Full Length Research Paper

Tracking sexual dimorphism in *Telfairia occidentalis* Hooker *f.* (Cucurbitaceae) with morphological and molecular markers

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Morphological (foliar features) and molecular (DNA fingerprinting) markers were used to characterize sexual dimorphism in *Telfairia occidentalis* Hook. *f.* (Cucurbitaceae). A total of thirty strains comprising fifteen males and fifteen females from market-sourced fruits bred to the third generation were used for the investigation. The amplified fragment length polymorphism (AFLP) technique was combined with silver staining to determine the level of DNA polymorphism in the strains. Results showed that the females were generally larger in size than the males in leaf morphological features, while DNA fingerprints revealed the existence of polymorphism in the plant. However, this genetic variability did not clearly correspond with presence of sexual dimorphism in the species. The implications of these results in understanding the genetics, breeding and conservation of the plant's germplasm are discussed.

Key words: Leaf morphology, DNA fingerprinting, sexual dimorphism.

INTRODUCTION

Amplified fragment length polymorphism (AFLP) can generate DNA profiles from biological materials that can be used to specifically mark them (Vos et al., 1995). The method has already been used to extensively in the molecular marking of previously uncharted genomes lacking sequence information (Hartwell et al., 2000). Furthermore, Singh (2004) noted that this approach may be applied in DNA fingerprinting of even strains or varieties of the same species to eliminate identity ambiguities. AFLP has been used in the genetic diversity studies of *Gossypium* spp. (Pillay and Myers, 1999), *Vigna angularis* (Yee et al., 1999) and *Salix* spp. (Barker et al., 1999).

Telfairia occidentalis Hook *f.*, a native of West Africa, commonly called fluted pumpkin, is one such uncharted genome. It belongs to the family Cucurbitaceae and is an important vegetable crop in West Africa. Apart from its dietary and industrial uses, recent reports indicate that it may be used in bioremediation of heavy metal polluted soils (Obute et al., 2001; Wegwu et al., 2002). The crop has been adequately characterized morphologically (Jeffrey, 1977; Okoli and Mgbeogu, 1983; Okoli, 1987). Nevertheless, it is known to exhibit sexual dimorphism. The sexes may be differentiated by differences in floral size, time of production of flowers, perianth colour variations as well as distribution of these floral attributes. In the light of the stringency required in germplasm marking for conservation and improvement, these morphological markers may not adequately meet this need. Therefore, we attempted here to apply molecular genetic techniques to fingerprint the genotype differences manifesting as the observed sexual morphs in the crop. This was against the backdrop of seeking out possible markers that can be used to track the sexes of *T. occidentalis* prior to flowering. The relevance of this approach to *T. occidentalis* germplasm description, registration, conservation, improvement and utility cannot

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be overemphasized in the light of the importance of the crop.

MATERIALS AND METHODS

Morphological features

Seeds of *T. occidentalis* were obtained from market-sourced fruits and bred up to F3 generation. The F3 plants grown in the University of Port Harcourt garden were used for the study. A total of 15 male and 15 female plants were selected. Vegetative morphological features like foliar dimensions and petiole length were measured with a transparent metre rule. Reproductive morphological features such as flowering time, size, colour and arrangement of the floral structures were visually assessed.

DNA extraction and purification

Leaves were collected from plants and ground in liquid nitrogen in a mortar. Samples of the ground tissue were treated with 15 ml of pre-heated extraction buffer (4% CTAB-hexadecyltrimethyl ammonium bromide, pH 8.0) and incubated at 65°C in oak ridge tubes for 30 min. The incubated samples were centrifuged at 6000 rpm. The resultant aqueous phase was transferred to new oak ridge tubes and equal volume of 24:1 (v/v) chloroform : isoamyl alcohol was added. To precipitate DNA, 2/3 volume of ice cold isopropanol was added to the samples and centrifuged at 6000 rpm for 5 min. The aqueous phase was then treated with RNase, 3 M sodium acetate, and cold ethanol and centrifuged at 6000 rpm for 5 min. DNA precipitates in form of pellets were washed in 70% ethanol, briefly dried and resuspended in 200 ml of Tris EDTA buffer. The concentration of extracted DNA was ascertained with a Pharmacia Gene Quant11 spectrophotometer (Pharmacia, England) at an absorbance wavelength of 260 nm while the stock solution of DNA extract was preserved at –20°C until required for further analysis.

DNA digestion and amplification

40 ng of purified DNA was digested by addition of EcoRI and MseI at 37°C for 2 h to obtain restriction fragments. Transference of the reaction tubes to 70°C for 10 min stopped the digestion. To the digest, 0.25 µl of T4 ligase in 8 µl adapter ligation solution together with EcoRI and MseI adapters were added and incubated at 20°C for 2 h. The sample was diluted by a factor of 1: 10 mM Tris EDTA.

PCR protocol

5 µl of diluted DNA solution was mixed with EcoRI+A and MseI+C primers in a reaction tube. Each sample composed of 0.18 µl of EcoRI primer, 4.5 µl of MseI primer, 2.0 µl of reaction buffer and 0.1 µl of Taq polymerase. On the whole eight primer pairs [E-AAC/M-CTC; E-AAG/M-CAG; E-ACG/M-CAA; E-AGC/M-CTT; E-ACT/M-CTG; E-AGG/M-CTA; E-ACA/M-CAA and E-ACA/M-CAG] from GIBCO BRL AFLP kit were used for selective amplification in a Perkin Elmer thermal cycler. This was programmed to run for 20 cycles at 94°C for 30 s; 56°C for 60 s and 72°C for 60 s.

Electrophoresis and staining

Samples so amplified were subjected to electrophoresis to separate the fragments while the gel was fixed in 10% (v/v) acetic acid for 20 min and rinsed in de-ionized water three times. It was stained with silver stain [0.1% (w/v) silver nitrate and 0.015% (v/v) formaldehyde] for 15 min. The stained gel was rinsed in de-ionized water for 10 s and developed in cold 4 - 10% developer [3% (w/v) sodium carbonate, 0.015% (v/v) formaldehyde and 0.002% (w/v) sodium thiosulphate]. Development was continued until the bands became visible then the gel was finally rinsed in distilled water and air-dried.

Scoring of DNA bands

DNA polymorphic bands were scored with the aid of a light box and a band was adjudged polymorphic if it was present in at least one genotype and absent in others. An absent/present matrix was generated and the results subjected to Jaccard's similarity coefficient analysis (Jaccard, 1908) using an NTSYS-PC software package Version 2.0 (Rohlf, 1998). The data generated from DNA fingerprinting were also subjected to phenetic analysis by pair-wise genetic similarity coefficient according to Jaccard (1908).

$$GSxy = a / (a + b + c)$$

where GSxy represents genetic similarity between two individuals x and y;
a indicates the number of polymorphic bands present in both individuals;
b is the bands present in x but absent in y; and
c is the number of bands present in y but absent in x.

Further analysis was carried out with unweighted pair group method of arithmetic average (UPGMA) and a similarity dendrogram was generated from this. This was designed to group the accessions into clusters based on genetic similarity.

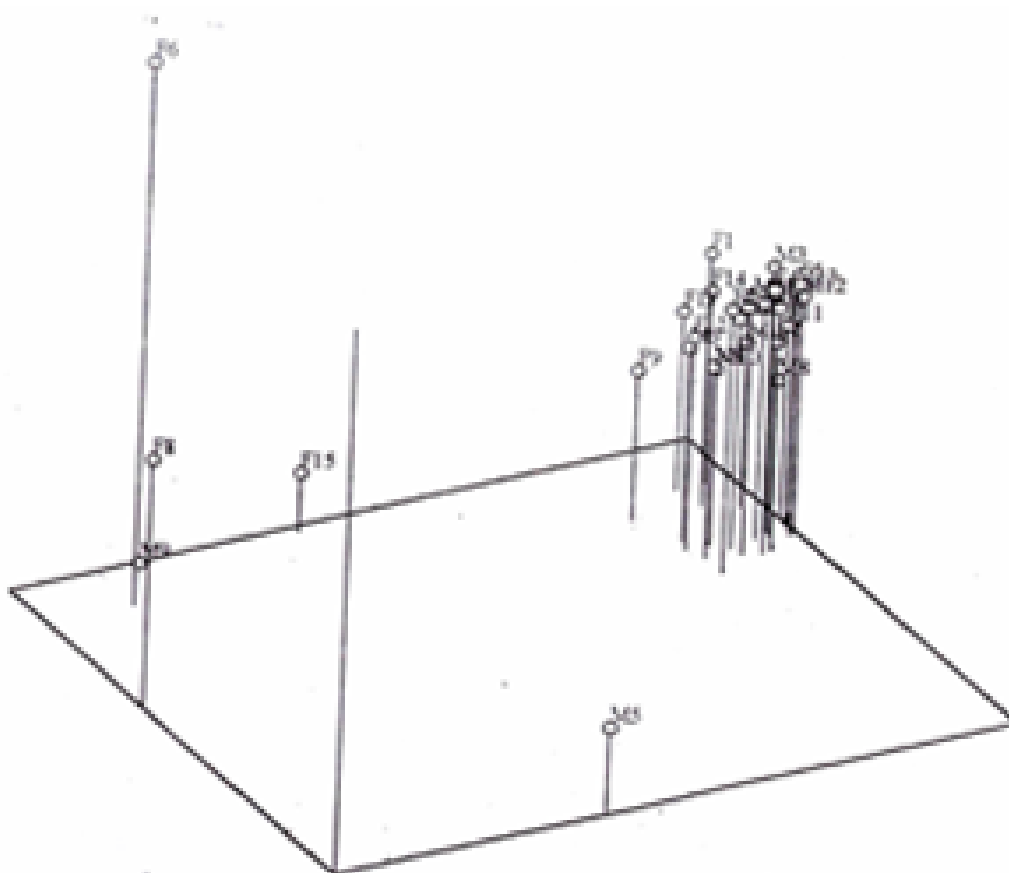## RESULTS

Foliar morphology

Leaf dimension observations on the plants showed that in spite of overlaps in the range of leaf dimension, leaves of the females appeared to be longer and wider than those of the males. Table 1 shows that length of leaves ranged from 20 – 31 cm in the male while it ranged from 23 – 37 cm in the females. Mean length was 25.74 ± 3.64 cm in male plants whereas it was 29.31 ± 3.62 cm in the females. Such differences were also noticed for the breadth of these leaves for the male and female plants. Not much variation was observed in the lengths of the petioles in both sexes. However, the observed mean differences in leaf linear dimensions were not significant when tested statistically ($P < 0.05$). For the reproductive parts, the male inflorescences were seen to project from the stem conspicuously and bore white small flowers; but the female flowers were solitary, short-stalked and borne on the axils of the leaves. Compared to the males the corolla lobes were cream to white in colour with the basal area purple in colour.

DNA fingerprints

Observations showed that while some DNA bands appeared in all accessions despite their sex, others were

Table 1. Showing the morphometric features of leaves on male and female *T. occidentalis* plants.

	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)
Male	25.74 ± 3.64^a (20.00 - 31.00)[b]	7.46 ± 0.65 (6.40 - 8.50)	10.36 ± 2.79 (7.00 - 14.75)
Female	29.31 ± 3.64 (23.00 - 37.00)	9.51 ± 1.19 (6.40 - 11.00)	10.01 ± 1.88 (6.50 - 14.00)

[a]Mean $\pm$ S.D.
[b]Range

Figure 1. Principal coordinate analysis of the 30 accessions of *Telfairia occidentialis*.

absent in some accessions. Since the electrophoretic gel was scored for differences in these bands across the accessions, a DNA band was recorded to be polymorphic if it was absent in at least one accession. Bands that appeared in all accessions were not scored, as these were monomorphic. Based on 165 AFLP bands that ranged from 80 – 1300 bp, the DNA fingerprint revealed variation of 53% but similarity of 94% between the male and female materials of *T. occidentalis*. The UPGMA clustering analysis assigned the materials into two major clusters as shown in Figure 1. While the first cluster had more plants, it included both male and female plants.

Similarly, the second cluster though made of just two plants, included one male and one female plant. Figures from these were used to construct a phenetic dendrogram (Figure 2) which showed that all the materials could not be sorted into exclusive lots based on the sex of the accessions.

DISCUSSION

Even when Okoli and Mgbeogu (1983) noted that dimensions of leaves are necessary in distinguishing
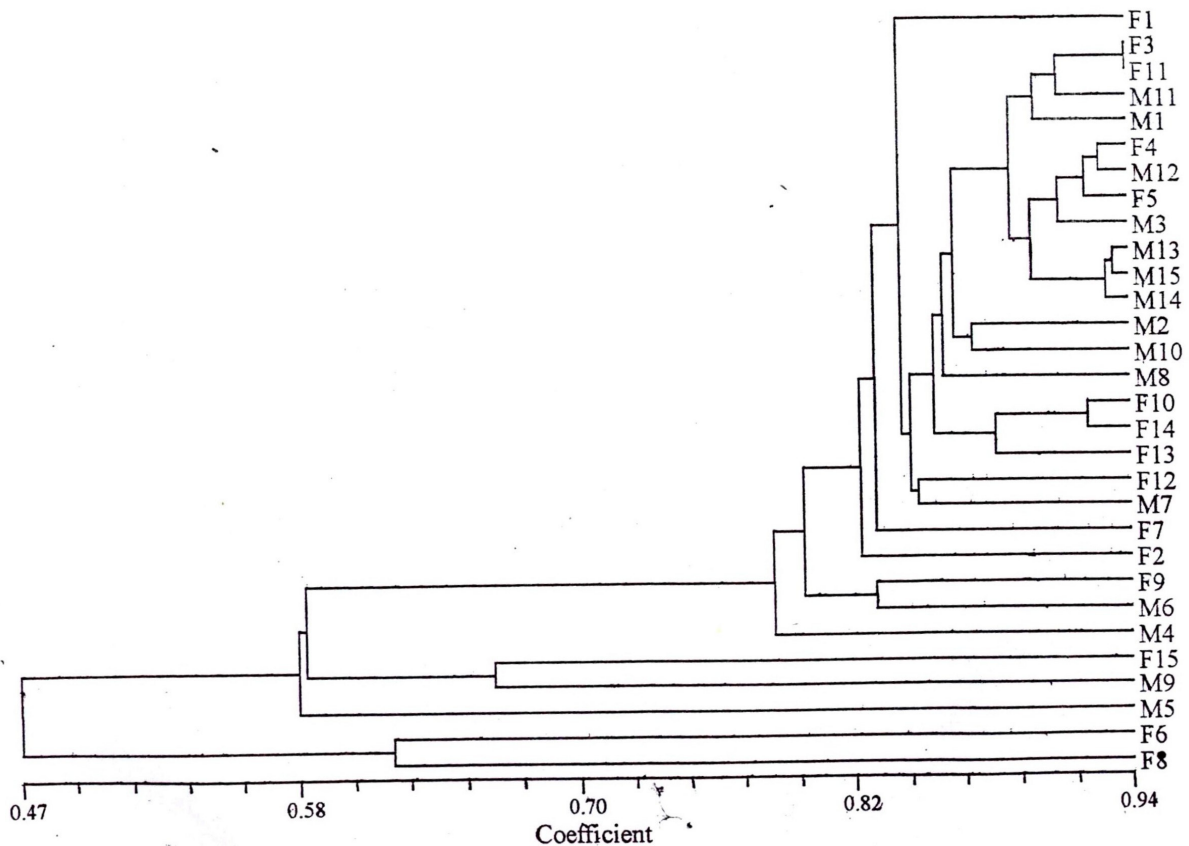
Figure 2. Genetic similarity of 30 accessions of *Telfairia accidentalis* based on AFLP analysis.

males from female plants of *T. occidentalis*, there were overlaps in the observed ranges of these features (Table 1) in this work. It is therefore, not advisable to attempt distinguishing the sexes in this plant based on leaf dimensions only. In addition, these workers reported that much of the diagnostic features for separation of the sexes were from the reproductive parts. Indeed the type, nature, colour and other parameters of the reproductive structures remain strong markers for detection of sexes of angiosperm groups in the field (Gledhill, 1972).

For the DNA fingerprinting, evidence is adduced here to show that polymorphism exists in several gene loci of the *T. occidentalis* genome. However, the phenomenon is distributed between the two sexual morphs in such a manner that could not discriminate between the sexes. Even so, the possibilities of further investigation with other techniques are only made apparent here. We conclude hence that though there is DNA polymorphism in *T. occidentalis* species, as revealed by AFLP technique, the approach could not adequately determine if the phenomenon is responsible for the sexual dimorphism observed in the plant. Meanwhile, as we await the outcome of more research in elucidating the incidence of sexual dimorphism in *T. occidentalis*, particularly in identifying appropriate diagnostic molecular

or chemical markers for the sexes; morphological attributes will continue to serve this end.

Explanation for the occurrence of the phenomenon in this plant may, therefore, be sought elsewhere. Indeed Berridge (1980) suggested that sexual dimorphism may be associated with hormonal differences in the sexual morphs under investigation. However, at the molecular level, most repeat sequences which lead to polymorphism are reported to occur in non-coding portions of DNA. Further investigation is still needed to adequately unravel the reasons for the occurrence of this phenomenon in this plant. Although both the morphological and AFLP methods could not clearly discriminate the sexual morphs of this plant, there still remains the possibility of finding molecular markers that can discriminate between the sexes of *T. occidentalis* with large scale amassing of data and extensive analyses.

ACKNOWLEDGEMENT

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