J. Sci. Engr. Tech. 4(2), 821-828, Oct. 1997

CHROMOSOME NUMBER AND KARYOTYPE ANALYSIS OF *SPHENOSTYLIS STENOCARPA* Linn AND *SPHENOSTYLIS* Linn *SCHWEINFURTHII* (African yam beans)

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ABSTRACT

The chromosome numbers of the two species of Sphenostylis, S. stenocarpa and S. shcweinfurthii were determined. A chromosome count of 2n = 20 (n=10) was recorded and the Karyotype analysis presented for the two species studied. The Karyotype analysis showed a significant variation between the genome of the two species. Within species variation in type of chromosome was also significant. There was a preponderance of large submetacentric chromosomes in S. stenocarpa, while S. schweinfurthii had predominance of medium sized metacentric chromosomes. It is suggested that speciation in the genus may be due mainly to chromosome reorganisation in structure with the number of chromosomes remaining unchanged.

INTRODUCTION

The genus *Sphenostylis* Linn is a small one belonging to the family Fabaceae. The genus contains mainly herbaceous and a few shrubby species (1). The genus has West African Savannah Zone as its centre of divergence. The complex is commonly called the African yam bean It is characterised by hard, long-cooking seeds which vary in colour from variety to variety.

The genus *Sphenostylis* is a source of a very proteinous food and various parts of the plant are said to have varying medicinal potentials (2 and 3). Some work has been done on the cultivation and improvement of the yield of varieties in the genus (4 and 5). Leakey, (4) suggested the improvement of the varietal yield through the process of selection based

on proper genotypic and phenotypic markers. The species recovered from a mixed growth of wild types and cultivated members gave higher growth rate and greater biomass consequently leading to better yield (5). Some work has also been done on the cytology of some species of the genus. Frahm-Leliveld (6) reported a chromosome number of 2n = 20 for *S. holoserica* and a chromosome number of 2n = 18 for *S. stenocarpa*. The present work is the first report on the Karyotype analysis as a basis for possible hybridisation programme.

MATERIALS AND METHODS

Five hundred and sixty (560) seeds of *Sphenostylis stenocarpa* and *Sphenostylis shweinfurthii* were collected from farmers in Akwa Ibom State. One thousand and twenty two (1022) seeds were also collected from the Akwa Ibom State Agricultural Development Project (AKADEP). One hundred (100) seeds were scarified in cone sulphuric acid for 5 minutes to break the dormancy. 10 seeds were placed on wet filter paper in petri-dishes to germinate while ten seeds were sown in pots. The rest of the seeds were planted out in beds. Root tips of the seedlings in the petri-dishes were harvested directly into acetic acid: ethanol (1 : 3 v/v) fixative and stored in a cool dry place for at least 24 hours before examination. The potted plants were maintained until they began to flower. Flower buds were then harvested at various levels of maturity and fixed the same way as the root tips. 210 root tips were collected from the seedlings. The root tips and 58 anthers from young flower buds were separately squashed in FLP Orcein stain and later examined under the microscope.

The Karyotype analysis was done using good slides of the root tips while the flower buds squashed provide the meiotic chromosome number. Forty (40) slides were prepared in each case. Measurement of the chromosomes were done using a stage micrometer. Chromosome lengths and arms were measured and their ratios calculated. Ideograms of the two species were constructed using the same scale for the two species. All the measurements were based on 10 cells and done when the chromosomes are most condensed. The chromosome arms were also measured with the same cells.

RESULTS

The two species of *Sphenostylis* showed a chromosome number of 2n = 20 (n = 10). This is the first report of Karyotype analysis for *S. stenocarpa* and *S. schweinfurthii*. The number was consistent in all the samples analysed. The variation in size and type of chromosomes within each species genome and between the species was very significant. The morphological distribution of the chromosomes is presented (Table 1). The mitotic chromosome of *S. stenocarpa* consists of 10 large, 6 medium and 4 small chromosomes. The genome of *S. schweinfurthii* consists of 4 large, 12 medium and 4 small chromosomes. Based on chromosome type, *S. stenocarpa* consists of 8 metacentric and 12 submetacentric chromosomes, while *S. schweinfurthii* has 12 metacentric and 8 submetacentric chromosomes. No acrocentric or telocentric chromosomes were recorded for either of the two species. The morphological distribution of the chromosome was also consistent between the stock from the farmers and the stock from AKADEP.

Between the two species, the large chromosomes measured between 62 - 83 μm, the medium chromosome measured between 40 - 49 μm and the small chromosomes measured 20 -22 μm. The total chromosome lengths and the chromosome arm ratios are presented (Table II). In *S. stenocarpa* the largest chromosome (chromosome 1) has the length of 72.4 ±2.5 μm and the smallest chromosome measured about 20.7±0.9 μm, (Chromosome 9). In *S. schweinfurthii* the largest chromosome (Chromosome 1) has the length of 81.3 ± 2.1 μm while the smallest chromosome is 26.5± 1.0 μm (Chromosome 10). The total chromosome length in *S. stenocarpa* (1020 μm) was greater than the total chromosome length in *S. schweinfurthii* (967 μm). Fig. 1 represents the Ideograms of the chromosomes of the two species. The variation in chromosomes composition within and between the species are clearly shown in the Karyograph.

In *S. stenocarpa*, chromosomes 1, 2, 6 and 9 are metacentric while 3, 4, 5, 7, 8 and 10 are submetacentric . In *S. schweinfurthii* chromosomes 1, 3, 4, 5, 6, and 9 are metacentric while the rest (2, 7, 8, 10) are submetacentric. In terms of size the chromosomes 1, 2, 3, 4, and 5 are large 6, 7, and 8 are medium and 9 and 10 are small in *S. stenocarpa* but in *S. schweinfurthii* chromosomes 1 and 2 are large, 3, 4, 5, 6, 7 and 8

are medium and 9 and 10 are small. This analysis was consistent in all the cells examined.

DISCUSSION

The consistency in the number of chromosomes in this genus is not unsuspected. This is so because chromosomes numbers of between 10 and 26 have been reported for the bean complex. Based on the description of Stebbins (7) 6 categories of chromosomes forms were identified for the genome of the two species. On examination of the karyotype, it was seen that asymmetry is of a low order, that is, there is little variation within the genome due to the predominance of metacentric to submetacentric components. On a relative scale, the chromosomes are generally large excepting chromosomes 1 and 2 which are extra large and 9 and 10 which are smaller. The chromosomes size is inversely proportional to the number of chromosome (8). The variation in symmetry that is karyotype composition between the two species probably resulted from structural changes and reconstruction of the original genome complex through translocation and deletions.

S. stenocarpa from the present study is a more advanced plant than *S. schweinfurthii* because of the variable chromosome content of the species. The older the genome in the evolutionary hierarchy, the greater the variation of its content. Because of the larger number of submetacentric chromosomes in *S. stenocarpa* and the greater number of the metacentric chromosome in the genome of *S. schweinfurthii* as already stated, speciation in the genus has occurred by changes or rearrangements in chromosome structure of the ancestral population coupled with changes in individual genes or gene complexes. The variation of total chromosome length between the two species may well be due to differential condensation resulting from structural rearrangement of the chromosomes (Table II). The chi-square result showed no significant difference between the total chromosome lengths of the two species.

Table 1: Chromosomes distribution of type and size in the two species

Species	Type of Chromosome	Number of Chromosome per Size Class			
		Large	Medium	Small	Total
S. stenocarpa	Metacentric	4	2	2	8
	Submetacentric	6	4	2	12
	Total	10	6	4	20
S. schweinfurthii	Metacentric	2	8	2	12
	Submetacentric	2	4	2	8
	Total	4	12	4	20

2n = 20

Table II: Chromosome Lengths and Arms Ratios of the 20 Chromosomes (Ten pairs each) of the two Species of *Sphenocentrum*

Species	Chromosome Type	Chromosome Lengths and Arm Ratios						
		Large		Medium		Small		Total Length
		Length	Arm Ratio	Length	Arm Ratio	Length	Arm Ratio	
S. stenocarpa	Metacentric	1.* 72.4 ± 2.5 2. 70.6 ± 2.2	1.00 ± 0.01 1.00 ± 0.01	6.* 43.7 ± 2.1	1.03 ± 0.02	9. 21.3 ± 0.9	1.01 ± 0.01	0**
	Submetacentric	3. 69.5 ± 2.1 4. 65.2 ± 1.9 5. 64.8 ± 2.0	1.67 ± 0.05 1.68 ± 0.09 1.62 ± 0.05	7. 41.5 ± 1.5 8. 40.4 ± 0.9	1.48 ± 0.04 1.45 ± 0.02	10. 21.+ ± 1.8	1.49 ± 0.02	10. 20.2 ± 18.7
S. schweinfurthii	Metacentric	1. 81.3 ± 2.1	1.02 ± 0.01	3. 49.1 ± 2.2 4. 45.9 ± 2.1 5. 44.2 ± 2.1 6. 43.1 ± 2.2	1.06 ± 0.02 1.01 ± 0.02 1.03 ± 0.02 1.02 ± 0.01	9. 27.0 ± 0.9	1.00 ± 0.00	
	Submetacentric	2. 79.2 ± 2.2	1.58 ± 0.04	7. 44.1 ± 2.1 41.2 ± 2.1	1.62 ± 0.03 1.59 ± 0.02	10.26 3 ± 1.0	1.48 ± 0.01	9. 63.3 ± 20.1

* Numbering of Chromosomes in order of descending size

** Total Chromosomes Length of all the 20 Chromosomes in each

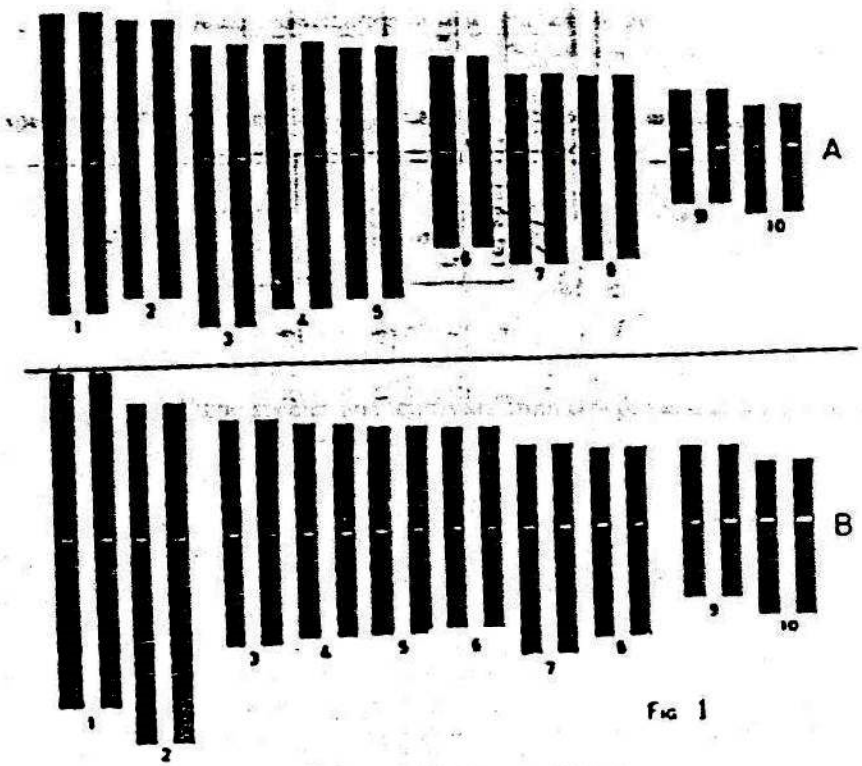

Fig. 1: Ideograms of the two species of Sphenostylis. The chromosomes are drawn from left to right in decreasing order of size. The chromosomes are aligned with their centromeres at the same level. A represents *S. stenocarpa* and B represents *S. schweinfurthii*.

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(Accepted 31 October 1996)