

# Gender in Fluted Pumpkin (*Telfairia occidentalis* Hook. F.)

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## ABSTRACT

A cytogenetic investigation was conducted on four lines of fluted pumpkin (*Telfairia occidentalis* Hook. F.) using the cold-induced pretreatment method as an integral part of the conventional technique. One major objective was to establish the chromosomal basis for sexual dimorphism in the crop. The results revealed a diploid chromosome number of  $2n = 22$ . The chromosomes are mainly metacentric and submetacentric with only chromosome pairs 10 and 11 being subtelocentric and telocentric, respectively. The karyotypic analysis revealed that chromosome pair number 5 is homomorphic in the female plant but heteromorphic in the male plant. The ideograms showed that the chromosome complement falls into 11 size class groups of one chromosome pair per class in the female and 12 size class groups with the 5<sup>th</sup> chromosome pair having two size groups arising from the non-identical lengths in the male. This would appear to implicate an XY system of sex chromosomes with homogametic XX female and heterogametic XY male in fluted pumpkin.

**Keywords:** dioecy, karyotype, heterogametic chromosomes, sex chromosomes, sexual dimorphism

## INTRODUCTION

Some plant species are sexually polymorphic, including dioecious species with separate males and females. Fluted pumpkin (*Telfairia occidentalis* Hook. F.) belongs to the family Cucurbitaceae (Irvine 1969; Purseglove 1984). It is a dioecious species with female plants producing only pistillate flowers and male plants producing only staminate flowers (Asiegbu 1985). This mechanism has made outbreeding mandatory and has, over the years, resulted in large genetic variability in fluted pumpkin. The dioecious nature of the crop has been found to affect the ratio of male to female plants. In a normal population, this ratio is approximately 1:1 (Anyim and Akoroda 1983; Akoroda and Adejora 1990).

The flower is entomophilous. The male flowers are borne on racemose inflorescence at the tip of the vines while the female flowers are solitary on short stalks in the leaf axils (Esiaba 1982). Akoroda *et al.* (1990) suggested that aphid, *Nomia tridentata* could be a pollinator of *Telfairia* because of its frequent presence on the female flowers, though some other insects like bees, dipteran flies, nymphs of bugs, black ants and carpenter bees thrive in *Telfairia* farms too. Fruits are formed 1-3 days after pollination and normally reach maturity after 3 months. Viable seeds are usually obtained from mature fruit after senescence.

Some attempts have been made to explain sexual dimorphism in fluted pumpkin with morphological and molecular markers (Ndukwu *et al.* 2005). Based on phenotypic observation, the authors reported that the female plants were generally larger in size than the male plants. They were also able to provide evidence of polymorphism using DNA fingerprints but could not provide any explanation for sexual dimorphism in fluted pumpkin. The present study was therefore initiated to address this lapse and provide convincing explanation for dioecy in fluted pumpkin which, to date, is unavailable in the literature.

## MATERIALS AND METHODS

Mitotic analysis of *T. occidentalis* was carried out using the cold-induced pretreatment method (Darlington and LaCour 1940) as an

integral part of the conventional techniques. Tender root tips of germinating seeds extracted from fluted pumpkin pods were harvested (excised) at 11.00 am when mitotic activities are believed to be high (Jackson 1962), using sterilized forceps and scalpel. The root tips, nearly 1 cm in length excised from the extracted seeds were washed with distilled water and cooled at 4°C for 48 hrs in a refrigerator (cold treatment).

The root tips were fixed with freshly prepared farmer solution (3 parts ethyl alcohol: 1 part glacial acetic acid) for 24 hrs and stored in 70% ethanol. They were rinsed with distilled water twice and hydrolysed in 18% HCl for 10 min at 60°C and rinsed in distilled water. The meristematic region of a young root tip was squashed in a drop of aceto-carmin (1%) on a slide. The cover slip was tapped gently with the end of a dissecting needle to spread the cells. The cells were then flattened by supplying firm pressure with the thumb on the slide that was placed between folds of filter paper. The slides were examined under a compound microscope. The slides that had well spread metaphase were photographed at X1000 using an Olympus, BX 51 model of photomicroscope equipped with Olympus C 4040 digital camera.

Chromosome measurements were made in  $\mu\text{m}$ . Measurements of the short arm (S), long arm (L) and the chromosome length (S+L) were made for each chromosome. The arm ratio ( $r = L/S$ ) was calculated and used to classify the chromosomes as proposed by Levan *et al.* (1961). The centromeric index, (S/S+L) X100 or (F %) was calculated as the ratio of short arm length to the total chromosome length expressed in percent (Huziwarra 1962). The total chromosome length in percent, TCL (%) was calculated using the formula described by Huziwarra (1962).

$$\text{TCL (\%)} = (S+L/\sum(S+L)) \times 100$$

where S = short arm length and L = long arm length.

The chromosomes were matched based on gross size, arm length and arm ratio measurements. The chromosomes were organized into metacentric, submetacentric, subtelocentric and telocentric according to their arm ratios. The images of paired chromosomes were cut and arranged in order of decreasing length to produce the ideograms. In assessing the chromosome morphology, centromeric position terminologies proposed by Abraham and Prasad (1983) were used. Depending on the relative length, the chromosomes were assigned to size classes according to Stebbins

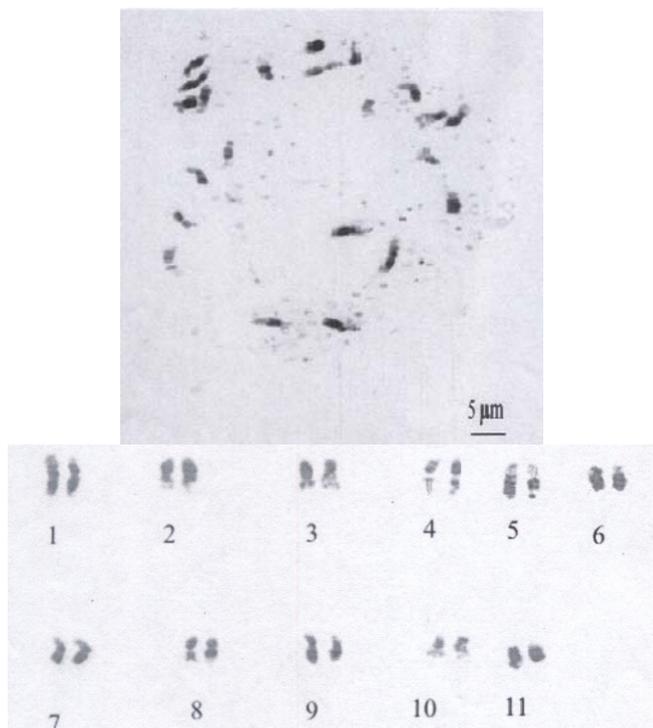


Fig. 1 Karyogram and karyotype of *T. occidentalis* showing non-identical pair of chromosome no. 5 in the male plant.

(1938) as follows:

- A. Short/small chromosomes (average length less than 2  $\mu\text{m}$ );
- B. Medium-small chromosomes (2-5  $\mu\text{m}$ );
- C. Medium-large chromosomes (5-9  $\mu\text{m}$ );
- D. Large/long chromosomes (average length more than 9  $\mu\text{m}$ ).

## RESULTS AND DISCUSSION

The photomicrographs showed clear evidence that the cultivated fluted pumpkin is a diploid with a somatic chromosome number of  $2n = 22$  (Figs. 1, 2). This finding is in agreement with an earlier report (Okoli and Mgbeogwu 1983). Table 1 shows the classification of the chromosomes according to Abraham and Prasad (1983) and Stebbins (1938). The chromosomes are mainly metacentric and submetacentric with only chromosome pairs 10 and 11 being subtelocentric and telocentric, respectively. The chromosome pairs 1-4 are classified as medium-large and chromosome pairs 5-11 classified as medium-small. The karyotypic formula proposed for *T. occidentalis* based on the morphological characteristics of the chromosomes is  $4C^m + 1B^m + 4B^{sm} + 1B^{st} + 1B^t$ . The superscripts, m, sm, st and t represent

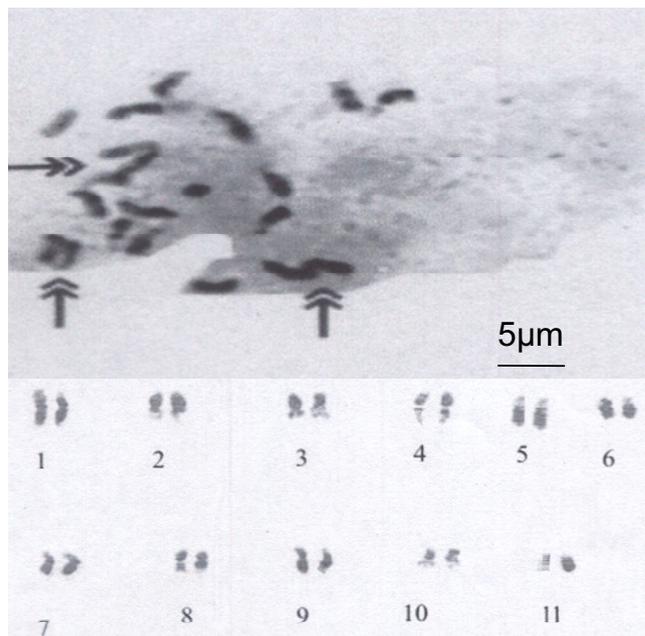


Fig. 2 Karyogram and karyotype of *T. occidentalis* showing identical pair of chromosome no. 5 in the female plant.

represent the metacentric, submetacentric, subtelocentric and telocentric positions of the centromere.

The ideograms (Figs. 3, 4) show the karyotype graphically. It is obvious from the ideogram of the female that the complement falls into 11 size class groups of one chromosome pair per size class. In contrast, the chromosome complement of the male showed 12 size groups (Fig. 1) with chromosome pair number 5 having two sizes arising from the non-identical lengths. The length of one of the chromosomes is 5.25  $\mu\text{m}$  corresponding with the length of the two chromosomes in the female. The length of the other chromosome is 4.89  $\mu\text{m}$  (Table 2). This scenario is suggestive that the chromosome pair number 5 is homomorphic in the female plant but heteromorphic in the male. These revelations would appear to implicate an XY system of sex chromosomes with a homogametic XX female and a heterogametic XY male in the fluted pumpkin.

Asiegbo (1985) reported that there were no evidence of monoecism, hermaphroditism or sex reversal in fluted pumpkin populations. The author also reported equal male and female ratios (1: 1) of *T. occidentalis* in the field. The existence of equal sex ratios (1: 1) is suggestive of equal possibilities in the separation of the sex chromosomes during gamete formation with no gamete recombination between the X and Y chromosomes. Equal sex ratios are unlikely in situations where dioecy is entirely genic without any cytological manifestation either in behaviour or struc-

Table 1 Karyotypic data of *Telfairia occidentalis*.

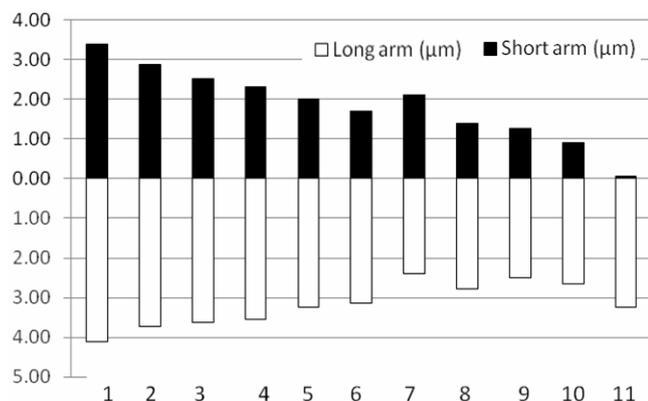
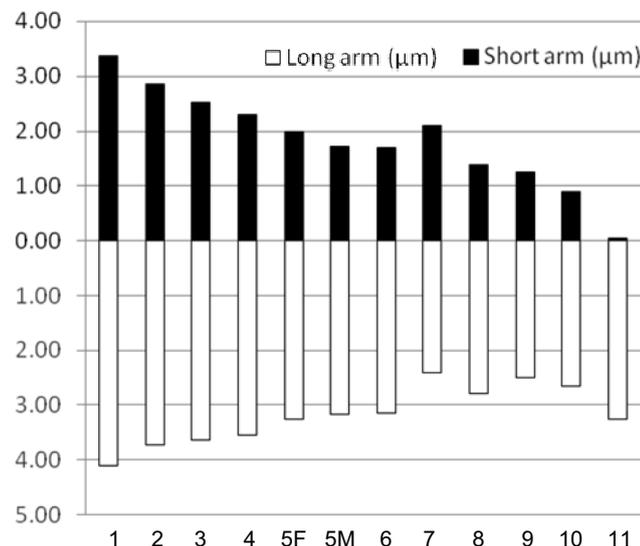
Chromosome pair number	S ( $\mu\text{m}$ ) chromosome	L ( $\mu\text{m}$ )	S+ L ( $\mu\text{m}$ )	TCL (%)	Arm ratio (L/S)	F%	Centromere position	Type
1	3.38 $\pm$ 0.17	4.10 $\pm$ 0.19	7.48 $\pm$ 0.29	13.49	1.21	45.19	M	C
2	2.87 $\pm$ 0.16	3.73 $\pm$ 0.15	6.60 $\pm$ 0.25	11.90	1.30	43.48	M	C
3	2.52 $\pm$ 0.15	3.63 $\pm$ 0.12	6.15 $\pm$ 0.18	11.09	1.44	40.98	M	C
4	2.30 $\pm$ 0.11	3.55 $\pm$ 0.15	5.85 $\pm$ 0.20	10.62	1.54	39.32	M	C
5	2.00 $\pm$ 0.07	3.25 $\pm$ 0.11	5.25 $\pm$ 0.12	9.47	1.63	31.05	SM	B
6	1.70 $\pm$ 0.16	3.15 $\pm$ 0.09	4.85 $\pm$ 0.11	8.75	1.85	5.05	SM	B
7	2.10 $\pm$ 0.14	2.40 $\pm$ 0.15	4.50 $\pm$ 0.16	8.12	1.14	46.67	M	B
8	1.38 $\pm$ 0.18	2.78 $\pm$ 0.20	4.16 $\pm$ 0.18	7.50	2.01	33.17	SM	B
9	1.25 $\pm$ 0.17	2.50 $\pm$ 0.18	3.75 $\pm$ 0.10	6.76	2.00	33.33	SM	B
10	0.90 $\pm$ 0.40	2.65 $\pm$ 0.16	3.55 $\pm$ 0.11	6.40	2.94	25.35	ST	B
11	0.05 $\pm$ 0.05	3.25 $\pm$ 0.10	3.30 $\pm$ 0.11	5.95	65.0	1.52	T	B
Total	20.45	34.99	55.44					
Mean	1.86	3.18	5.04					

S : Short arm length ( $\pm$  S.E), L: Long arm length ( $\pm$  S.E), S+L : Total chromosome length ( $\pm$  S.L), TCL(%) : Total chromosome length percentage  $(S+L/\sum(S+L)) \times 100$

**Table 2** Karyotypic data of *Telfairia occidentalis*.

Chromosome pair number	S ( $\mu\text{m}$ ) chromosome	L ( $\mu\text{m}$ )	S+L ( $\mu\text{m}$ )	TCL (%)	Arm ratio (L/S)	F%	Centromere position	Type
1	3.38 $\pm$ 0.17	4.10 $\pm$ 0.19	7.48 $\pm$ 0.29	13.49	1.21	45.19	M	C
2	2.87 $\pm$ 0.16	3.73 $\pm$ 0.15	6.60 $\pm$ 0.25	11.90	1.30	43.48	M	C
3	2.52 $\pm$ 0.15	3.63 $\pm$ 0.12	6.15 $\pm$ 0.18	11.09	1.44	40.98	M	C
4	2.30 $\pm$ 0.11	3.55 $\pm$ 0.15	5.85 $\pm$ 0.20	10.62	1.54	39.32	M	C
5	(F) 2.00 $\pm$ 0.07 (M) 1.72 $\pm$ 0.15	3.25 $\pm$ 0.11 3.17 $\pm$ 0.10	5.25 $\pm$ 0.12 4.89 $\pm$ 0.18	9.47 8.88	1.63 1.84	31.05 35.17	SM SM	B B
6	1.70 $\pm$ 0.16	3.15 $\pm$ 0.09	4.85 $\pm$ 0.11	8.75	1.85	35.05	SM	B
7	2.10 $\pm$ 0.14	2.40 $\pm$ 0.15	4.50 $\pm$ 0.16	8.12	1.14	46.67	M	B
8	1.38 $\pm$ 0.18	2.78 $\pm$ 0.20	4.16 $\pm$ 0.18	7.50	2.01	33.17	SM	B
9	1.25 $\pm$ 0.17	2.50 $\pm$ 0.18	3.75 $\pm$ 0.10	6.76	2.00	33.33	SM	B
10	0.90 $\pm$ 0.40	2.65 $\pm$ 0.16	3.55 $\pm$ 0.11	6.40	2.94	25.35	ST	B
11	0.05 $\pm$ 0.05	3.25 $\pm$ 0.10	3.30 $\pm$ 0.11	5.95	65.0	1.52	T	B
Total	20.17	34.91	55.01					
Mean	1.83	3.17	5.01					

S : Short arm length ( $\pm$ S.E), L: Long arm length ( $\pm$ S.E), S+L : Total chromosome length ( $\pm$ S.L), TCL(%) : Total chromosome length percentage  $(S+L/\sum(S+L)) \times 100$ , F : Female, M : male

**Fig. 3** Ideogram of *T. occidentalis* showing female sex chromosomes.**Fig. 4** Ideogram of *T. occidentalis* showing male sex chromosomes.

ture of chromosomes as reported for some members of the family, Cucurbitaceae: *Luffa echinata*, *Momordica dioica*, *Edgaria darjeelingensis* and *Melothria heterophylla* (Westergaard 1958). It is also unlikely in situations where dioecy arose from nascent epigenetic changes due to cytosine methylation (Gorelick and Osborne 2002; Gorelick 2003). Thus, the most elegant explanation for the equal sex ratio of male and female plants is the presence of a distinct pair of male and female sex chromosomes in *T. occidentalis*. Apparently from the foregoing, it is reasonable to conclude that sex in fluted pumpkin is genetically determined. How-

ever, it is difficult at this stage to determine the number of loci that determine maleness. A detailed mapping of the fluted pumpkin chromosome carrying the sex determining genes would reveal structural details about the sex determining region. Gene sequencing would help uncover the candidate sex determining gene or genes. Therefore, the sex mechanism should be followed further for a better understanding of the nature of genes on the Y chromosome as well as its male determining potentiality. Genomic technology could be employed to characterize the male specific region involved in sex determination at the molecular level.

## REFERENCES

- Abraham Z, Prasad PN (1983) A system of chromosome classification and nomenclature. *Cytologia* **48**, 95-101
- Akoroda MO, Adejora MA (1990) Patterns of vegetative and sexual development of *Telfairia occidentalis* Hook. F. *Tropical Agriculture* **67** (3), 243-247
- Akoroda MO, Ogbechi O, Adebayo NI, Uguro MI, Fuwa B (1990) Flowering, pollination and fruiting in fluted pumpkin (*Telfairia occidentalis*). *Scientia Horticulturae* **43**, 179-206
- Anyim OA, Akoroda MO (1983) Determination of optimal sex ratio for maximum fruit yield in *Telfairia occidentalis*. Annual Report of NIHORT, Ibadan, Nigeria, pp 116-119
- Asiegbe JE (1985) Characterization of sexes in fluted pumpkin: Growth and yield in the male and female sexes. *Horticultural Science* **50** (6), 251-255
- Darlington CD, la Cour LF (1940) Nucleic acid starvation of chromosomes in *Trilium*. *Journal of Genetics* **40**, 1850
- Esiaba RO (1982) Cultivating the fluted pumpkin in Nigeria. *World Crop March/April*, 70-72
- Gorelick R (2003) Evolution of dioecy and sex chromosomes via methylation driving Muller's ratchet. *Biological Journal of the Linnean Society* **80**, 353-368
- Gorelick R, Osborne R (2002) Inducing sex change and organogenesis from tissue culture in the endangered African cycad *Encephalartos woodii* (Cycadales, Zamiaceae). *South African Journal of Science* **98**, 114-117
- Huziwara Y (1962) Karyotype analysis in some genera of Compositae. III. Further studies on the chromosomes of aster. *American Journal of Botany* **49** (2), 116-119
- Irvine FR (1969) *West African Crops*, Oxford University Press, 272 pp
- Jackson RC (1962) Interspecific hybridization in *Haplopappus* and its bearing on chromosomes evolution in the *Blepharodon* section. *American Journal of Botany* **49** (2), 119-132
- Levan A, Fredga K, Sandberg AA (1961) Nomenclature for centromeric position on chromosomes. *Hereditas* **52**, 201-220
- Ndukwu BC, Obute GC, Wary-Toby IL (2005) Tracking sexual dimorphism in *Telfairia occidentalis* Hooker f. (Cucurbitaceae) with morphological and molecular marker. *African Journal of Biotechnology* **4** (11), 1245-1249
- Okoli BE, Mgbegwu CM (1983) Fluted pumpkin *Telfairia occidentalis*. West African vegetable crops. *Economic Botany* **3** (2), 145-149
- Purseglove JW (1984) *Tropical Crops. Dicotyledons* (Vols 1, 2) English Language Book Society and Longmans, London, 100 pp
- Stebbins GL (1938) Cytological characteristics associated with growth habits in the dicotyledons. *American Journal of Botany* **25**, 189-198
- Westergaard M (1958) The mechanism of sex determination in dioecious plants. *Advances in Genetics* **9**, 217-281