH-425	Sirsi, Karnataka, India
24.	<i>D. horrida</i> var. <i>concanensis</i>	Climber	DH-480	Belgaum, Karnataka, India
25.	<i>D. rubiginosa</i>	Climber	DR-428	Castle Rock, Karnataka, India
26.	<i>D. rubiginosa</i>	Climber	DR-466	Sirsi, Karnataka, India
27.	<i>D. rubiginosa</i>	Climber	DR-429	Siddapur, Karnataka, India
28.	<i>D. malabarica</i>	Climber	Dm-426	Londa, Karnataka, India
29.	<i>D. malabarica</i>	Climber	Dm-482	Sirsi, Karnataka, India
30.	<i>D. malabarica</i>	Climber	Dm-481	Sahasralinga, Karnataka, India

termine the optimum time of hydrolysis and to obtain correct staining of nuclear DNA, the root apices of *Dalbergia* and *Pea* were hydrolyzed at different time interval of 10 min to 100 min (GREILHUBER and BARANYI 1999; DOLEZEL and NOVAK 1984). The highest optimum absorbance was noticed at 60 min for both *Dalbergia* and *Pea* with 5N HCl at 20°C ± 0.5°C. The hydrolyzed root tips were given a distilled water wash, and then stained in leucobasic fuchsin (pH 2.2) for 90 minutes in total darkness at 25°C. Basic fuchsin (Pararoseanniline) used was from Sigma, USA. CI 42500. Root apices were then given three subsequent washes of 10 min each in freshly prepared SO₂ water (2.0 gm Potassium metabisulphite in 400 ml distilled water and 20 ml. of 1N HCl) followed by a brief rinsing in distilled water. The root tips were squashed in a drop of glycerol on a glass slide. Three replications of each material were prepared. Measurement of DNA was made using Leitz MPV compact cytophotometer at 541 nm wavelength. The cytophotometer is interphased to a computer and stored by a relevant programme. Measurement of at least 25 metaphase spreads per replicate was made. Simultaneously similar numbers of 4C readings were also taken for *Pisum sativum* cv. Minerva mapple, which was used as an internal standard. In each case the mean of arbitrary units were converted into absolute value (pico grams) of DNA using the mean of identical number of readings from *Pisum sativum* roots processed simultaneously in the same tube as the material. The 4C DNA content of *Pisum sativum* is 19.46 pg (BENNETT and LEITCH 1995) and it was used as a standard for calibrating DNA content of *Dalbergia* species in pico grams. Analysis of variance (ANOVA) was performed using statistical software SPSS- ver-9.0.

RESULTS AND DISCUSSION

Genome size of a species is fairly stable and is an useful character in taxonomic and evolutionary studies (PRICE 1976; OHRI and KHOSHOO 1986; GREILHUBER and EHRENDORFER 1988; RAINA 1990; BENNETT and LEITCH 1995; BENNETT *et al.* 2000). The 4C DNA content has been determined in 10 species of *Dalbergia* involving 30 collections (Table 2). In each species a minimum of three populations have been studied.

Dalbergia is an interesting genus, which comprises of trees, shrubs and climbers and occupy diverse habitats ranging from dry / moist deciduous to semi-evergreen to evergreen forest. It is an exclusively diploid genus with 2n=20 chromosomes. The 4C DNA content in *Dalbergia* species varies considerably. The lowest DNA is 5.85 pg in *D. lanceolaria* and highest is 7.88 pg in *D. horrida* (Fig-1). About

Table 2 — 4C Nuclear DNA amount variation in *Dalbergia* species.

Sl. No.	Acc. No.	Species	Mean 4C DNA amount ± S.E
1.	DL-433	<i>D. latifolia</i>	6.86 ±0.0023
2.	DL-450	<i>D. latifolia</i>	6.87 ±0.0043
3.	DL-456	<i>D. latifolia</i>	6.86 ±0.0013
		<i>D. latifolia</i> Mean	6.86 ±0.0026
4.	DS-406	<i>D. sissoo</i>	6.47 ±0.0282
5.	DS-402	<i>D. sissoo</i>	6.47 ±0.0197
6.	DS-404	<i>D. sissoo</i>	6.45 ±0.0098
		<i>D. sissoo</i> Mean	6.46 ±0.0107
7.	Ds-475	<i>D. sissoides</i>	7.24 ±0.0353
8.	Ds-476	<i>D. sissoides</i>	7.20 ±0.0286
9.	Ds-477	<i>D. sissoides</i>	7.20 ±0.0240
		<i>D. sissoides</i> Mean	7.22 ±0.0157
10.	DL-407	<i>D. lanceolaria</i>	5.86 ±0.0032
11.	DL-479	<i>D. lanceolaria</i>	5.82 ±0.0227
12.	DL-461	<i>D. lanceolaria</i>	5.84 ±0.0093
		<i>D. lanceolaria</i> Mean	5.85 ±0.0094
13.	DP-412	<i>D. paniculata</i>	6.34 ±0.0255
14.	DP-415	<i>D. paniculata</i>	6.33 ±0.0271
15.	DP-417	<i>D. paniculata</i>	6.33 ±0.0228
		<i>D. paniculata</i> Mean	6.33 ±0.0127
16.	DM-416	<i>D. melanoxylon</i>	7.37 ±0.0090
17.	DM-410	<i>D. melanoxylon</i>	7.40 ±0.0151
18.	DM-485	<i>D. melanoxylon</i>	7.35 ±0.0120
		<i>D. melanoxylon</i> Mean	7.37 ±0.0095
19.	DV-441	<i>D. volubilis</i>	7.74 ±0.0086
20.	DV-436	<i>D. volubilis</i>	7.74 ±0.0112
21.	DV-435	<i>D. volubilis</i>	7.81 ±0.0122
		<i>D. volubilis</i> Mean	7.76 ±0.0117
22.	DH-424	<i>D. horrida</i> var. <i>concanensis</i>	7.86 ±0.0092
23.	DH-425	<i>D. horrida</i> var. <i>concanensis</i>	7.87 ±0.0217
24.	DH-480	<i>D. horrida</i> var. <i>concanensis</i>	7.91 ±0.0125
		<i>D. horrida</i> var. <i>concanensis</i> Mean	7.88 ±0.0099
25.	DR-428	<i>D. rubiginosa</i>	7.37 ±0.0202
26.	DR-466	<i>D. rubiginosa</i>	7.35 ±0.0211
27.	DR-429	<i>D. rubiginosa</i>	7.37 ±0.0092
		<i>D. rubiginosa</i> Mean	7.36 ±0.0093
28.	Dm-426	<i>D. malabarica</i>	7.52 ±0.0177
29.	Dm-481	<i>D. malabarica</i>	7.52 ±0.0178
30.	Dm-482	<i>D. malabarica</i>	7.55 ±0.0199
		<i>D. malabarica</i> Mean	7.54 ±0.0092

1.3 fold variation is apparent at a constant chromosome number. Analysis of variance (ANOVA) of DNA content variation in different species of *Dalbergia* is significant (Table 3). Like most woody genera, *Dalbergia* also exhibits a narrow range of genome size variation (OHRI and KHOSHOO 1986). Woody angiosperms have been believed to possess a small and relatively uniform genome size because of the implied constraints on maximum nuclear size by the small cambial cells, which form wood fibres (DARLINGTON 1937; STEBBINS 1950).

Like other woody angiospermic taxa {e.g. *Acacia melanoxylon* (6.5 pg), *Pongamia glabra* (7.2 pg) etc.},

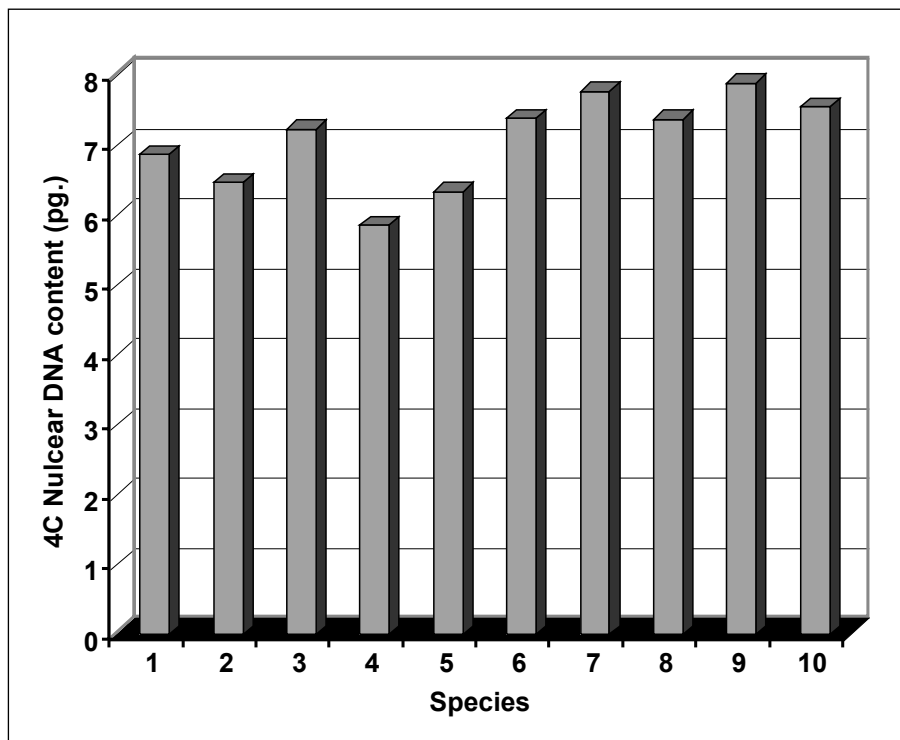


Fig. 1 — Genome size variation in *Dalbergia* species. 1 - *D. latifolia*. 2 - *D. sissoo*. 3 - *D. sissoides*. 4 - *D. lanceolaria*. 5 - *D. paniculata*. 6 - *D. melanoxyylon*. 7 - *D. volubilis*. 8 - *D. rubiginosa*. 9 - *D. horrida*. 10 - *D. malabarica*.

Table 3 — Analysis of Variance (ANOVA) of interspecific variation in *Dalbergia* species.

Source of variation	D.F.	Sum of Squares	Mean Squares	F Ratio	F Prob.
Between Groups	9	12.0595	1.340	3603.34	2.62*
Within Groups	20	0.00744	0.00037		
Total	29	12.0669			

* Significant at 5% level

tree species of *Dalbergia* exhibit small genome size ranging from 5.85 pg in *D. lanceolaria* to 7.22 pg in *D. sissoides*. In contrast, the shrubs and climbers of this genus also reveal a narrow range of genome size variation from 7.36 pg in *D. rubiginosa* to 7.88 pg in *D. horrida*. Apparently, climbers have higher amount of DNA than their ancestral tree species. It is generally accepted that the climbers have evolved from the tree species (LAWRENCE 1973). Thus, lianas or climber species of *Dalbergia* occupying evergreen / semi evergreen forests have evolved from tree species of *Dalbergia* through evolutionary increase in genome size.

In fact, many studies have shown that variation in DNA amount is under strong selection pressure with respect to various phenotypic and phenological factors (BENNETT 1972; OHRI and KHOSHOO 1986). Morphological and anatomical studies reveal that climbers are derived from trees and shrubs (RAD-

FORD *et al.* 1974). Anomalous secondary growth and greater diameter of vessel elements is a derived feature. Both having adaptive value for climbers related to flexibility and more efficient transport (CARLQUIST 1991; LOMBELLO and FORNI-MARTINS 1998). Chromosomal studies in different genera of Sapindaceae indicate that the climbing habit is derived from non-climbing habit (LOMBELLO and FORNI-MARTINS 1998). Thus, diversification of climber species in *Dalbergia* from their ancestral tree taxa is accompanied by increase in genome size.

Economically important timber tree species shisham *D. sissoo*, Indian rosewood, *D. latifolia* and malabar blackwood *D. sissoides* exhibit 6.46 pg, 6.86 pg and 7.22 pg of DNA respectively. These three tree species are closely related and form one genetic assemblage. From the distributional point of view, *D. sissoo* is a primitive species, as it is the most widely distributed taxon of *Dalbergia* in India. In

comparison to this species, *D. latifolia* is less widely distributed and appears to be evolved from it. Endemic south Indian species, *D. sissooides* occupying semi-evergreen forests of western ghat area is morphologically similar to *D. latifolia* and appears to be evolved from it. It appears that species differentiation in these closely related tree species *D. sissoo* (6.46 pg), *D. latifolia* (6.86 pg) and *D. sissooides* (7.22 pg) have occurred through small increase in genome size.

D. lanceolaria and *D. paniculata* are morphologically similar with DNA content of 5.85 pg and 6.33 pg respectively. Obviously, latter species has 7.6% more DNA than former taxon. THOTHATHRI (1987) has proposed that *D. paniculata* may be merged with primitive taxon *D. lanceolaria* with subspecies status. Apparently, *D. paniculata* appears to have evolved from *D. lanceolaria* through an evolutionary increase in genome size. Present studies on evolution of genome size in *Dalbergia* clearly shows that, like most woody angiosperms the genome size in *Dalbergia* is small and exhibits a narrow range of variations among its species.

Lianas or climber species like *D. volubilis*, *D. horrida*, *D. rubiginosa* and *D. malabarica* have evolved from primitive tree species through evolutionary increase in genome size. Species differentiation among tree species *D. sissoo*, *D. latifolia*, *D. sissooides* and also in *D. paniculata* has occurred through small increase in genome size. Thus, an increase in DNA amount seems to be the predominant evolutionary trend during species differentiation in *Dalbergia*.

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