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Full Length Research Paper

Cytological studies on some accessions of African yam bean (AYB) (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich. Harms)

Popoola, J. O[1] , Adegbite A. E [2] and Obembe O. O[1]

[1]Department of Biological Sciences, Covenant University PMB 1023, Ota, Nigeria.
[2]Department of Biological Sciences, University of Agriculture, PMB 2240, Abeokuta, Nigeria.

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Mitotic, meiotic and pollen studies were carried out on selected accessions of African yam bean (AYB). Presented results confirmed diploid status of the species. Somatic chromosome counts of 2n= 22 (n=11) were made in most of the accessions except TSs3 in which counts of 2n= 18 were made in some cells. The somatic chromosomes were very small, mostly metacentric and submetacentric with distinct centromeres. The smallness of the chromosomes made it very difficult for *karyotype*. Meiosis was regular in most of the accessions with formation of 11 bivalents, except in TSs3 and TSs23 where some univalents erratic chromosome movements were observed. The studies revealed a correlation between meiotic irregularities and low pollen fertility. The limitations of the results of this study arose from the notorious difficulty of studying legume chromosomes.

Keywords: Chromosomes counts, meiotic irregularities, pollen fertility, *Sphenostylis stenocarpa*.

INTRODUCTION

African yam bean (*Sphenostylis stenocarpa* Hochst. Ex. A. Rich.Harms.) is a dicotyledonous plant belonging to the family Fabaceae and the genus *Sphenostylis* (Okigbo, 1973; Allen and Allen, 1981). Among members of the genus *Sphenostylis* (E. Meyer), *S. stenocarpa* has been reported to be the most economically important (Rachie and Roberts, 1974; Potter, 1991, Adewale, 2010). Klu et al. (2001) and Azeke et al. (2005) documented and reviewed many uses of African yam bean (AYB) including medicinal importance though with reported cases of under – development and utilization in its native and introduced areas (Moyib et al., 2008; Bioversity International, 2009; Popoola et al., 2011). Scientific information on *S. stenocarpa* is scanty when compared to other major food legumes such as cowpea, soy beans etc. due to its underutilization, low exploitation and cultivation. The low level of acceptability and adoption has been partly attributed to the presence of nutritional secondary metabolites such as saponins, flavonoids and alkaloids in the seeds and vegetative parts of the crop (Asuzu and Undie, 1986; NRC, 2006). The crop has also undergone little or no genetic improvement to boost its agronomic and nutritional qualities.

Most species of the genus *Vigna* which belong to the same family as AYB have been reported to be diploids with 2n = 22 chromosomes number. Diploid status of AYB with somatic chromosome counts of 2n = 18 and 22 have been speculated over the years (Peter and Davidse, 1977; Bandoin and Mergaei, 2001). The available literature on the species reveals that research efforts have focused on morphological characterization, genetic diversity, and evaluation of the nutritional and chemical components. Available reports on the cytology of the crop are scanty and not precise. The need for studies in this area becomes imperative to provide information on the basic biology and cytology of the crop. The major aim of this paper is therefore to ascertain somatic chromosome number(s), meiotic chromosome behaviour and provide basic information on the pollen characters which are crucial to overall genetic manipulation and improvement of this valuable crop.

*Corresponding author Email:
pjay_gbenro2008@yahoo.com;
gbenro.popoola@gmail.com +234 80 57 1085 66

MATERIALS AND METHODS

The seeds of the AYB accessions used for this study were obtained from the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Flower buds, pollen grains and seeds used for root generation were derived from plants raised in the experimental field of IITA under standard cultural practice.

Cytological studies

Mitotic and meiotic studies were carried out on some selected accessions of AYB that showed distinct characteristics in their morphology and growth habit. The selected accessions are TSs10, TSs3, TSs11, TSs16, TSs23, TSs104B and TSs119.

Mitotic studies

Seeds harvested from the selected accessions were plated on moistened germination paper in specially made germination plastics. The root generated from the sprouted seeds were pretreated with 0.04% colchicine solution when their radicles were about 1 cm long for 3 h between the hours of 9.00am and 12.00 noon, after which they were rinsed in clean tap water and fixed in 1:3 acetic acid / ethanol (v/v) for 24 h. The roots were hydrolyzed in 1NHCL for five minutes, squashed and stained with FLP- orcein using the squashing techniques described by Adegbite and Olorode (2002).

Meiotic Studies

Pollen mother cells from young flower buds were fixed directly in 1:3 acetic acid / ethanol (v/v) between the hours of 9.00am and 12.00 noon at room temperature. The fixed flower buds were dissected to extract the young anthers. Anthers excised from the flower buds were squashed and stained with FLP- orcein. Photomicrographs of good mitotic and meiotic stages were taken at X1000 magnification under oil immersion, using a Leica 2000 phase contrast microscope.

Pollen Studies

Pollen grains were obtained from opened flowers collected at 0630 - 0930 GMT. Slides were prepared by dusting pollen grains from the opened flowers in a drop of cotton blue in lactophenol on a clean slide and applying a cover slip or by squashing mature anthers from unopened flowers in the stain. Five slides from five different flowers were prepared for each of the accession.

Pollen fertility was estimated by counting pollen grains from at least ten fields on each of the five slides prepared for each accession at X100 magnification. Pollen grains with full cytoplasm were considered fertile while those with half or shrink cytoplasms were considered sterile based on the protocols of Jackson (1962) and Olorode and Baquar, (1976). The percentage of pollen fertility was estimated by expressing the number of fertile pollen grains as a percentage of the total pollen grains counted. Pollen size was determined by measuring the diameter of forty full and deeply stained randomly selected pollen grains on the five slides prepared for each accession at X 400 magnification using ocular micrometer. The ocular measurements were later converted to microns using the stage micrometer. Means and standard deviations were calculated for the measurements. Photomicrographs of pollen grains stained with FLP – orcein were taken at X 400 to show the pollen structure and texture.

Pollen grains were obtained from opened flowers collected at the early hours of the morning. Slides were prepared by dusting pollen grains from the opened flowers in a drop of cotton blue in lactophenol on a clean slide and applying a cover slip or by squashing mature anthers from matured unopened flower buds in the stain. Five slides from five different flowers were prepared for each of the accessions.

Pollen fertility was estimated by counting pollen grains from at least ten fields on each of the five slides prepared for each accession at X100 magnification. Pollen grains with cytoplasmic content stained deep blue were considered fertile while those that were not stained or only partially stained or with collapsed outline were considered as sterile (Jackson, 1962; Olorode and Baquar, 1976). The percentage pollen fertility was estimated by expressing the number of fertile pollen grains as a percentage of the total pollen grains counted. Pollen size was determined by measuring the diameter of forty full and deeply stained randomly selected pollen grains on the five slides prepared for each accession at X 400 magnification using ocular micrometer. The ocular measurements were later converted to microns using the stage micrometer. Mean and standard deviations were calculated for the measurements. Photomicrographs of pollen grains stained with FLP – orcein were taken at X 400 magnification to show the pollen structure and texture.

RESULTS

Cytological studies

Somatic chromosome counts of 2n = 22 were made for all the selected accessions except TSs3 in which counts of 2n = 18 were made in some cells. The somatic chromosomes are small, mostly metacentric and

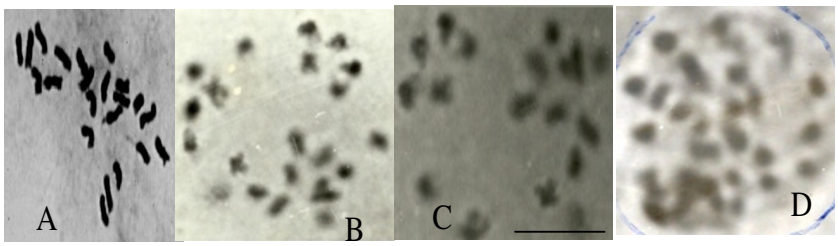

Figure 1. Mitotic chromosomes in the accessions studied. A: 2n = 22 in TSs10 and TSs104B; B: 2n = 22 in TSs7; C: 2n = 18 in TSs3 and TSs23; D: Pre metaphase in TSs104B (2n=22). Scale line = 0.50 µm.

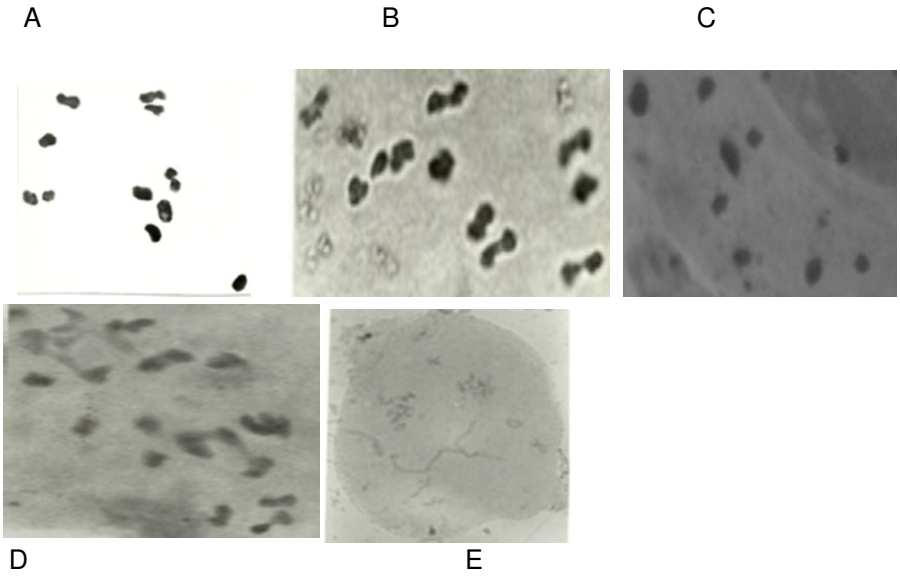

Figure 2: Meiotic Chromosome behavior of the accessions studied. A: Metaphase n= 11 in TSs10 and TSs104B; B: Metaphase I (n=11 in TSs7); C: Metaphase1 n= 9 in TSs3 and 23; D: Anaphase 1 (erratic) in TSs23; E: Anaphase in TSs16. Scale = 0.50 µm

submetacentric with one or two pairs of satellited chromosomes. Figure 1 shows mitotic metaphase and other stages in some of the AYB accessions studied.

Meiosis was observed to be regular in most of the accessions studied with formation of eleven bivalents except in TSs3 where nine bivalents were observed in some cells. However, univalents and erratic movement of chromosomes at anaphase were observed in TSs23. Figure 2 shows meiotic divisions in some of the accessions studied.

Pollen studies

All the accessions of *S. stenocarpa* studied possess tricolporate fenestrate pollen grains with scabrate exine. The pollen grains are reticulate, gently rounded without sharp corners with spinous cover that are interrupted by three protuberances (germ pore) in a fixed geometrical pattern (Iversen and Troels – Smith, 1950; Moore and Webb, 1978; De Leonardis et al., 1993). Photomicrographs showing the pollen structure, texture

Table 1. Pollen data, seed set %, seed germination and % moisture.

S/No.	AN	NPC	PF (%)	PS ± (µm)	SS %	SG %	% MC
1	TSs3	2655	66.43	77.50 ± 3.33	96.08	96.67	8.90
2	TSs7	2158	87.58	68.13 ± 3.18	91.10	66.60	9.23
3	TSs10	2754	83.17	74.50 ± 2.85	94.53	90.00	11.11
4	TSs11	2349	89.61	71.00 ± 2.93	82.63	83.30	8.62
5	TSs16	1972	83.87	73.46 ± 3.18	90.91	80.00	8.20
6	TSs23	1873	56.45	67.00 ± 3.87	89.10	60.00	8.33
7	TSs104A	2347	89.48	71.25 ± 4.89	87.43	80.00	9.80
8	TSs104B	2321	85.48	70.25 ± 4.48	87.44	75.00	10.17
9	TSs119	2785	95.30	82.75 ± 2.75	90.00	90.00	11.43

AN – Accession number; NPC – Number of pollen counted; PF – Pollen fertility; PS – Pollen size; SS% - Seed set percentage; SG% - Percentage seed germination; %MC – Moisture content rate.

and fertility have been shown and reported in earlier publication (Popoola et al., 2011) while Table 1 shows the data on pollen characters, seed set percentage, seed germination rate and moisture content. Pollen fertility was observed to be very high in most of the accessions (usually above 80%), except in accessions TSs 3 and TSs23 with pollen fertility of 66.43 and 54.45% respectively, in which meiotic irregularities were observed. Seed set and seed germination percentages were observed to be high for all the accessions.

DISCUSSION

The importance of chromosome number, meiotic behavior and pollen grain morphology in the characterization of plant species have been stressed by many authors. The meiotic process and chromosome behavior (gametogenesis) and the resulting pollen grains (male gametes) are very vital in the determination of fertility of any species. The present chromosome counts of 2n = 22 and 2n=18 in the studied accessions of AYB corroborate the previous counts reported for the genus *Sphenostylis* and a related species *S. marginata* (Peter and Davidse, 1977).

The metaphase chromosomes of AYB studied are largely metacentrics and submetacentrics that are very small in size which makes their measurement and characterization difficult. This factor is partly responsible for the rarity or absence of karyotypic data and descriptions for the species. The limitations of the results therefore arose from the notorious difficulty of studying legume chromosomes.

The occurrence of 2n=22 counts in majority of the accessions confirms the chromosome number of the species to be 2n=22, with n=11 being the gametic and basic chromosome number for the species and the genus respectively. The 2n=18 counts made in some cells are products of aneuploid decrease from 2n=22 consequent upon the observed meiotic irregularities, such as univalents and erratic movement and distribution of chromosomes to the poles observed in some of the accessions. The effects of these meiotic abnormalities resulted in relatively low pollen fertility and high pollen size variation in the affected accessions. However, pollen fertility was observed to be very high and pollen size variation low in all the accessions with normal meiotic division.

The meiotic mechanism operating in some of the accessions resulting in variable chromosome numbers could be of evolutionary importance in the species by creating genetic variations that could lead to evolution of new species or new varieties of the species. It could also lead to the evolution of new chromosome number for the species. All these therefore call for concerted and focused research efforts on the crop especially using recent molecular tools to circumvent some of the constraints to the development and improvement of the crop. AYB is crucial to food availability and security in the sub-Sahara Africa.

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