KARYOTYPE ANALYSIS AND ESTIMATION OF NUCLEAR DNA CONTENT IN SIX SPECIES OF ACACIA (FABACEAE)

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Karyotype analysis, determination of somatic chromosome number, total chromosome length and volume, estimation of 4C DNA content and Interphase Nuclear Volume (INV) were carried out in 6 species of *Acacia* of the family *Fabaceae*. Somatic chromosome number 2n=26 in *A. auriculiformis, A. catechu, A. dealbata, A. decurrens, A. suma* and 2n=52 in *A. mollisima* were recorded for the first time. Significant interspecific variations in nuclear DNA amount was noted. The 4C DNA content varied from 2.28 pg in *A. catechu* to 4.82 pg in *A. mollissima*. The INV varied from 210.22 μ^3m in *A. suma* to 356.23 μ^3m in *A. decurrens*. Correlation coefficient studies showed positive correlation between the genomic chromosome lenght, chromosome volume and INV. No interdependency was found between 4C DNA content and chromosome lenght or volume and INV. The structural alterations in the chromosomes as well as loss or addition of highly repetitive sequences in the genome caused variations in the nuclear DNA at interspecific level indicating a macro- and micro- evolution of the species.

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تجزیه و تحلیل کاریوتیپ و تخمین مقدار DNA هسته‌ای در ۶ گونه آکاسیا

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تجزیه و تحلیل کاریوتیپ، شمارش کروموزومی در مرحله میتوز، تعیین حجم و طول کلی مجموعه کروموزومها، تخمین مقدار 4C DNA و تعیین حجم هسته در مرحله بین تقسیم برای ۶ گونه آکاسیا از تیره نخود انجام شده است، شمارش کروموزومی $2n=26$ برای گونه‌های *A. catecho, A. decurrens, A. dealbata, Acacia aurculiformis,* و *A. suma* $2n=52$ برای گونه *A. mollisima* برای اولین بار گزارش می‌گردد. تنوع قابل ملاحظه‌ای در مقدار DNA هسته دیده می‌شود. مقدار 4C DNA از ۲/۲۸pg (پیکوگرم) در گونه *A. catecho* تا ۴/۸۲pg در گونه *A. mollissima* تغییر می‌نماید.

حجم هسته در مرحله بین تقسیمی $210/22\mu^3m$ در گونه *A. suma* تا $356/23\mu^3m$ در گونه *A. decurrens* تغییر می‌نماید. مطالعات ضریب همبستگی، همبستگی مثبت بین طول و حجم کروموزومی و حجم هسته در مرحله بین تقسیمی را نشان می‌دهد. ارتباطی بین مقدار 4C DNA و طول یا حجم کروموزوم و حجم هسته در مرحله بین تقسیم مشاهده نمی‌گردد. تغییر ساختار کروموزوم‌ها و همچنین کاهش و یا افزایش در توالی ژنوم که موجب تنوع DNA هسته در گونه‌های مختلف می‌شود، نشانه تغییر و تحول بزرگ و کوچک در گونه‌ها است.

INTRODUCTION

The genus *Acacia* Willd. of the family *Fabaceae* and subfamily *Mimosoideae,* of xerophytic habitat constitutes of an approximately 800 tree species mostly from Australia. The leaves are often bipinnate and the flowers are regular with the petals valvate in bud. Acacias have flowers with numerous stamens, yields a number of valuable products. The Australian Black Wattle (*Acacia decurrens*) and Golden Wattle (*A. pycnantha*) are the sources of wattle bark, which is used in tanning. A number of species, including the Australian Black Wood (*A. melanoxylon*) and *A. visco,* are used as timber. The species of *Acacia* including *A. stenocarpa* and *A. senegal* yield gum arabic (Heywood 1985). The somatic chromosome number 2n=26 was reported for *Acacia auriculiformis, A. catechu, A. dealbata, A. suma* and 2n=26, 27 in *A. mollissima* (Atchison 1948, Berger et al. 1958, Datta 1971). Cytophotometric estimation of nuclear DNA content was reported only in *A. catechu* (Ohri and Kumar 1986). Detailed karyotype analysis, cytophotometric estimation of 4C DNA content and INV in different species of *Acacia* have not yet been reported. In order to ascertain precisely the importance of DNA in genetic diversity and phylogeny, an understanding of the genetic behaviour at specific level, is necessary. The present study principally deals with somatic chromosome number, DNA content in 6 species of *Acacia* to find out the extent to which the values have a selective advantage in development of new species.

MATERIAL AND METHODS

Seeds of *Acacia auriculiformis* A. Cunn. ex Benth., *A. catechu* (Linn. f.) Willd., *A. decurrens* Willd., *A. dealbata* Link., *A. mollisima* Willd. and *A. suma* Buch.-Ham. obtained from the experimental garden of Regional Plant Resource Centre, Bhubaneswar. Root tips were pretreated in saturated aquous paradichlorobenzene and aesculine mixture (1:1) for three and half hours at 14° C followed by overnight fixation in 1:3 propionic ethanol. Chromosome staining was made in 2% lacto-propionic orcein after cold hydrolysis in 5N HCl for 7 min. Root-tips were squashed in 45% propionic acid. Ten well scattered metaphase plates were selected for karyotype analysis of each species. Total chromosome length and volume of the

genome was ascertained following the method of Das and Mallick (1993 b). Form percentage (F%) of individual chromosomes was calculated following the method of Levine et al (1964). Total form percentage (TF%) was the average of sum total of F% of a karyotype.

For scoring of INV, the root-tips of about 2.5 mm length were fixed in 1:3 acetic acid: ethanol for 24 h at 25 °C, hydrolysed in 1N HCl at 4 °C for 15 min. After thorough washing, root-tips were put into Schiff's reagent for 1 h at 20 °C and kept in dark for staining. Squash preparation was done in 45% acetic acid. Ten randomly selected nuclei were scored from each root-tip; sample size was 20 root-tips per species. Under oil immersion objectives the mean of the two diameters of nuclei, obtained by measuring at right angles to each other, was used to calculate the volume using the formula, volume=4/3 πr^3, where r is the radius of the nuclei.

For Feulgen cytophotometric estimation of 4C DNA, ten fixed root-tips from each species were hydrolysed in 1N HCl for 12 min at 60 °C, washed in distilled water and stained in Schiff's reagent for 2 h at 14 °C, each root-tip squash was prepared in 45% acetic acid. Ten scoring were made from each slide and 4C DNA was estimated from metaphase chromosomes using Nikon Optiphot microscope with microspectrophotometer following the plug method of Sharma and Sharma, (1980) with monochromatic light at 550 nm. *In situ* DNA values were obtained on the basis of optical density which were converted to picograms (pg) by using Bennet and Smith's, (1991) 4C nuclear DNA value (79.46 pg) for *Pisum sativum* cv. Minerva Maple as standard. The correlation coefficient analysis between different chromosomal parameters was done to find out the genomic characteristics. ANOVA were performed among the nuclear DNA values following Duncan's multiple range test (Harter 1960).

OBSERVATIONS

Chromosome characteristics

The somatic chromosome number 2n=26 was observed in *Acacia auriculiformis, A. catechu, A. decurrens, A. dealbata, A. suma* and 2n=52 was noted in *A. mollissima.* On the basis of the size of chromosome and the position of the constrictions, a number of chromosome types were found to be

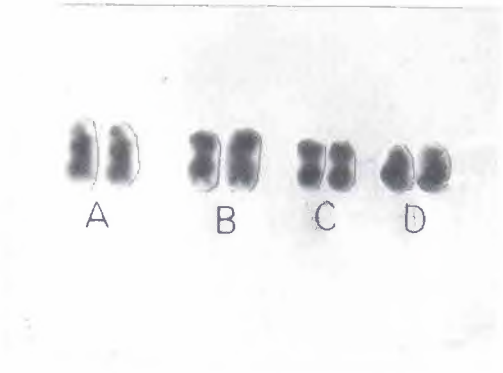


Fig. 1. Types of chromosomes in *Acacia* species.

common among the species studied though they differed from each other in the minute details of the karyotype. A general description of the representative types of chromosomes is as follows (Fig. 1):

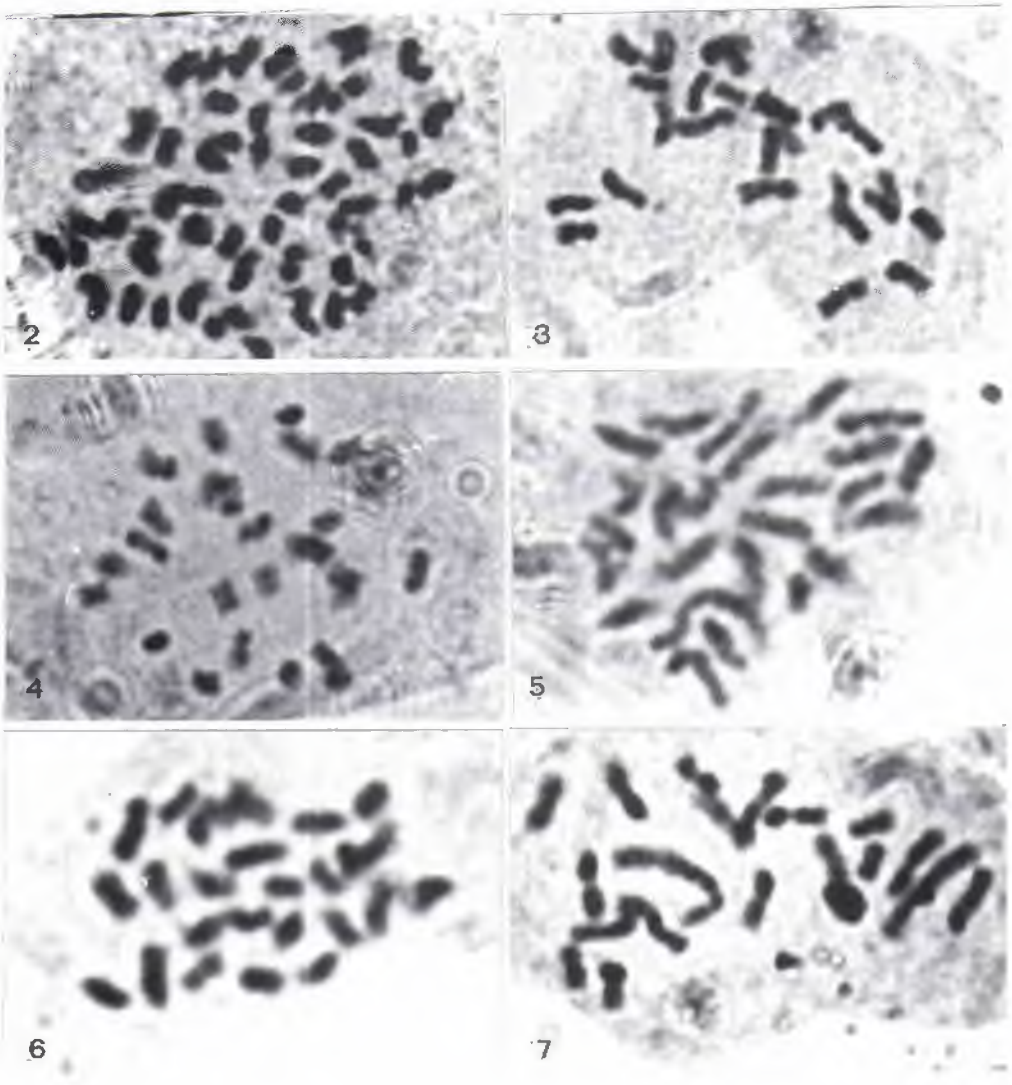
Type A: Large to medium sized chromosome with two constrictions. The primary constriction is nearly median to median in position, the secondary constriction is nearly submedian to subterminal in position.

Type B: Large to medium sized chromosome with two constrictions. The primary constriction is nearly submedian to submedian in position and the secondary constriction is nearly subterminal in position on the long arm.

Type C: Medium sized chromosome with nearly median to median primary constriction.

Type D: Medium sized chromosome with nearly submedian primary constriction.

The karyotype formula of all the species revealed definite differences in the chromosome structure (Figs. 2-7). All the types of chromosomes i. e. A, B, C and D were found in the genome of *A. auriculiformis, A. catechu, A. suma* having the chromosome number 2n=26 and *A. mollissima* with 2n=52. Type A chromosome was found in all the species except *A. dealbata* with 2n=26 chromosomes. Secondary constricted chromosome type B was absent in *A. decurrens*. A minimum six number of D type chromosome were also obtained in *A. decurrens*. Furthermore, dose differences in the submedian and the median constricted type C and D chromosomes were noted in all the studied species. The total genomic chromosome length ranged from 45.30 - 85.95 μm and chromosome volume 62.32 - 95.24 μm^3 in *A. dealbata* and *A. mollissima* respectively (Table 1). The total form percentage (TF%) varied from 40.70 in *A. suma* to 44.85 in *A. decurrens* respectively. Significant variations in chromosome lenght, volume and TF% were observed among the studied species of *Acacia*.



Figs. 2-7. Somatic metaphase plates of different species of *Acacia*; 2. *A. mollissima* (2n=52); 3. *A. auriculiformis* (2n=26); 4. *A. dealbata* (2n=26); 5. *A. decurrens* (2n=62); 6. *A. suma* (2n=26); 7. *A. catechu* (2n=26). x 2940.

Table 1. Nuclear DNA content in different species of *Acacia* along with the values of other cytological parameters.

Species	Chromosome Number (2n)	Karyotype formula	NSC	Genomic chromosome length (μm)±SE	Genomic chromosome volume (μm3)±SE	4CDNA content (pg)±SE	INV (μm3±SE)	TF%	ACL (μm)	ACV (μm3)	ANDC (pg)	AINV (μm3)
A.auriculiformis	26	4A+2B+14C+6D	6	54.14±2.13	72.28±1.67	2.311±0.11	222.44±2.99	42.43	2.08	2.78	0.088	8.55
A.catechu	26	2A+4B+12C+8D	6	58.80±1.20	78.04±1.98	2.284±0.09	210.22±2.56	43.81	2.26	3.00	0.087	8.08
A.dealbata	26	4B+14C+8D	4	45.30±2.45	62.32±1.98	2.420±0.14	310.56±3.28	40.70	1.74	2.39	0.093	11.94
A.decurrens	26	2A+18C+6D	2	57.36±1.12	75.42±1.03	2.355±0.08	250.20±3.25	44.85	2.20	2.90	0.090	9.62
A.mollissima	52	4A+6B+30C+12D	10	85.95±2.09	95.24±2.13	4.821±0.15	356.23±6.20	42.46	1.65	1.83	0.092	6.85
A.suma	26	4A+2B+8C+12D	6	50.38±1.76	66.98±1.11	2.362±0.13	233.66±4.00	42.63	1.93	2.57	0.090	8.98

NSC = Number of secondary constricted chromosome, ACL = Average chromosome length, ACV = Average chromosome volume, ANDC = Average nuclear DNA content, AINV = Average Interphase Nuclear Volume.

INV and 4c nuclear DNA amount

Interphase nuclear volume (INV) varied with species. The minimum 210.22 μm^3 INV was recorded in *A. catechu* and maximum 348.40 μm^3 was found in *A. mollissima*. The frequency polygon of INV in different species showed variatinos in the distribution around the mean keeping a constant sharp peak at its mean value (Fig. 8). The data on nuclear DNA amount and other cytological parmameters have been presented in Table 1. The 4C DNA varied significantly in different species of *Acacia* from 2.284 pg in *A. catechu* to 4.821 pg in *A. mollissima*. The average nuclear DNA content varied from 0.087 pg in *A. catechu* to 0.093 pg in *A. dealbata*. 4C DNA content was not directly proportional with other cytological parameters. The ANOVA and Duncan's multiple range test showed significant variations in the nuclear DNA among the species of *Acacia* at 1% level (Tables 2 and 3).

DISCUSSION

Karyotype, genome length and nuclear DNA amount

The karyotype studies of six species of *Acacia* revealed some interesting facts. The

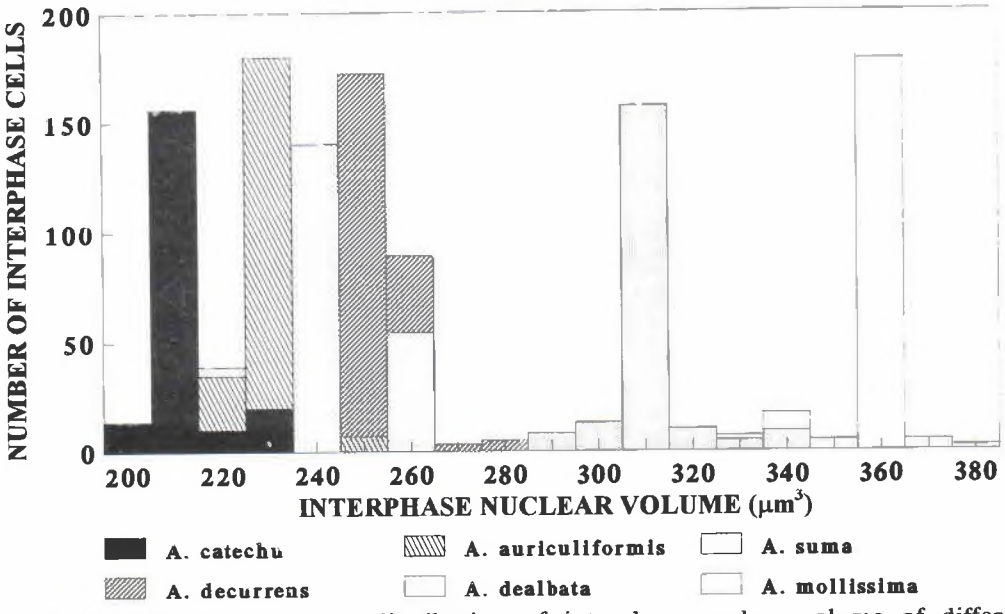


Fig. 8. Comparative frequency distribution of interphase nuclear volume of different species of *Acacia.*

metaphase chromosome number 2n=26 was noted in 5 out of 6 species studied which was in accordance with earlier findings of Atchison (1948), Datta (1971) and Bir and Kumari (1975) in *A. auriculiformis;* Sharma and Bhattachara (1958), Mehra and Sareen (1937) in *A. catechu;* Khan (1951) in *A. suma.* Somatic chromosome number 2n=52 was recorded in *A. mollissima* against 2n=26, 27 reported by Atchison (1948) and Datta (191). The number of secondary constricted chromosomes ranged from 2 in *A. decurrens* to 10 in *A. mollissima.* Type A and B chromosomes having secondary constrictions were present in all 4 species except *A. dealbata* and *A. decurrens* whereas type A was absent in the former and B type in the latter. However, dose variations in type A, B, C and D chromosomes were important feature in different *Acacia* species. There were more number of C type chromosomes as compared to the D type in all the species except in *A. suma.* Evidently, the structural changes as well as the changes in the parts of heterochromatins might have played a vital role (Mukhopadhay and Sharma 1987, Das et al.

Table 2. ANOVA of 4C DNA content in six species of *Acacia*

Source	DF	SS	MS	F
Between species	5	232.884	46.576	42.887**
Within species	54	58.650	1.086	
Total	59			

** = Significant at P ≥ 0.01, DF = degrees of freedom, SS = sum of sqares, MS = mean squars, F = variance ratio.

1995, 1996) in interspecific differences. The gradual shifting and alteration of TF% values from 40.70% to 44.85% might be due to the structural alterations in the genome (Table 1). The structural alterations as well as variations in the secondary constricted chromosomes of different species might be due to duplication or translocation between the chromosomes with or without secondary constrictions at a very early stage of evolution (Das 1991, Das and Mallick 1993a, b, Das and Das 1994, Das et al. 1995, 1996). The average chromosome lenght varied from 1.65 to 2.26 μm in *A. mollissima* and *A. catechu* respectively. The correlation coefficient between genome lenght and genomic DNA content did not show significant relationship (r=0.372). This clearly suggests that the DNA content is not positively correlated with the total chromosme length. Chromosome volume and INV showed positively significant correlation with chromosome length. Evidently, the differences in lenght may be attributed to the differential condensation and spiralization of chromosome arms.

Nuclear DNA amount in relation to genomic chromosome volume and INV

A detail analysis revealed significant variations in the average chromosome volume ranging from 1.83 μm^3 in *A. mollissima* to 3.00 μm^3 in *A. catechu* (Table 1). The DNA content did not show any significant correlation with the genome volume (r=0.233). The species specific compaction of DNA threads along with nucleosomes or the additional gene sequences (Das and Mallick 1989 a) with

Table 3. Multiple comparisons of the means of 4C DNA amounts for 6 species of *Acacia* (values for difference between pairs of mean in pg).

Species	*A. auriculiformis*	*A. catechu*	*A. dealbata*	*A. decurrens*	*A. mollissima*
A. acatechu	0.027*				
A. dealbata	0.109*	0.136*			
A. decurrens	0.044*	0.071*	0.065*		
A. mollissima	2.510**	2.537**	2.401**	2.466**	
A. suma	0.051*	0.078*	0.058*	0.007ns	2.459**

ns = non significant, * = significant at 5% level, ** = significant at 1% level, C. D. values at 5% = 0.025, C. D. values at 1% level 0.159.

altered non-histone proteins (Chattopadhyay and Sharma 1990) in the chromosome played an important role for chromosomal architecture of the species. INV was not significantly correlated with DNA content (r=0.123) whereas it showed a positive correlation with the chromosome length and charomosome volume of the species. Perhaps, the differential interaction of genomic characteristics lead to genomic DNA variation (Yamaguchi and Tsunoda 1969, Das and Mallick 1989 b, c.)

Diversification in DNA amount

The estimated 4C DNA values were reported for the first time in 5 out of the 6 studied species of *Acacia*. The nuclear DNA content through cytophotometric analysis showed 2.284 pg of DNA in *A. catechu* which was in accordance with the earlier report (Ohri and Kumar 1986). Significant differences of 4C DNA were recorded at interspecific level; such variations are in agreement with the findings of other workers (Furuta et al. 1975, Narayan and Rees 1976, Price et al. 1980, Resslar et al. 1981, Raina and Rees 1985, Banerjee and Sharma 1987, Das and Mallick 1989 c, 1993 a, b, Das and Das 1994. Das et al. 1995, 1996). The constancy in the DNA amount at the species level in repeated experiments confirmed the stable 4C DNA content in each species. The DNA amount, though, differed significantly at species level, the differences in the DNA content, however, greatly depended on the repetitive DNA amount (Flavell et al. 1977). The maximum (4.0821 pg) 4C DNA

content was noted in *A. mollissima* and the minimum (2.284 pg) in *A. catechu* with all the A, B, C and D type of chromosomes among the studied *Acacia* species. The chromosome volume, however, showed a high correlation with chromosome length (0.640) and INV (0.842). The variability in the stable DNA content in different species might be attributed to the loss or addition of many repeats in the genomes through alteration of the micro- and macro-environment during evolution of species (Price et al. 1980, Das and Das 1994, Das et al. 1995, 1996). The variability of DNA amount has often been attributed to loss or addition of highly repitive DNA sequences in a genome which reached a certain level and got stabilized during microevolution and gradual selection.

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