J. of Genetic Eng. & Biotechnol. (NRC) 3, No. 1, pp 89- 102(2005)

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Karyotype Analysis in Three Species of Acacia

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Detailed karyotype, and somatic chromosome numbers were studied in three species of the genus *Acacia*: *A. ehrenbergiana* Hayne, *A. etbaica* Schweinf. and *A. gerrardii* Benth. ssp. *negevensis* Zoh. var. *negevensis*. The analysis of Karyotype showed that most of the chromosomes were metacentric or submetacentric except for *A. ehrenbergiana* which contained one pair of telocentrics. There was a major difference in the number of satellites and their locations among the species , especially the secondary constriction on long arms of the third chromosome in all species .The present results demonstrate significant differences in chromatin length , genome size among the three species, and karyotype differences among species play an important role in cytotaxonomy. Based on these results, the taxonomic positions for these species were verified where *A. ehrenbergiana* is best situated in subg. *Acacia*, *A. etbaica* in subg. *Aculeiferum* and *A. gerrardii* var. *negevensis* in subg. *Heterophyllum* .

Key word: karyotype –DNA- Acacia

The genus *Acacia* comprises more than 1200 species .It is the largest in the subfamily *Mimosoideae* and the second largest within the family *Leguminosae* (*Fabaceae*).This genus is widely distributed throughout the dry tropics of the world, (Ross, 1979) .It was taxonomically complex because; all *Acacia* species being naturally cross-pollinated, the presence of genetic variations within the species and the occurrence of natural *Acacia* hybrids are quite common. It is due to this considerable genetic and consequent morphological variations that the status of many *Acacia* species and varieties has been a disputed matter and delimitation of subdivisions and clarification of relationships between groups and between species are very often beset with difficulties (Muhammad, 1951; Guinet, and Vassal, 1978;Harrier et al.,1997). This genus is ecologically very important especially in arid areas, where most species of this genus enriched soils in nitrogen due to symbio (Sharma and Bhattacharyya, 1958; Coulaud *et al.*, 1995; tic nitrogen fixationBukhari, 1997b, c) .The species of this genus provide a variety of useful products and services. In addition to their ecological and economical importance, they are regarded as medicinal plants (Mossa *et al.*, 1987).

The majority of previous work used taxonomic evidences to classify this genus and to understand the relationships among species within this genus.

Cytological evidences which were employed have notable role particularly the chromosome number and the karyotype because of the genetic variations within species in addition to cytological characters specialization. This genus has high basic chromosome numbers and variable levels of ploidy with small size of chromosomes

Atchison, 1(948; Muhammad, 1951; Sharma and Bhattacharyya, 1958; Vassal and Lescanne, 1976; Guinet and Vassal, 1978; Bukhari, 1997a, b).

In the area of cytological evidences, the genus *Acacia* has been subjected to many investigations which proved that the chromosome number, ploidy , levels of total chromatin , karyotype and on other cytological characters play an important role in identification of generic and subgeneric grouping of *Acacia* and were used as taxonomic tools. In genus *Acacia* the divergence and evolution of its species were associated with significant variation in the amount of DNA, therefore the DNA estimation is a useful mean to differentiate the taxonomic groups of genus *Acacia* and to define the subgenus and it is also useful for the development of the comparative taxonomic divisions (Mukherjee and Sharma, 1993, 1995; Bukhari, 1997c). The objective of the present study is the investigation and analysis of the karyotype, total chromatin length of the haploid set, chromosomal number, and nuclear DNA content estimation in three species of *Acacia A. ehrenbergiana* Hayne, *A. etbaica* Schweinf. and *A. gerrardii* Benth. ssp. *negevensis* Zoh. var. *negevensis* ,and to classify these species according to these taxonomic evidences.

Materials and Methods

The seeds used in this study were kindly provided by the National Wildlife Research Center (NWRC), Taif, Saudi Arabia (Table 1). The seeds were scarified with a sharp scalpel soaked in warm water overnight and then transferred onto moistened filter papers set in Petri dishes for germination. Prefixing treatments started when the primary roots had grown to about 12 to 15 mm. Pretreatment and fixation steps were conducted according to the method of Bukhari (1997a, b). The fixed root tips were hydrolyzed in 5N HCl for 60-90 minutes at room temperature, study of somatic chromosome presented extreme difficuty due to their very weak stainability especially *A. ehrenbergiana* increase this shortcoming the root tips were stained in feulgen for 3 hours and squash preparations were made in 3% acetocarmine and carbolic fuchsin. Cells with well spread chromosome were partly indentified under the 100X objective were photographed the photograph were magnified and then sketched by hand.

Chromosome measurement:

The chromosome number was ascertained for each species by counting 100 well-spread mitotic metaphases. For chromosomal description and nomenclature for centromeric position, the system of Levan *et al.* (1964) was followed. The satellites were also measured and considered in determining the total chromosomes length and arm ratio.

Micrometer scales were used to measure the chromosome length. These parameter were assessed statistically using an unpaired T test with significant level of $P<0.05$.

DNA measurement:

For DNA measurement, the germination seeds were transplanted into plastic pots containing 80% peat and 20% sand. Then tee seedling were raised in the greenhouse .for each accession , about 0.4-0.5 mg of tissue from the youngest fully expanded

leaf of 1 month old seedling was arrested , the DNA was isolation from at lest ten different individuals for each species by Ultra Clean plant Kit (Mo Bio Laboratories,Inc. Solana Beach,USA).

TABLE 1. Localities of *Acacia* species used.

Species	Locality
A.ehrenbergiana	KSA, Jeddah
A. gerrardii var. *negevensis*	KSA, 30 km east of the city of Taif, open field.
A. etbaica	KSA,Raydah prorected area in forester Asir mountains, open filed.

Results

Chromosome number:

The somatic chromosome number determination in these species showed a diploid number of 2n= 2X = 26 (Fig. 1 A,B ,C).

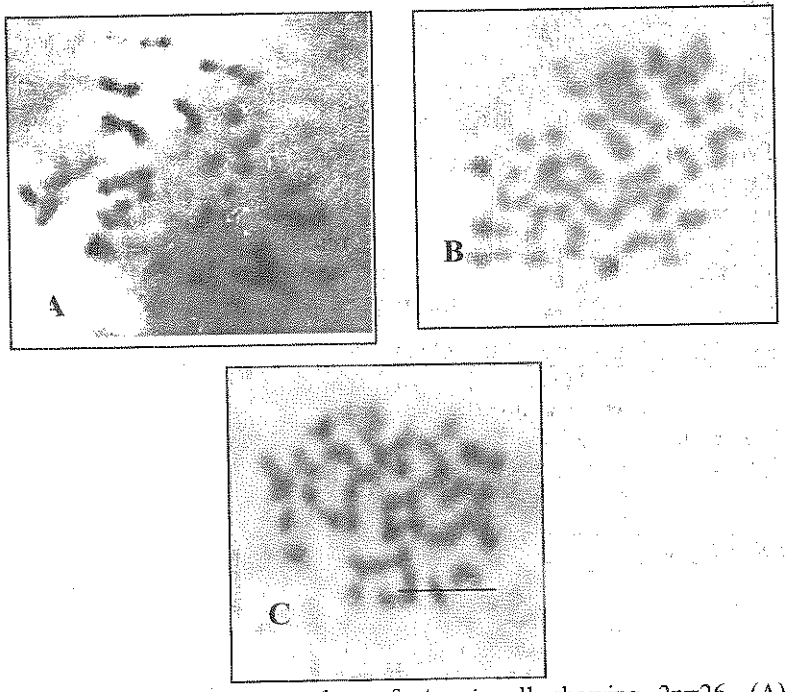

Fig. 1. Somatic chromosome number of *Acacia* all showing 2n=26. (A) *A. ehrenbergiana* and (B) *A. gerrardii* sp. *negevensis* var. *Negevensis* and (C) *A. etbaica*.
Bar = 5 μm.

Karyotype analysis :

For Karyotype analysis the chromosome were described in this study by AL jabarti on the basis of morphological type and vale of their absolute length. Divided to four sizes of chromosome on the basis of mean of their absolute length to long chromosome having absolute length from 3.7μm to 2.8μm , medium sized

chromosome having absolute length from 2.7μm to 1.8μm. small chromosome rang from 1.7 μm to 1 μm. and very small chromosome having absolute length below 1 μm. that is known as microchromosome . and the chromosomes were divided on the basis of morphology to:

Type A: long chromosome with nearly mediam primary constriction and secondary constriction located on longer arm in subterminal position.

Type B: long chromosome having primary and secondary constrictions both located nearly on submedian position at opposite ends of the chromosome.

Type C: long chromosome sized with medium primary constriction.

Type D: long chromosome sized possessing two constriction, primary and secondary, one nearly median and the other close to the former at the middle of the chromosome.

Type E: large sized chromosome with submedian primary constriction and secondary constriction position at median of the short arm.

Type F: medium sized chromosome with middle primary constriction and secondary constriction in subterminal position of long arm.

Type G: medium sized chromosome with submedian centromere.

Type H: medium sized chromosome with two constrictions primary is median and the secondary close to former.

Type I: medium sized chromosome primary and secondary constrictions in submedian position at the opposit ends of the chromosome.

Type J: medium sized chromosome with median primary constriction.

Type K: small chromosome with median primary constriction.

Type L: small chromosome with Centromere not clear.

Type M: short chromosome with subterminal centromere.

Type N: very small chromosome known as microchromosome.

Karyotypic data for each species are presented in Table 2(a&b).

TABLE 2(a). Measurement (μm) of somatic chromosome of *Acacia* species

Chromosome pair no.	Mean chromosome length arms		Mean satellait length	Total length	Arm ratio (r)	Relative length (%)	Centromeric position*
	Long arm (L)	Short arm (S)					
A. ehrenbergiana							
1	1.2	1.09	0.76	3.05	1.72	13.36	sm
2	1.2	1.2	0.33	2.73	1.28	11.96	m
3	0.76	0.54	1.3	2.61	1	11.43	m
4	1.09	0.98	---	2.07	1.11	9.07	m
5	1.09	0.87	---	1.96	1.25	8.59	m
6	0.87	0.87	---	1.74	1	7.61	m
7	0.81	0.76	---	1.58	1.07	6.92	m
8	0.81	0.65	---	1.48	1.25	6.84	m
9	0.76	0.71	---	1.47	1.07	6.44	m
10	---	---	---	1.2	---	5.43	---
11	0.98	0.11	---	1.09	8.90	4.77	t
12	---	---	---	0.98	---	4.29	---
13	---	---	---	0.87	---	3.81	---
Total chromosome length				22.83			

*** m= metacentric; sm= submetacentric; t= acrocentric.**

TABLE 2(b). Measurement (μm) of somatic chromosome of *Acacia* species

Chromosome pair no.	Mean chromosome length arms		Mean satellait length	Total length	Arm ratio (r)	Relative length (%)	Centromeric position*
	Long arm (L)	Short arm (S)					
A. gerrardii spp. *negevensis* var. *negevensis*							
1	1.59	0.84	1.02	3.45	1.01	12.69	m
2	2.05	1.14	---	3.19	1.8	11.73	m
3	1.02	0.97	0.89	2.88	1.97	1.95	sm
4	1.93	0.77	---	2.7	2.51	9.93	sm
5	1.48	1.14	---	2.62	1.30	9.64	m
6	1.47	1.14	---	2.61	1.29	9.91	m
7	1.48	1.02	---	2.50	1.45	8.96	m
8	1.24	0.78	---	2.02	1.59	7.43	m
9	0.63	0.59	---	1.16	1.07	4.27	m
10	---	---	---	1. 14	---	4.19	---
11	---	---	---	1.01	---	3.71	---
12	---	---	---	1	---	3.85	---
13	---	---	---	0.91	---	3.35	---
Total chromosome length				27.19			
A. etbaica							
1	1.85	0.98	0.87	3.7	1	15.97	M
2	1.52	0.92	0.82	3.26	2.54	14.07	sm
3	1.09	0.71	0.88	2.68	2.77	11.57	sm
4	0.93	0.71	0.98	2.62	2.69	11.31	sm
5	1.15	0.65	---	1.79	1.77	7.73	sm
6	0.87	0.81	---	1.68	1.07	7.25	m
7	0.83	0.81	---	1.64	1.02	7.08	m
8	0.82	0.54	---	1.36	1.52	5.78	m
9	0.77	0.43	---	1.21	1.79	5.22	m
10	---	---	---	0.85	---	3.67	---
11	---	---	---	0.83	---	3.85	---
12	---	---	---	0.83	---	3.85	---
13	---	---	---	0.72	---	3.11	---
Total chromosome length				23.17			

*** m= metacentric; sm= submetacentric; t= acrocentric.**

A. ehrenbergiana

Karyotype, ideogram, karyograme showed one pair of large chromosome sized, 5 pairs of medium sized chromosome and 5 pairs of small chromosome and two pairs of microchromosome. The chromosome length varies from 3.5μm to 0.78μm and relative length varies from 13.36μm to 3.81μm. the total chromain length of haploid set of chromosome is 22.83μm. (Fig. 2).

The centromere medium in 8chromosomes, submedian in one pair of chromosome and subtermenal in one pair of chromosome, pairs of satellite were presented in first, second and third chromosome pairs on the long arm. The diameter of the nucleus was 13μm.

A. gerrardii spp. *negevensis* var. *negevensis*

Karyotype details showed three pairs of large chromosomes, 5 pairs are medium sized, 4 pairs are small and one pair microchromosome. (Fig. 3).

The chromosome length range from 3.45μm to 0.91μm. and the tota; chromatin length is 27.19μm per haploid genome.

The centromere in most chromosomes was metacentric except chromosome 3 and 4 was submetacentric.

Two pairs of satellites showed one on short arm of first chromosome pairs and another on long arm of third chromosome pairs. The nucleus diameter was 14 μm.

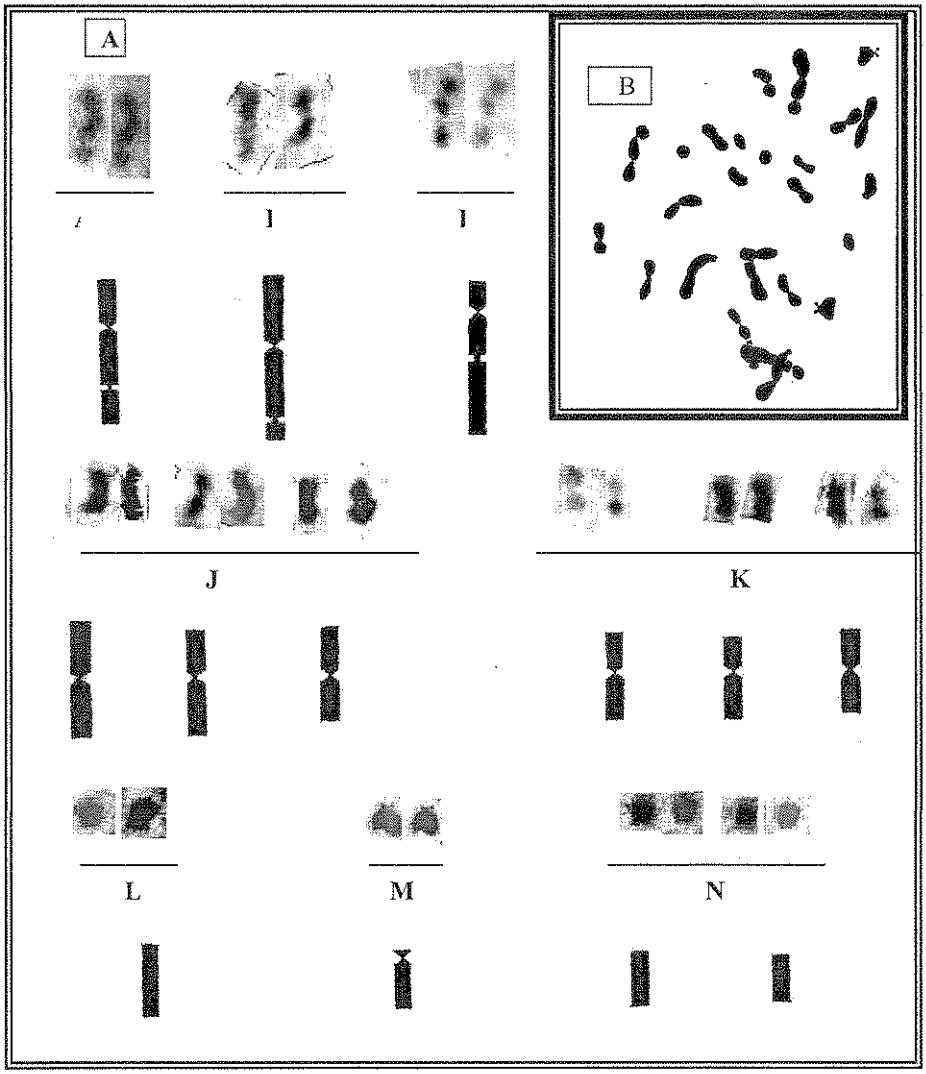

Fig.2: Showing A. karyotype (4600X) and ideogram (5000X) of A. *ehrenbergiana* and B. Karyogram in *A. ehrenbergiana* (4000X).

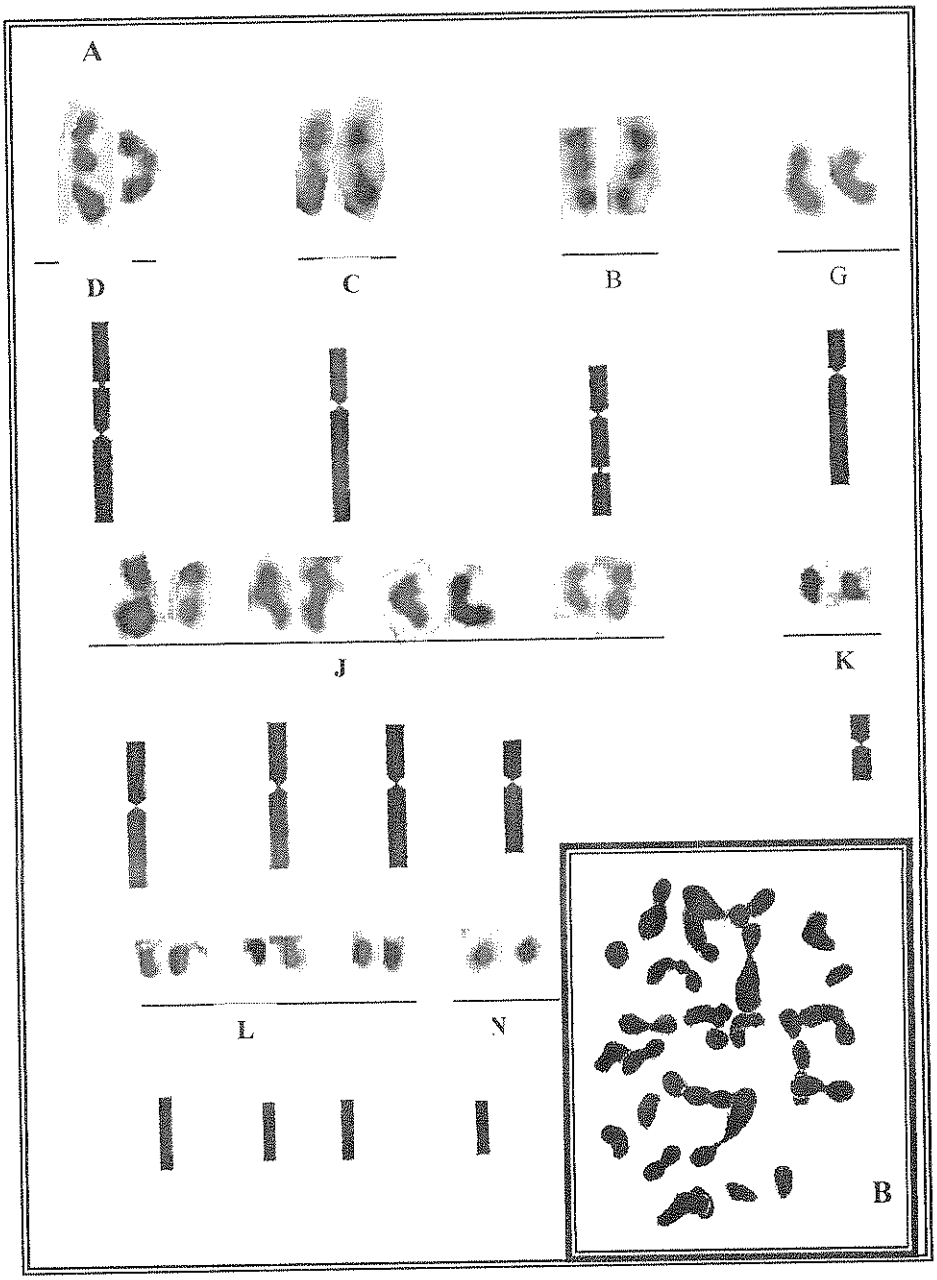

Fig.3: Showing A. karyotype (4400X) and ideogram (5000X) of *A. gerrardii* var. *negevensis* and B. Karyogram in *A. gerrardii* var. *negevensis* (4200X).

A. etbaica

Karyotype, karyogram and ideogram, showed two pairs of large chromosome, three pairs of medium sized chromosome, and four pairs are small and four micro chromosomes. (Fig. 4).

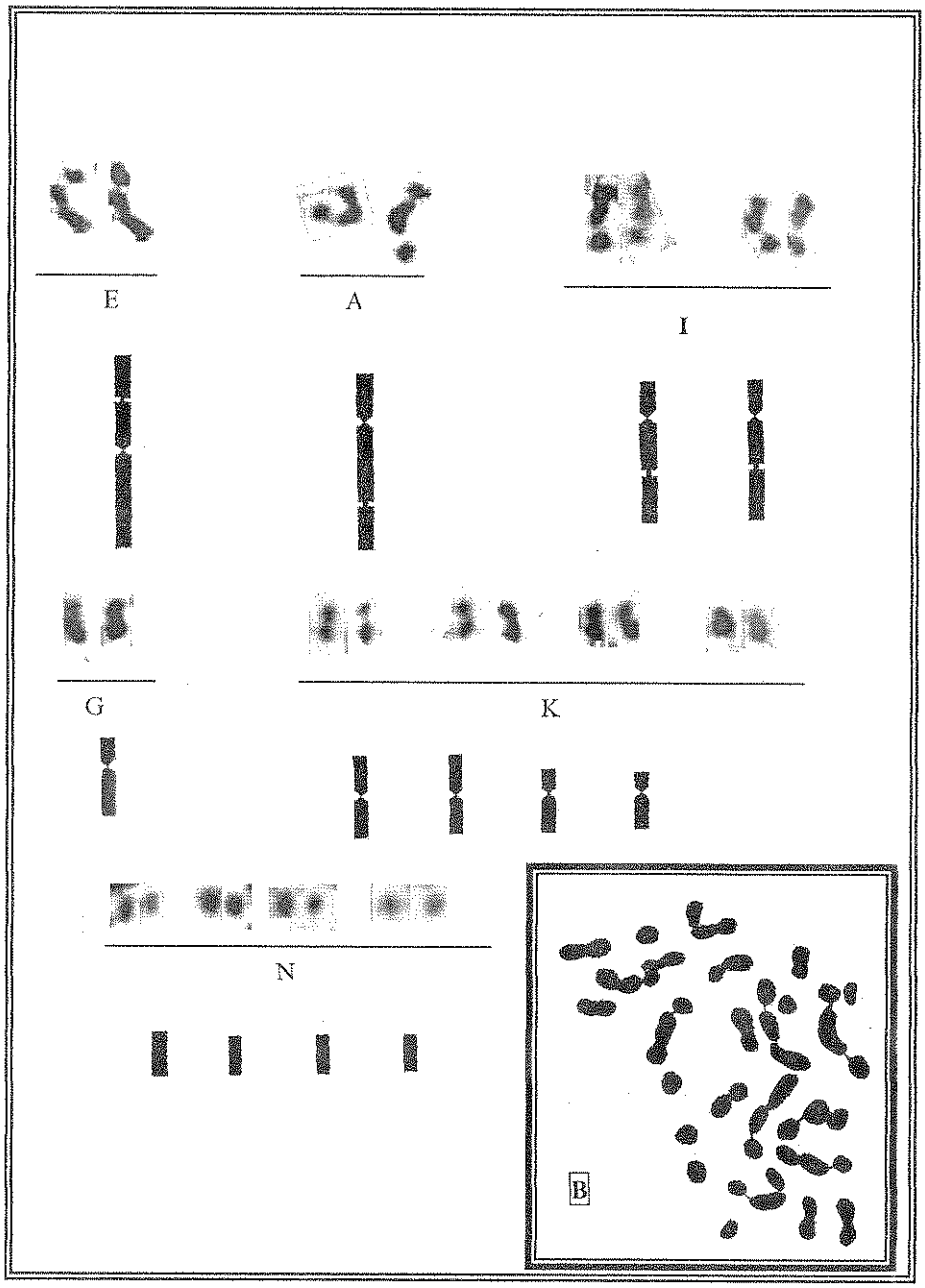

Fig. 4: Showing A. karyotype (4600X) and ideogram (5000X) of A. *ethaica* and B. Karyogram in *A. ethaica* (4600X).

The size of the complement ranged between 3.7 μm and 0.72 μm. the total chromatin length is 23.17 μm per haploid genome.

The relative lengths of the chromosome decreases from 15.97% to 3.11% in this species, 5 pairs are metacentric and 4 submetacentric. A satellite is observed on the short arm of chromosome 1 and on the long arm of chromosomes 2, 3 and 4. The nucleus diameter was 15 μm.

The detailed comparison of Karyotypes between *Acacia* species (Table 3) had showed different specific between it in spite of the centromeres were medium and submedium in chromosomes which have distinct centromere on it except one pairs of chromosome was telocentric in *A. ehrenbergiana*, also there was a major difference in the number of satellites and their locations among the species. There were six satellites in *A. ehrenbergiana* and eight and four satellites in the two species *A. etbaica* and A. *gerrardii* var. *negevensis*, respectively. The third chromosome pair in *A. etbaica* has their secondary constriction situated more distally than these of *A. gerrardii* var. *negevensis* and *A. ehrenbergiana*. The same applies to half of the corresponding chromosome in *A. ehrenbergiana* and the chromosomes sized in A. gerrardii var. negevensis are larger than *A. ehrenbergiana* and *A. etbaica* (Fig. 5). The shorter long total length of chromosome was found in *A. ehrenbergiana* and the longest total length of chromosome was in *A. gerrardii* var. *negevensis* (Fig. 6).

TABLE 3. Comparison of cytogenetically characters between species

Species	Karyotype formula*	Range of mean chromosome length (μ)	Total chromatin length (μ)
A. ehrenbergiana	$1L^{s}$+ 3M+ $2M^{s}$+ 5S + 2microchr.	3.05 - 0.78	22.83
A. gerrardii var. *negevensis*	$2L^{s}$+1L+5M+4S+1 microchr.	3.7 - 0.72	23.17
A. etbaica	$2L^{s}$+$2M^{s}$+1M+4S+4 microchr.	3.45 - 0.91	27.19

* (L) Large chromosome, (M) medium chromosome,
(S) small chromosome, (microchr.) microchromosome.
(L^{s}, M^{s}) long and medium chromosome respectably with satellites.

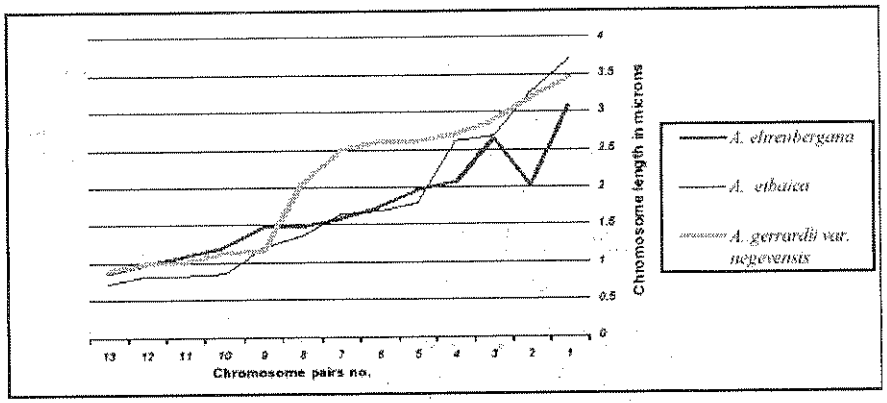

Fig. 5 Ideogram showing the differences between the chromosomes sized in three species.

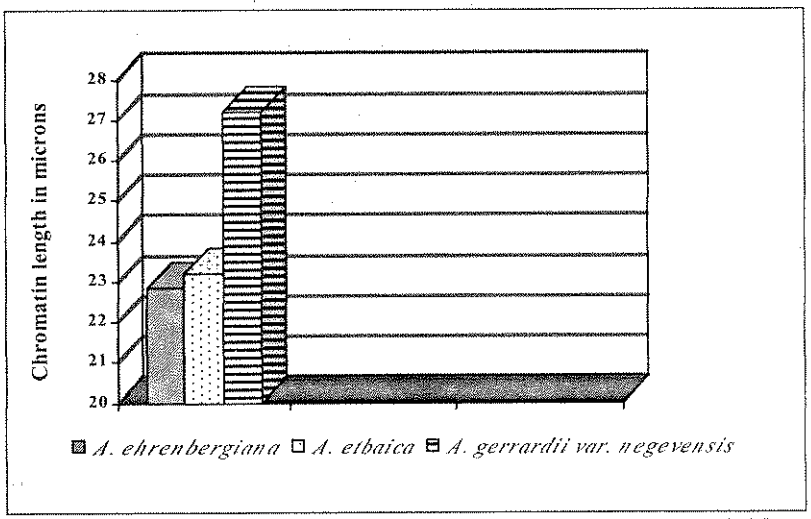

Fig. 6: Histogram showing the total chromatin length of the haploid complement in the three species of *Acacia*.

The "t" test of average chromosome length in 5 cell was found to be significant different (P<0.05) between *A. ehrenbergiana* and *A. gerrardii* var. *negevensis* only. And significant different (P<0.05) was also found between the three species in the total chromatin (Table 4.)

TABLE 4. Significant difference between species.

Significant difference between species	(t) average chromosome length	(t) average chromatin length	(t) average nucleus diameter
A. ehrenbergiana - A. gerrardii	- 2.470*	- 57.114*	- 0.686 ns
A. ehrenbergiana - A. etbaica	- 0.233 ns	- 5.672*	- 1.232 ns
A. etbaica - A. gerrardii	1.902 ns	86.789*	- 0.724 ns

*** Significantly different at P<0.05 ns: no significant differences**

DNA amount

Results showed that the mean value of DNA content in *A. ehrenbergiana* cells was (6.46 ±1.57). While, in A. *etbaica* and *A. gerrardii* var. *negevensis* cells the mean values content were (4.52 ± 0.73 and 4.44 ± 0.44) respectively. The variance between the species revealed significance (Fig.7) significant different (P<0.05) was found between this three species (Table5).

TABLE 5. Showing significant differences between species in DNA amount

Significant Difference Between Species	(t) Average DNA amount
A. ehrenbergiana - A. gerrardii	3.919*
A. ehrenbergiana - A. etbaica	3.539*
A. etbaica - A. gerrardii	- 0.297*

*** Significantly different at P<0.05**

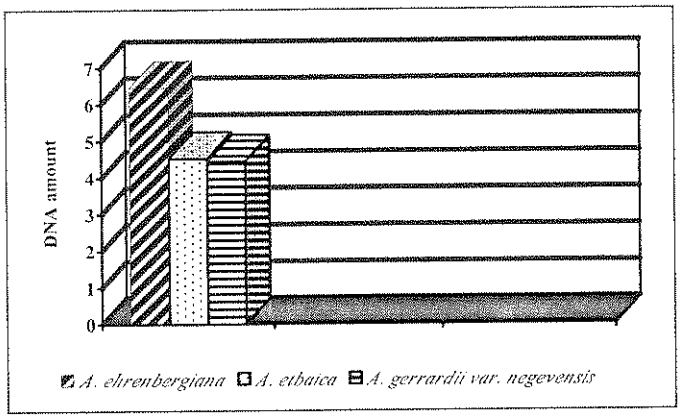

Fig.7: Histogram showing the total DNA amount in the three species of *Acacia*.

Discussion

All the members of the three species in this investigation were diploid 2n = 2X = 26 chromosomes which confirm previous reports which pointed out that this chromosome number was very common in this genus onb), (Atchis 1948; Muhammad, 1951; Sharma and Bhattacharyya, 1958; Vassal and Lescanne, 1976; Bukhari, 1997a.

Karyotype and chromosome number in *A. etbaica* and *A. gerrardii* var. *negevensis* were reported in this study for the first time. While our present result about chromosome number and karyotype in *A. ehrenbergiana* conflict with Bukhari (1997b) which found 2n = 52 chromosomes and this difference may be due to the species which Bukhari studied it belongs to knew taxa varied of this species. Our results support this proposal since we found one piairs of chromosome telocentric in karyotype while Bukhari found all the chromosome metacentric and submetacentric. To detect these taxa more detailed cytological and molecular work are needed to elucidate this taxa and also comparative study between these two simples must be curried out.

Karyotype analysis showed that most chromosomes had the primary constrictions in submedian to median position and this results confirm the previous reports about primary constrictions position in *Acacia* species (Muhammad, 1951; Sharma and Bhattacharyya, 1958; Bukhari, 1997a, b) and the presence of one telocentic pairs of chromosome pair is in agreement with Bukhari (1997b) who found that *A. nubica* only has telocentric. Chromosome

The secondary constrictions in *A. ehrenbergiana* and *A. gerrardii* var. *negevensis* which belong to subgenus *Acacia* in this study rang from four to six which disagree with Sharma and Bhattacharyya (1958) Bukhari (1997 b) but in accordance with Muhammad (1951) who detected in six satellites. *A. farnesiana* Accordingly, there are species of subgenus *Acacia* have secondary constrictions less than eight. Sharma and Bhattacharyya (1958) Bukhari (1997 b) designated the

species belong to subgenus *Aculeiferum* characters with eight satellites our results arein agreement with previous studies.

The absolute size differences among the three species showed limited as a method for taxonomic identification in this study and also nuclear diameter is limited as taxonomic tools.

Comparative cytological study reveals that the length of chromosomes and gross appearance of the karoytype show a variation in all the species investigated, in spit of the gross resemblances in the morphology of the chromosomes of the different species, minute differences in karyotypes between one species and another have been clearly brought out. The difference in the karytype of different species is mainly due to differences in their chromosome size as well as in the number of chromosome type belonging to the different groups. They mainly differ with respect to the number and position of secondary constrictions. The position of secondary constriction on third chromosome in all species was helpful in discriminating between these three species. The present observations therefore emphasize the fact that species of *Acacia* can be classified on the basis of their karyotypes. The difference between the karyotyps of different species indicates that considerable structural changes of chromosomes have occurred during evolution. This deduction is in agreement with Muhammad (1951); Sharma and Bhattacharyya (1958); Vassal and Lescanne (1976); Guinet and Vassal (1978) and Bukhari (1997a, b).

The DNA content was estimated in the three species for the first time. The results of the estimation of DNA content in *Acacia* indicate its potential as a useful parameter of genetic diversity in this genus.

The lower DNA content in *A. gerrardii* var. *negevensis* indicates that this species was highly specialized in comparison with species of *Acacia* in this investigation. Where obscurity evolution and specialization of species associated often with lower amount of nuclear DNA (Rees and Jones, 1977; Crawford , 1990; Bennett *et al.* , 2000). Also Mukherjee and Sharma (1995) indicated that the amount of nuclear DNA can be used as a trait for selection of well adapted fast productive species for plantations and afforestation in genus *Acacia*.

According to the presented results, it was concluded that the karyotype has obvious importance in the differentiation among the three species particularly the satellites and their lacations. The results also confirmed that each of the chromatin length and the DNA content constitutes one of the important evidences that can be used independently to differentiate among the species that belong to the genus *Acacia*. In contrast, the diameter of the nucleus is one of the evidences with limited role in the differentiation among the species and can be disregarded in the cytological studies in this genus.

It was also concluded from this study that the current taxonomy of the species investigated in the current research is valid, the results obtained in the present study are strongly supported the taxonomic position,i.e;. *A. ehrenbergiana* belong to subg. *Acacia*, *A. etbaica* belong to subg. *Aculeiferum* but *A. gerrardii* var. *negevensis* showed cytogentic characters identical to cytogentical marked in subg. *Heterophyllum*

Acknowledgment

The authors are grateful to Curtaor of NWRC for supplying *Acacia* seeds. The help of Marwah N. Baeshen with the statistical analysis is gratefully acknowledged.

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تحليل الطرز الكروموسومي وتقدير كمية السدنا في ثلاثة انواع من جنس الاكاشيا

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تناولت هذة الدراسة العدد الكروموزمى الجسدى و الطرز الكروموسومي فى ثلاثة أنواع من جنس الأكاشيا هى: أ. ايهرينبيرجيانا ؛ أ. إيتبايكا و أ. جيراردى ، تحت نوع نيجفينسس ، سلالة نيجفينسس . أظهر تحليل الطرز الكروموسومي أن معظم الكروموزومات وسطية السنترومير أو تحت وسطية ما عدا زوج كروموزومى واحد طرف السنترومير فى أ. ايهرينبيرجيانا وظهرت اختلافات واضحة فى عدد التوابع وموضعها بين الأنواع ، خاصة الإختناق الثانوى على الذراع الطويل للزوج الكروموزومى الثالث فى جميع الأنواع . واثبتت النتائج الحالية وجود اختلافات معنوية فى طول الكروماتين وحجم الجينوم بين الأنواع الثلاثة ، و اختلاف الطرز الكروموسومي بين الأنواع يلعب دورهام فى التقسيم الخلوى . وعلى أساس هذة النتائج تم مناقشة الوضع التقسيمى لهذة الأنواع بما يؤكد انتماء النوع أ. ايهرينبيرجيانا لتحت الجنس أكاشسيا و أ. إيتبايكا لتحت جنس أكيوليفيرم و أ. جيراردى ، سلالة نيجفينسس تحت الجنس هيتروفيليم .