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CYTOGENETIC STUDIES ON BAMBARA GROUNDNUT (*VIGNA SUBTERRANEA* (L.) VERDC)

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ABSTRACT

The study reports the result of analysis of chromosomes of Vigna subterranea performed in the mitotic prometaphase and metaphase stages using conventional techniques. The somatic chromosome study was done using the shoot meristem as the root tips persistently showed very low mitotic indices. The result revealed somatic chromosome number of 2n = 22. There was no evidence of polyploidy in any of the accessions. The chromosome morphology was described on the basis of the centromere position. From the F% value, it was evident that the different accessions showed wide range of variation in their karyoptypes. The minimum F% range of 33.33 to 50.00 was recorded for Ac-01, Ac-02 and Ac-03. They had metacentric and submetacentric chromosomes. Ac-04 had F% range of 25.00 to 50.00. With the exception of the 9th chromosome pair that was telocentric, the chromosomes were mostly metacentric and submetacentric. Because the accessions were diploid, the uniformity in chromosome number of the four accessions investigated is a clear indication that they may have evolved from a common ancestor, forming a homogeneous assemblage.

Key words: Cytogenetics, chromosome, Bambara groundnut**,** *Vigna subterranea*

Interest on the search for minor and lesser known legumes in tropical West Africa

INTRODUCTION

Interest on the search for minor and lesser known legumes in tropical West Africa is attributable to a number of factors viz: increased utilization of most commercially grown pulses as supplements in livestock feed (Uguru and Madukaife, 2001), usefulness as a source of cheap quality protein for the local diet (Nnanyelugo *et al*, 1985; Akubuo and Uguru, 1999); and the prohibitive cost of animal protein (Ene-Obong and Carnovile, 1992). Bambara groundnut is not a very popular legume and literature on its genetics especially in relation to its cytology is scanty. To date, there is paucity of information on the karyotype of Bambara groundnut and breeding efforts to improve it have received very limited attention. Investigations in this respect have centred mainly on other *Vigna species* such as cowpea (Pignone *et al*. 1990) and Phaseolus (Sinha and Roy 1979). Successful hybridization as a means of creating variability has not been achieved. The reasons for this are yet to be established, partly because many of the ecotypes and landraces are yet to be studied. A detailed knowledge of the cytogenetics of the crop will

be useful in designing ways of overcoming existing hybridization barriers. The present study was initiated to determine the cytological status of Bambara groundnut lines collected from different agro-cologies of Nigeria and, as a corollary, establish how such cytogenic information would be utilized in achieving successful hybridization in bambara groundnut.

MATERIALS AND METHODS

Dry, dormant, pure line seeds of four accessions of Bambara groundnut, Ac-01, Ac-02, Ac-03 and Ac-04 characterised by the seed seed size and eye colour (plate 1) were collected from four ecological zones of Nigeria. The somatic chromosome study was done using the shoot meristems as the root tips persistently showed very low mitotic indices.

Young shoots were harvested at emergence and pre-treated in 0.002M aqueous solution of 8-hydroxyquinoline for one hour at room temperature. The pre-treated shoots were washed, sieved under running water and fixed in Canoy's solution (3: l ethanol acetic acid) for 24 hours. The samples were hydrolysed in 18% HCl for about 1 ½ hours at room temperature and finally washed with distilled water.

The meristematic end of the young shoot was teased into the slide, stained and squashed in a drop of F.L.P.O stain (Olorode, 1974). A cover slip was placed on the squashed material and allowed to 2stand for 2 hours to allow the chromosomes absorb enough stain. A firm pressure was applied with the thumb and gentle tapping was made on the cover slip with the base of a ballpoint pen to help flatten the cells.
Good cells with well spread metaphase chromosomes were used for the karyotypic studies. Photomicrographs were taken using Leitz DIALUX research microscope. The chromosomes were measured in microns and classified on the basis of the length (Sinha and Roy, 1970; Barone and Saccardo, 1990) as follows:

Type	**Chromosome length**
A	2µm - 3µm
B	Iµm to less than 2µm.
C	< 1µm.

Measurements of the short arm, long arm and the chromosome length were taken. The arm ratio was calculated as the ratio of the long to the short arm. The F% was calculated as the ratio of the total sum of short arm length to the total sum of the chromosome length expressed as a percent (Huziwara, 1962). The karyotypes were constructed by organizing chromosomes into metacentric, submetacentric and telocentric chromosomes according to their arm ratios. The following measurements were taken for each chromosome: short arm (s), long arm (l) and the total chromosome length (cl) and the arm ratio (r = l/s) were calculated and used to classify the chromosomes. The sum of short arm length/total chromosome length relation (TF %) was also calculated.

RESULTS

The characteristics of Bambara groundnut accessions used are presented in Table1. The principal distinguishing features are the seed coat colour, eye colour and seed size as estimated with 100-seed weight. Because of the cleistogamous nature of Bambara groundnut, out-crossing is very uncommon and hardly influences the crop purity. This unique characteristic has made seed coat colour a reliable feature for the identification and local classification of the various genotypes. AC-01 and AC-03 had seeds with cream colour; AC-02 and AC-04 had red and black seed coat colour, respectively (plate 1). With the exception of AC-03 with dark ring around the white eye, all the other accessions are endowed with white eye colour. With respect to the seed size, AC -01 and AC -03 are large seeded with mean 100 – seed weights of 167.10g and 146.05g, respectively.

Plate 1: Seeds of the four Accessions of Bambara Groundnut

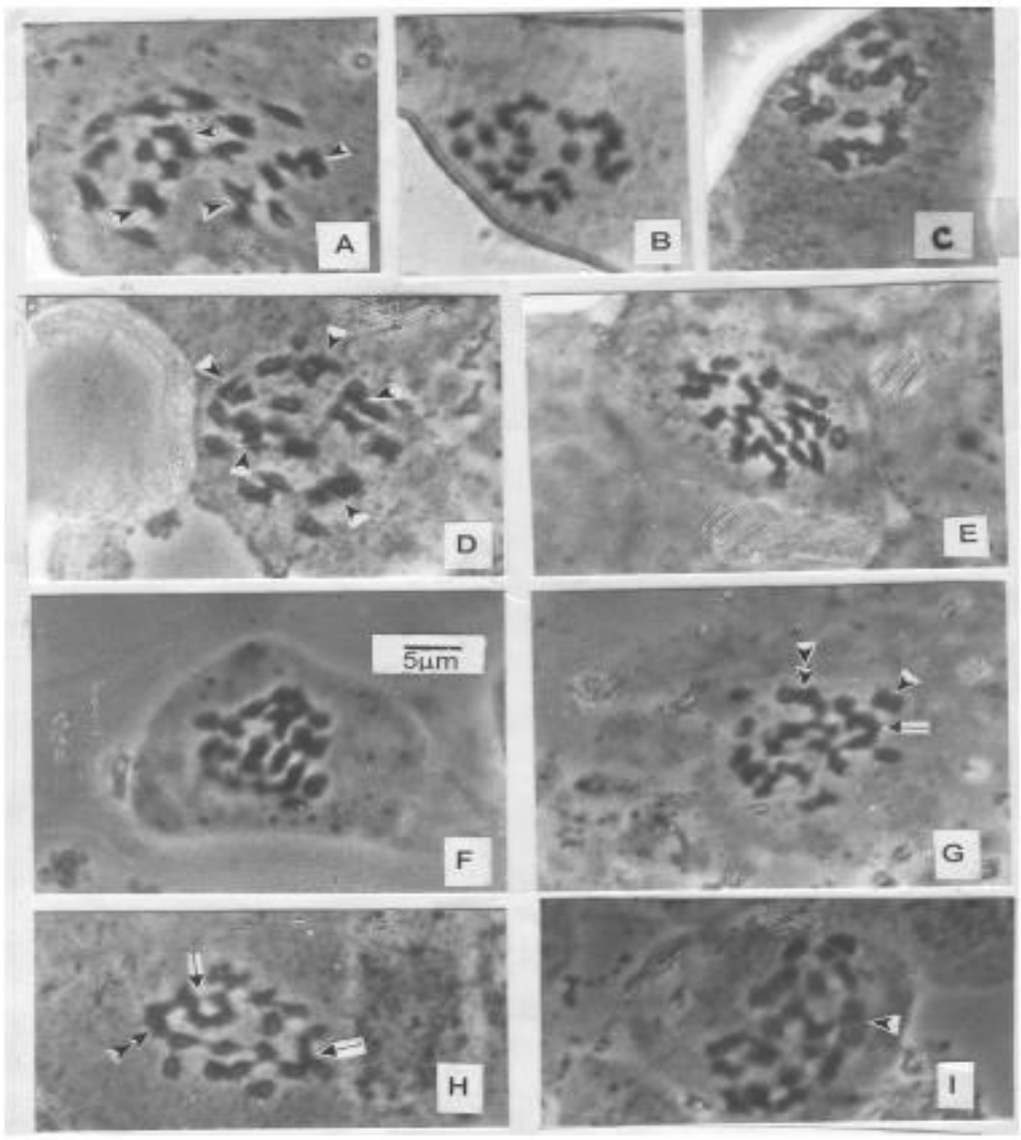

Plate 2: karytopye of the four bambara groundnut genotypes

▲ ----overlap of 2 chromosomes

▲▲ ----overlap of 3 chromosomes

→ ----overlap of 4 chromosomes

Table 1: Seed characteristics and collection site of the four bambara groundnut Accessions.

Accession	Seed Coat Colour	Eye colour	100-seed wt	Collection Site	Ecological Zone	State
AC-01	Cream	White	167.10g	Long. $11^0 15^1$ Lat. $10^0 30^1$	Sudan Savannah	Gombe
AC-02	Red	White	85.90g	Long. $12^0 45^1$ Lat. 9^0	Sudan Savannah	Adamawa
AC-03	White	White with Dark ring around eye	146.05g	Long. $8^0 50^1$ Lat. $7^0 05^1$	Southern Guinea Savannah	Benue
AC-04	Black	White	96.52g	Long. $7^0 20^1$	Derived Guinea Savannah	Enugu

Table 2: Measurements of 11 pairs of somatic chromosomes of AC-01 genotype of Bambara groundnut in respect of chromosome length, arm ratio, F% and centromere position.

Chromosomes pair number	AC-01					
	Total chromosomes Length	Long arm (µm)	Short arm (µm)	Arm ratio	F%	Centromere position
1	4.00	2.50	1.50	1.67	37.50	SM
2	4.00	2.00	2.00	1.00	50.00	M
3	3.50	2.00	1.50	1.33	42.86	M
4	3.50	1.75	1.75	1.00	50.00	M
5	3.00	2.00	1.00	2.00	33.33	SM
6	3.00	1.50	1.50	1.00	50.00	M
7	3.00	1.50	1.50	1.00	50.00	M
8	2.50	1.50	1.00	1.50	40.00	SM
9	2.50	1.50	1.00	1.50	40.00	SM
10	2.50	1.25	1.25	1.00	50.00	M
11	2.50	1.00	1.00	1.00	50.00	M
Total	33.50	18.50	15.00			
Mean	3.05	1.68	1.36			
SD	0.65	0.42	0.34			

Mitotic stages at promataphase are presented in Plate 2. Plates 2 A, B and C represent AC-01; D and E, AC-02; F and G, AC–03; H and I, AC-04. The photomicrographs show a somatic chromosome number of 2n = 22 for Bambara groundnut. The details of the karyotype of each genotype with regard to the chromosome length, the lengths of the long and short arms, arm ratio, F% are presented in Tables 2 -5. In AC–01, the length of the chromosome pairs ranged from 2.0µm to 4.0µm
($\overline{X}$ =3.05 and SD = 0.65). The chromosome lengths in AC–02 varied from 2.0µm to 3.5µm ($\overline{X}$ = 2.77 and SD = 0.41). The Accession, AC – 03 had chromosomes varying from 1.5µm to 3.5µm ($\overline{X}$=2.64µm and S.D=0.64) in length.

Table 3: Measurements of 11 pairs of somatic chromosomes of Ac-02 genotype of bambara groundnut in respect of chromosome length, arm ratio, F% and centromere position.

Chromosome pair Number	AC-02					
	Total chromosome length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio	F.%	Centromere position
1	3.50	2.00	1.50	1.33	42.86	SM
2	3.00	2.00	1.00	2.00	33.33	SM
3	3.00	2.00	1.00	2.00	33.33	SM
4	3.00	1.50	1.50	1.00	50.00	M
5	3.00	1.50	1.50	1.00	50.00	M
6	3.00	1.50	1.50	1.00	50.00	M
7	2.50	1.50	1.00	1.50	40.00	SM
8	2.50	1.50	1.00	1.50	40.00	SM
9	2.50	1.25	1.25	1.00	50.00	M
10	2.50	1.25	1.25	1.00	50.00	M
11	2.00	1.00	1.00	1.00	50.00	M
Total	30.50	17.00	13.50			
Mean	2.77	1.55	1.23			
SD	0.41	0.33	0.24			

The chromosome lengths of AC-04 ranged from 1.5 -3.0µm ($\overline{X}$ = 2.45µm and SD = 0.35). Arm ratios did not vary much, being 1.0 to 2.0 in AC – 01, AC -02 and AC–03, and 1.0 to 3.0 in AC – 04. Table 6 shows the karyotype and total chromosome lengths of the four Bambara groundnut genotypes.When the total chromatin lengths were considered, its minimum (27.0um) and maximum (35.5µm) values were recorded in AC–04 and AC-01, respectively. The chromatin lengths in the rest genotypes fell within this range.

Table 4: Measurement of 11 pairs of somatic chromosomes of Ac-03 genotype of bambara in respect of chromosomes length, arm ratio, F% and centromere position.

Chromosome Pair Number	AC-03					
	Total chromosome length (µm)	Long arm (µm)	Short arm (µm)	rm ratio	F.%	Centromere Position
1	3.50	2.00	1.50	1.33	42.86	SM
2	3.50	2.00	1.50	1.33	42.86	SM
3	3.00	2.00	1.00	2.00	33.33	SM
4	3.00	2.00	1.00	2.00	33.33	SM
5	3.00	1.50	1.50	1.00	50.00	M
6	2.50	1.50	1.00	1.50	40.00	SM
7	2.50	1.25	1.25	1.00	50.00	M
8	2.50	1.25	1.25	1.00	50.00	M
9	2.00	1.00	1.00	1.00	50.00	M
10	2.00	1.00	1.00	1.00	50.00	M
11	1.50	1.00	0.50	2.00	33.33	SM
Total	29.00	16.50	12.50			
Mean	2.64	1.50	1.14			
SD	0.64	0.43	0.30			

Table 5: Measurements of 11 pairs of somatic chromosomes of AC-04 genotype of bambara groundnut in respect of chromosome length, arm ratio, F% and centromere position.

Chromosome Pair number	AC-04					
	Total chromosome length (µm)	Long arm (µm)	Short arm (µm)	Arm ratio	F.%	Centromere position
1	3.00	2.00	1.00	2.00	33.33	SM
2	3.00	1.50	1.50	1.00	50.00	M
3	2.50	1.50	1.00	1.50	40.00	SM
4	2.50	1.50	1.00	1.50	40.00	SM
5	2.50	1.50	1.00	1.50	40.00	SM
6	2.50	1.50	1.00	1.50	40.00	SM
7	2.50	1.25	1.25	1.00	50.00	M
8	2.50	1.25	1.25	1.00	50.00	M
9	2.00	1.50	0.50	3.00	25.00	T
10	2.00	1.00	1.00	1.00	50.00	M
11	2.00	1.00	1.00	1.00	50.00	M
Total	27.00	15.50	11.50			
Mean	2.45	1.41	1.05			
SD	0.35	0.28	0.25			

TABLE 6: Karyotype of the four bambara groundnut genotypes.

Accession	Total chromosome (µm)	Range of chromosome length (µm)	Mean length of chromosome (µm)	SD	Range of F (%)	T.F. (%)	Karyotype formula
AC-01	33.50	2.00 - 4.00	3.05	0.65	33.33-50.0	45	2n=22=6m + 5sm
AC-02	30.50	2.00 – 3.00	2.77	0.41	33.33–50.0	44	2N=22=6M + 5sm
AC-03	29.00	1.50 - 3.50	2.64	0.64	33.33-50.0	43	2N=22=5m + 6sm
AC-04	27.00	2.00 – 3.00	2.45	0.35	25.00-50.0	43	2n=22=5m+5sm+1t

The F % in the different accessions ranged from 33.33 to 50.0 percent in AC-01, AC–02, and AC–03, and from 25 – 50 percent in AC–04. The range of T.F percentage in the different accessions was between 43 to 45 percent. The Karyograms of AC-01 and AC– 02 was composed of 6 metacentric and 5 submetacentric chromosomes. The somatic chromosome complement of AC–03 was composed of 5 metacentric and 6 submetacentric pairs while that of AC–04 comprised 5 metacentric pairs, 5 submetacentric pairs and one telocentric pair. There was no discernable evidence of satellited chromosomes in all the genotypes studied.

DISCUSSION

The cultivated Bambara groundnut is autogamous (Baudoin and Mergeai, 2001) endowed with very strong tendency to maintain its natural characteristics owing to the cleistogamous nature (Cobley and Stede, 1976: Purseglove, 1976: Tindall, 1983) of its flowers. Typical among the morphological attributes are the seed coat colour and seed size. These hardly change and are therefore used by the local growers as the basis for classification.

The photomicrographs showed clear evidence that the cultivated bambara groundnut is a diploid with a somatic chromosome number of 2n=22. This record is in agreement with earlier reports (Purseglove, 1976; Tindall, 1983;

Baudan and Mergeai, 2001). There was no indication of polyploidy in any of the genotypes. The karyotype which hitherto had not been critically analysed by previous workers was analysed with some interesting features revealed. A mere record of the chromosome number of bambara groundnut by earlier workers (Purseglove, 1976; Tindall, 1983; Baudoin and Mergeai, 2001) is not of any major value except when taken together with additional information on the morphology of the chromosomes. While the basic karyotype remains constant, there were however, variations in the morphological characteristics of the chromosome complements of the genotypes. The uniformity in the chromosome number of all the genotypes is a clear indication that irrespective of the discernable morphological differences, they may have evolved from a common ancestor thereby classifying them as a homogeneous block. The genotypes were characterised by a graded karyotype into medium and small sized chromosomes. With respect to the total chromatin length, the genotypes AC–01 and AC–02 had total lengths of 33.50µm and 30.50µm while AC–03 and AC–04 had total lengths of 29.00µm and 27.00µm, respectively. There appears to be no perfect agreement between the total chromatin lengths and the morphological features (seed coat colour, eye colour and seed size) used in classifying the genotypes. The reduced chromatin lengths in some of the genotypes may be attributed to chromosomal aberrations and the role of heterochromatic segments during the process of evolution.The genetic and evolutionary relevance of heterochromatin had also been reported by Darlington and Mather (1950).The deletion of heterochromatic parts might have occurred in natural selection resulting in the decrease of the chromosomes. The chromosome size ranged from 1.5µm to 4.0µm. This is quite small when compared to the range of chromosome lengths of 14.1µm to 85.5µm reported for cowpea (*Vigna ungiculata)* (Barone and Saccardo, 1990). Going by the classification criteria of Sinha and Roy (1979), bambara groundnut chromosomes can be grouped into the A type (2µm - 3µm), B type (1µm – less than 2µm) and C type (<1µm). Apart from the 11th chromosome pair of AC – 03, the values of the entire chromosome length of all the genotypes can be classified as A type chromosomes. The genotype having greater chromosome lengths and high DNA contents are primitive whereas the ones with reduced chromosome lengths and low DNA contents are regarded as advanced lines (Stebbins, 1971). Their karyotypes were uniformly formulated as 2n=22=6m+5sm for AC–01 and AC–02; 2n= 22 = 5m+5sm for AC–03 and 2n=22=5m+5sm+1tc for AC–04. The F% values indicated that the different genotypes showed wide range of variations in their karyotypes. The minimum range (33.33 – 50 %) was ecorded in AC – 01, AC – 02 and AC – 03. These three genotypes also had chromosomes with mostly metacentric and submetacentric centromeres. The genotype AC – 04 had the maximum range (25 – 50%) and chromosomes with metacentric, submetacentric and telocentric centromeric positions. Thus, on the basis of F % values and the position of primary constrictions, the genotypes AC–01, AC–02, and AC–03 appear to manifest primitive conditions in contrast to AC–04 with wider F % value and chromosomes with metacentric, submetacentric and telocentric centromeres. The telocentric position of the centromere may have been brought about by deletion and consequent reduction in the size of one of the arms of the chromosomes, causing the shifting of centromere position and reduction in the absolute size of the chromosome (Sinha and Roy, 1979). Of the four accessions investigated, AC–01, AC–02 and AC–03 had symmetrical karyotypes. AC–04 had asymmetrical karyotypes since it had, in addition to the median and submedian chromosomes, a telocentric chromosome pair. It is well known that symmetrical karyotypes are more primitive than the asymmetrical ones. As such, the asymmetrical karyotypes are said to have evolved from symmetrical ones. Such conclusions had also been drawn by previous workers (White, 1945; Babcock, 1942; Stebbins, 1971; Sinha and Roy, 1979) in different animal and plants species. The T.F percentage value was between 43% and 45%. F% and the T.F% are two important parameters used in

determining the position of the genotypes in evolution (Sinha and Roy, 1979). In both parameters, the short arm length is a common factor in the computation of their values. The available results would tend to suggest that the genotypes of the cultivated Bambara groundnut have developed over the years. The evolutionary trends may have progressed from metacentric to submetacentric and telocentric centromeres, from large to relatively small chromosomes; and from symmetric to asymmetric in its karyotype. The perfect uniformity in chromosome number among the Bambara groundnut genotypes could have a number of implications:

1. The morphological divergence in respect of seed coat colour, eye colour and seed size amongst the genotypes of the Bambara groundnut species can be attributed to structural changes in the chromosome complements and gene pool.

2. That the cultivated bambara groundnut may have evolved from a common ancestor forming a homogeneous assemblage. Karyotypic evolution brought about by re-patterning of chromosome is the prime evolutionary factor for speciation within a genus where all species have the same somatic chromosome number (Sinha and Ray, 1977). On this premise, it is reasonable to conclude that karyotypic evolution arising from re-patterning of chromosomes may have been in force in the cultivated bambara groundnut as all the genotypes have 2n=22.

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