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Cytological Investigations in Some Important Tree Species of Rajasthan I. Karyomorphological Studies in some Species of *Anogeissus* (DC.) Guill., Perr. & A. Rich.

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Summary

Karyomorphological studies have been done in ten different accessions belonging to three different species of *Anogeissus* (*A. pendula*, *A. latifolia* and *A. sericea*) an important hardwood tree of Rajasthan. The somatic chromosome number of 2n=24 has been observed in all the species/accessions with distinct interspecific variation in the arm ratio of respective homologous pairs of chromosomes. Nucleolar chromosomes are reported in two taxa (*A. pendula* BSJO 19564 and *A. sericea* var. *sericea* BSJO 19568) and heteromorphism is recorded in *A. pendula* BSJO 19563, BSJO 19564, *A. latifolia* BSJO 19570 and *A. sericea* var. *sericea* BSJO 19569. The karyotypes of all the species/accessions were more or less symmetrical. The role of karyotypic variation in speciation and evolution of the genus *Anogeissus* has been discussed in detail.

Key words: Anogeissus, species, hardwood tree, karyotype analysis, nucleolar chromosomes, symmetry.

Introduction

The data on chromosome numbers and comparative karyology is fundamental to overall understanding of genome in different species or in morphologically diverse populations within a species (STACE, 2000). Karyological studies in hardwoods are hampered by the difficulty in obtaining well spread metaphase plates and optimum staining thereby obscuring the morphological details. These problems have earlier been pointed out by GILL and SINGHAL (1998a and b); DAS et al. (1995). Hence very little information is available on tropical hardwood species especially of arid and semiarid regions. The present study therefore, deals with karyological details of some taxa of the genus *Anogeissus* (Combretaceae) which is important as timber, fuel wood and fodder tree in the arid ecosystem of western Rajasthan, India.

Material and Methods

Extensive surveys were conducted in different areas in the state of Rajasthan to locate various populations of *Anogeissus*.

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The trees have been marked and appropriately labeled before seeds were collected from them, which formed the basic material for detailed mitotic studies. Herbarium specimens for all the trees under study were prepared and voucher specimens were deposited at Botanical Survey of India, Jodhpur. For obtaining actively growing root tips, seeds were germinated on moist filter paper in petri-plates kept at 25±2°C in dark in BOD incubator. The root tips of about (0.5–1 cm) long were excised and pretreated with 0.025% colchicine (Himedia) for three hours followed by fixation in freshly prepared carnoy's fluid for 24 hours. Root tips were hydrolysed with 1 N HCl for 8–10 minutes at 60°C and stained in 0.5% leuco-basic fuchsin. The stained tips were squashed in 1% aceto-carmine. At least five clear preparations of chromosome complements of each accession were analyzed for the karyotypes. Idiograms were prepared from photomicrographs by cutting out individual chromosomes, arranging them in descending order of their length and matching on the basis of morphology. BATTAGLIAS's (1955) classification of median (V), submedian (L), subtelocentric (J) and telocentric (I) based on the arm ration of 1:1; >1:1<1:3; >1:3<1:0; and 1:0 respectively was used for comparison. The degree of symmetry was estimated as per the scheme proposed by STEBBINS (1971).

Results

Mitotic data on 10 accessions of *Anogeissus* are summarized in *Tables 1–2*.

Chromosome Complements

10 accessions belonging to three species of *Anogeissus* viz. *A. pendula*, *A. latifolia*, *A. sericea* (var. *nummularia* and var. *sericea*) have been studied in detail for somatic chromosome number and karyomorphological details. All the accessions collected from various locations showed 2n=24 chromosomes which were clearly resolved into 12 pairs forming a series from the longest to shortest pair with in the complement.

A. pendula JNVU/RI 2001

Five pairs were metacentric and rest of the seven pairs were submetacentric in nature. Longest chromosome was almost two times longer than the smallest one. No heteromorphic or nucleolar chromosomes were recorded. Karyotypic formula was resolved into 10 V + 14 L (*Table 1*).

A. pendula BSJO 19563

Five pairs were metacentric and seven pairs of chromosomes were found to be submetacentric in nature. The ratio of longest to smallest chromosome was 1.5. The seventh pair was found to be heteromorphic in nature and no nucleolar chromosomes were recorded (*Figs. 1a, 2a*). Karyotypic formula was resolved into 10 V + 14 L (*Table 1*).

A. pendula BSJO 19564

Four pairs were metacentric and seven pairs were submetacentric. The remaining one pair (12^{th}) was found to be

Table 1. – Karyomorphology and arm ratio in various taxa of *Anogeissus* (BATTAGLIA, 1955).

S.No.	Taxa	2n	r-index in different chromosomes												Karyotypic formula
			I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
	Anogeissus														
1.	*A.. pendula* JNVU/RI 2001	24	1.05 L	1.5 L	1.0 V	1.0 V	1.0 V	1.0 V	1.1 L	1.15 L	1.0 V	1.1 L	1.5 L	2.0 L	10V+14L
2.	*A. pendula* BSJO 19563	24	2.05 L	1.0 V	1.5 L	1.5 L	1.0 V	1.6 L	2.0* $\underline{1.7}$ LV	1.5 L	1.0 V	1.0 L	1.0 V	1.1 L	10V+14L
3.	*A. pendula* BSJO 19564	24	$\overline{2.1}$ L	1.0 V	1.5 L	1.65 L	1.7 L	1.5 L	1.3 L	1.5 L	1.0 V	1.0 V	1.0 V	1.1 $\underline{1.0}$ LV	9V+14L+1Ln*
4.	*A. latifolia* BSJO 19571	24	1.0 V	1.5 L	1.0 V	1.05 L	1.1 L	1.2 L	1.0 V	1.0 V	1.1 L	2.0 L	1.0 V	1.0 V	12V+12L
5.	*A. latifolia* BSJO 19570	24	1.5 L	2.05 L	1.05 L	1.0 V	1.5 L	1.0 V	2.0 L	2.0 L	1.0 V	1.1 L	1.1 $\underline{1.0}$ LV	1.0 V	9V+15L
6.	*A. sericea var. nummularia* BSJO 19566	24	1.6 L	1.05 L	1.0 V	2.5 L	1.0 V	1.2 L	1.0 V	1.1 L	1.0 V	1.2 L	2.0 L	1.0 V	10V+14L
7.	*A. sericea* var. *nummularia* BSJO 19567	24	1.5 L	1.0 V	1.1 L	1.15 L	1.5 L	1.0 V	2.0 L	2.0 L	1.0 V	1.0 V	1.0 V	1.0 V	12V+12L
8.	*A. sericea* var. *sericea* BSJO 19565	24	1.05 L	1.0 V	1.1 L	1.0 V	3.0 J	2.7 L	1.5 L	2.05 L	1.0 V	1.0 V	1.0 V	2.0 L	10V+12L+2J
9.	*A. sericea* var. *sericea* BSJO 19568	24	$\overline{1.6}$ L	1.05 L	1.5 L	1.5 L	1.0 V	1.0 V	3.0 J	1.0 V	1.0 V	1.05 L	1.1 L	1.1 L	8V+13L+1Ln*+2J
10.	*A. sericea* var. *sericea* BSJO 19569	24	1.5 L	1.4 L	1.0 V	1.0 V	1.0 V	1.0 V	1.3 L	1.2 L	1.4 V	1.0 L	1.5 L	1.9 L	10V+14L

Heteromorphic pairs are underlined and nucleolar pairs are lined above the value. * Nucleolar Chromosomes

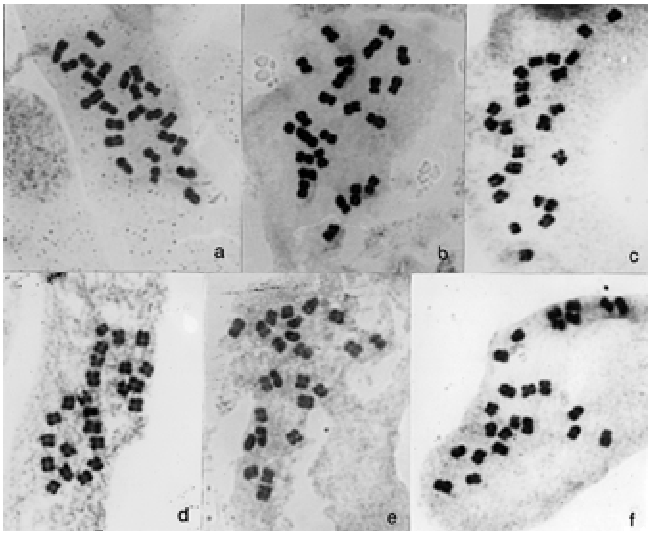

Figure 1. – Mitotic complements of *Anogeissus* species; (a) *A. pendula* BSJO 19563, 2n=24; (b) *A. pendula* BSJO 19564, 2n=24; (c) *A. latifolia* BSJO 19570, 2n=24; (d) *A. sericea* var. *nummularia* BSJO 19567, 2n=24; (e) *A. sericea* var. *sericea* BSJO 19568, 2n=24; (f) *A. sericea* var. *sericea* BSJO 19569, 2n=24.

heteromorphic in nature constituting a metacentric and submetacentric chromosome (*Figs. 1a, 2b*). The karyotypic formula was resolved into 9 V+14 L +1 Ln (*Table 1*). Interestingly the longest pair with nucleolar organizer is almost three times longer than the smallest chromosome in the complement.

A. latifolia BSJO 19571

Six pairs were metacentric and the remaining were submetacentric in nature. No heteromorphic or nucleolar chromosomes were observed. The ratio of longest to smallest chromosomes was 1.6. Karyotypic formula was resolved into 12 V + 12 L (*Table 1*).

A. latifolia BSJO 19570

Four pairs were metacentric and seven pairs were submetacentric. The remaining one pair (11th) was heteromorphic in nature and no nucleolar chromosomes were observed in the complement (*Figs. 1c, 2c*). The ratio of longest to smallest chromosomes was 1.3. Karyotypic formula was resolved into 9 V + 15 L (*Table 1*).

A. sericea var. *nummularia* BSJO 19566

Five pairs were metacentric and seven pairs were submetacentric. Heteromorphic and nucleolar organizer chromosomes were not observed. Ratio of longest and smallest chromosomes was about 1.6 times. Karyotypic formula was resolved in to 10 V + 14 L (*Table 1*).

A. sericea var. *nummularia* BSJO 19567

Six metacentric and six submetacentric pairs were observed. Heteromorphic and nucleolar chromosomes were not observed (*Figs. 1d, 2d*). The ratio of longest to smallest chromosomes was 1.3 and karyotypic formula was recorded as 12 V + 12 L (*Table 1*).

A. sericea var. *sericea* BSJO 19565

Five pairs were metacentric and six were submetacentric while one of the pairs (5th) was subtelocentric in nature. No

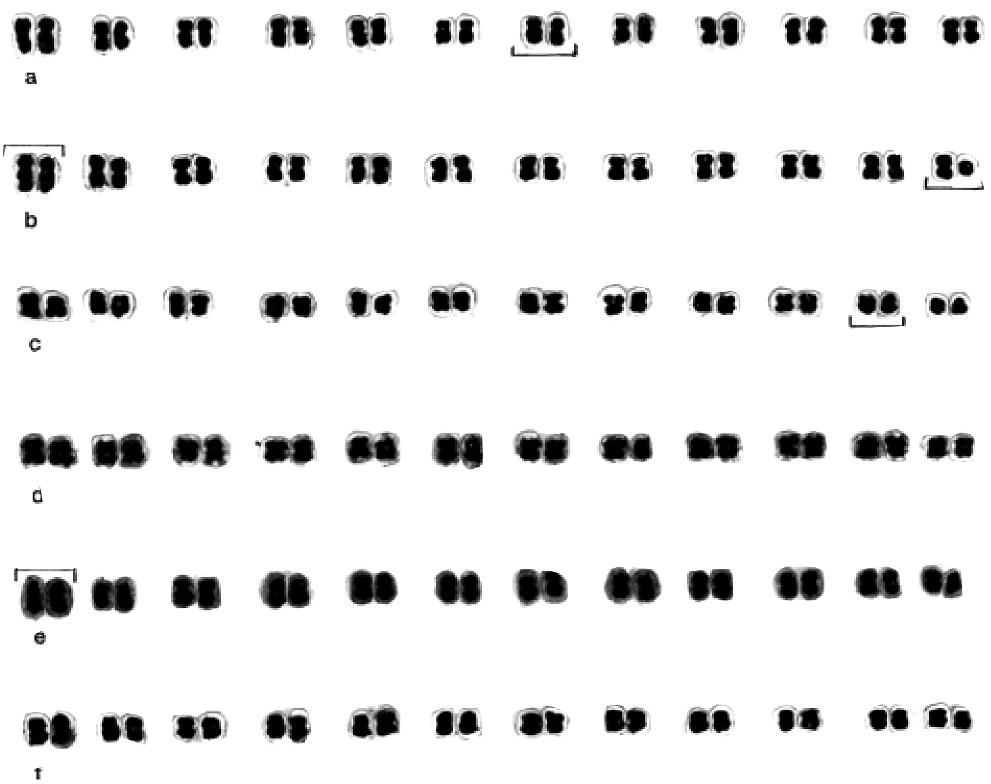

Figure 2. – Photo-idiograms of *Anogeissus* species; (a) *A. pendula* BSJO 19563; (b) *A. pendula* BSJO 19564; (c) *A. latifolia* BSJO 19570; (d) *A. sericea* var. *nummularia* BSJO 19567; (e) *A. sericea* var. *sericea* BSJO 19568; (f) *A. sericea* var. *sericea* BSJO 19569. Nucleolar chromosomes are marked above the short arm. Heteromorphic pair marked below the long arm.

heteromorphic or nucleolar organizer chromosomes were recorded in this accession. Ratio of the longest and smallest chromosome was about 1.6 times and karyotypic formula resolved into 10 V + 12 L + 2 J (*Table 1*).

A. sericea var. *sericea* BSJO 19568

Four pairs were metacentric and six were submetacentric in nature. The first pair was found to be nucleolar while the seventh pair was subtelocentric (*Figs. 1e, 2e*). Karyotypic formula resolved into 8 V + 13 L + 1 Ln + 2 J (*Table 1*).

A. sericea var. *sericea* BSJO 19569

Majority of the cells observed in this accession were having the somatic chromosome number of 2n=24 (*Figs. 1f, 2f*). However, in about 20 percent of cells a variant chromosome number of 2n=22 was recorded. In this variant chromosome complement, four pairs were observed as metacentric and six pairs were found to be submetacentric in nature. One pair (1^{st}) was found to be heteromorphic in nature. Karyotypic formula resolved into 10 V + 14 L (*Table 1*).

Karyotypic details

Data on chromosome morphology in various accessions of *Anogeissus* has been summarized (*Table 1*). Variation was observed with respect to number of metacentric and submetacentric chromosomes, presence or absence of nucleolar chromosomes and/or heteromorphic pairs in the complements. The taxa belonging to *A. pendula* and *A. latifolia* as well as *A. sericea* var. *nummularia*, are characterized by the presence of either metacentric or submetacentric chromosomes whereas three accessions of *A. sericea* var. *sericea* are distinct in having at least one pair of subtelocentric chromosomes in the complement.

The chromosome morphology with regard to a particular pair in the karyotype has shown significant variation both at inter- and intraspecific level (*Table 1*). For example the twelfth pair in *A. pendula* and *A. sericea* var. *sericea* is submetacentric in all of their accessions whereas it is distinctly metacentric in various accessions of *A. latifolia* and *A. sericea* var. *nummularia* . Such observations can be extended even to other pairs as well. Interestingly, in some cases the variation in the chromosome morphology is noticeable even at intraspecific level. In *A. pendula* as many as 7 different pairs had shown inconsistency with regard to chromosome morphology. A pair of heteromorphic chromosomes are recorded in two (BSJO 19563 and BSJO 19564), one (BSJO 19570) and one (BSJO 19569) accessions of *A. pendula, A. latifolia* and A. *sericea* var. *sericea* respectively.

Symmetry

Following the classification of Stebbins (1971) the karyotypes in different accessions of the genus *Anogeissus* studied were resolved into 1A (*A. pendula* BSJO 19563, *A. latifolia* BSJO 19571, BSJO 19570 and *A. sericea* var. *nummularia* BSJO 19566, BSJO 19567), IB (*A. pendula* JNVU/RI 2001), IC (*A. pendula* BSJO 19565), 2A (*A. sericea* var. *sericea* BSJO 19565) and 2 B (*A. sericea* var. *sericea* BSJO 19668 and BSJO 19569) (*Table 2*).

Nucleolar Chromosomes

Nucleolar chromosomes were observed only in two (*A. pendula* BSJO 19564 and *A. sericea* var. *sericea* BSJO 19568) out of ten accessions belonging to the genus *Anogeissus* (*Table 1*). Interestingly in both these taxa, the first pair in the complements was found to be nucleolar in nature and in both the cases there was prominent secondary constriction.

Table 2. – Number of subtelocentric chromosomes, ratio of largest/smallest chromosomes and degree of symmetry in various taxa of *Anogeissus.*

S. No.	Taxa	2n	Number of subtelocentric chromosomes	Ratio of largest/ smallest chromosome length	Category of symmetry
1.	*A. pendula.* JNVU/RI 2001	24	-	2.0	1B
2.	*A. pendula* BSJO 19563	24	-	1.5	1A
3.	*A. pendula* BSJO 19564	24	-	3.0	1C
4.	*A. latifolia* BSJO 19571	24	-	1.6	1A
5.	*A. latifolia* BSJO 19570	24	-	1.3	1A
6.	*A. sericea* var. *nummularia* BSJO 19566	24	-	1.6	1A
7.	*A. sericea* var. *nummularia* BSJO 19567	24	-	1.3	1A
8.	*A. sericea* var. *sericea* BSJO 19565	24	2	1.6	2A
9.	*A. sericea* var. *sericea* BSJO 19568	24	2	2.0	2B
10.	*A. sericea* var. *sericea* BSJO 19569	24	-	2.1	2B

Discussion

Except for the single report by Gill et al., (1979) on *A. sericea* (2n=24) no other information is available on cytological details of *Anogeissus* species. The other reports by Singh (1992), Kuar (1992), Yusuf (1996) and Agarwal (1996) in *A. sericea* var. *nummularia*, *A. sericea* var. *sericea*, *A. pendula* and *A. latifolia* respectively, are all pertaining to *in vitro* regenerants. The more striking feature of their observations has been that except for a meagre proportion of 3–5 percent, all the cells analysed had shown the 2n number of chromosome as 24. The present investigation involving representative taxa of different populations in all the species of *Anogeissus* did confirm the somatic chromosome number as 24, without any indication regarding existence of polyploidy/aneuploidy or any numerical variation in the natural populations.

The present data together with available details from the literature confirms that *Anogeissus* is a monobasic genus with x=12. According to Gill et al. (1982) most genera belonging to family Combretaceae (*Calycopteris, Conocarpus, Guiera, Terminalia,* besides *Anogeissus*) are based on x=12. Similarly, in six species of *Terminalia* three diploid (2n=2x=24), tetraploid (2n=4x=48) and triploid (2n=3x=36) numbers based on x=12 were reported by Ohri (1996). This information further draws support from the estimations of 2C DNA amount, which has shown 3.5 fold difference in diploid and tetraploid species of *Terminalia*.

In the absence of detailed information regarding basic number of the genus *Anogeissus*, it is quite imperative to have a comparison with other genera of the family Combretaceae, to which *Anogeissus* belongs. The available information on chromosome counts (Kumar and Subramaniam, 1987; Gill and Singhal, 1998a and b; Ohri, 1996) in various genera viz. *Combretum, Getonia, Quisqualis*, *Terminalia* and their constituent species, shows the existence of various basic numbers ranging from 7 in *Terminalia tomentosa* to 14 in *Combretum coccineum* (Nanda, 1962). However, majority of the genera has the basic number of 12 or 13. More interestingly the polyploids reported in *Combretum coccineum* (2n=4x=56), *Getonia* (2n=4x=48), *Quisqualis* (2n=4x=52) and *Terminalia* (2n=4x=48) are all based on these two basic numbers only. Hence it can be safely concluded that various species of *Anogeissus* viz. *A. pendula*, *A. latifolia*. *A. acuminata* and *A. sericea* (var. *nummularia*, *sericea*) with a 2n number of 24, are all evolved from x=12. This is also supported by Mehra and Bawa (1968). Majority of the woody angiosperms have been reported to have evolved from x=12 (Mehra, 1972; Khosla, 1975; Khosla and Sareen, 1978; Morawetz, 1986; Singhal and Gill, 1989; Gill et al., 1990).

Polyploidy is absent in *Anogeissus*, unlike other genera of family Combrataceae viz., *Terminalia* and *Quisqualis*. It is important to mention here that all the *Anogeissus* species studied are propagated through seed and natural vegetative propagation methods are almost unknown (Deora, 1993). Even propagation by root suckers (Dogra, 1995) has very little scope, leaving seeds as the sole means of propagation. The complete absence of vegetative means of propagation and the absence of polyploidy in any of the species studied suggests a correlation between these two aspects as suggested by Mehra and Bawa (1968), and Gill and Singhal (1998b). A genetic mechanism which possibly favours the formation of polyploid complexes with predominant asexual reproduction by vegetative means might not be operative in arid zone tree species in general and *Anogeissus* species in particular. It may be mentioned here that many of the predominant tree species of arid and semiarid regions of Rajasthan viz. *Salvadora persica, S. oleoides, Capparis decidua, Tecomella undulata, Prosopis cineraria, Butea frondos, Striculea urens, Wrightia tinctoria* etc. which are devoid of polyploidy are also known for lack of any vegetative means of propagation.

Characteristic differences have been recorded in karyotypes, both at inter and intraspecific level, of the genus *Anogeissus*. In general, first, seventh, eighth, ninth and twelefth pairs show uniformity with respect to the chromosome morphology at interspecific level in all the species. However, moderate to greater degree of variation was recorded in the remaining pairs. In case of *A. pendula* a lesser degree of variation in three accessions has been observed with a notable exception of heteromorphism in two accessions viz. *A. pendula* BSJO 19563, BSJO 19564. However, these two accessions differed from each other in exhibiting heteromorphism in different pairs. *A. sericea* var. *sericea* was characteristic in having one pair of subtelocentric chromosomes (J), though its position with in the karyotypes differed in all the three accessions studied, while it was totally absent in other species. Such variation with respect to V,L,J types of chromosomes may result due to structural changes in chromosomes viz. duplication, deletions, interchanges and inversions (Rao and Chandel, 1991; Stebbins, 1971). In addition to this, most of the accessions studied had either median or submedian chromosomes with subtelocentrics forming an insignificant proportion. Further it was also observed that submedian types outnumbered median types of chromosomes in all but two accession of *Anogeissus* studied. One accession each belonging to *A. latifolia* and *A. sericea* var. *nummularia* had equal number of median and submedian chromosomes. The range of submedian/median types among various taxa was 12–15 and 8–12 respectively.

The karyotypes in all the taxa studied were found to be symmetrical according to Stebbins (1971) classification. Further the ratio of longest and shortest never exceeded more than 2.3. Subtelocentric/telocentric chromosomes which were recorded in only one in *A. sericea* var. *sericea* (all the three taxa) did not alter overall symmetry of the karyotypes because their number never exceeded two.

The absence of any deviant chromosome numbers other than x=12 and overall symmetry suggest that the diversification at both inter and intraspecific level has occurred without any significant numerical and structural changes.

A pair of nucleolar organisers observed in the form of secondary constriction in only two taxa of the total ten studied may be attributed to technical difficulties arising out of small size of chromosome. The *in situ* hybridization techniques like fluorescent in situ hybridization (Fish) and multicolour fluorescent in situ hybridization (McFish) may be of great help to resolve these problems and may provide an idea about nucleolar organizer chromosomes based speciation.

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