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Research Paper

Early Fluted Pumpkin (*Telfairia occidentalis* Hook. f.) Sex Identification Based on Biochemical Criteria

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The present field study was conducted to investigate the possibility of using plant physiological parameters as indices for fluted pumpkin sex identification during the vegetative growth stage of the plant – 8 weeks after planting. Thirty plants were sampled during the vegetative phase of the plant's growth (8 weeks after planting) and analyzed for biochemical indices including protein, fat, carbohydrate, iron, moisture, dietary fibre, ash, tannin, oxalate, and trypsin inhibitor content of their leaves. The results showed that of the eleven biochemical parameters upon which prediction was based, leaf protein content was the most reliable index for early sex prediction in the crop plant, with the accuracy value of 65.52%.

Keywords: Male, female, accuracy, protein

INTRODUCTION

Fluted pumpkin (*Telfairia occidentalis* Hook.f.) is a leafy vegetable of great economic importance. It belongs to the family, Cucurbitaceae and originated from tropical West Africa (Irvine, 1969; Esiaba, 1983). It is a herbaceous perennial crop although it is usually cultivated as an annual, especially under the traditional farming systems in West Africa. It is cultivated across the low-land areas. Fluted pumpkin is found in abundance during the rains, in areas with high rainfall such as West Africa. During the dry season, the plant is watered frequently for the production of good quality leaves and fruits. The crop plant is dioecious. The female plant produces bigger leaves than the male plant (Asiegbu, 1987). It produces tendrils with which it climbs. The main uses of *Telfairia occidentalis* Hook. f. is as a leaf and seed vegetable. The tender shoots, succulent leaves and immature seeds are cooked and consumed in several cuisines. They are often cooked with fish, meat and tapioca. Broad, succulent leaves attract higher premium in the vegetable market. Immature seeds are cooked or roasted. Seeds can also be fermented for several days and eaten as slurry or used as a condiment. The leaves have high protein content, low levels of anti-nutrients such as phytic acid, tannin and saponin, with an excellent ratio of essential amino acids to total nitrogen (Ladeji *et al*., 1995).

Fluted pumpkin is dioecious and the sex of a given individual plant cannot be known until after flowering which takes about 4 months (16 weeks) after planting for the male, and about 4 ½ months (18 weeks) for the female. The appearances of flowers in male plants mark the commencement of senescence in those individuals. The leaves of female plants are preferred for culinary purposes, and are therefore in higher demand. This, coupled with the fact that the female also produces the pods which bear seeds for future planting, which gives additional monetary returns to farmers, results in the observation that both producers (i.e., farmers) as well as consumers of the vegetable prefer females to males (Willie and Amaechi, 2017). The inability to know the sex of any given individual of the plant is a major constraint to its production. It would therefore be helpful to evolve ways of determining the sex of each plant at an early (i.e., pre-flowering) stage. This would arm farmers with useful information with which they could control the ratio of males to females in a way that boosts production. As fluted pumpkin is dioecious, vegetatively propagating any given individual would produce plants that express the sex of that particular individual. Tissue culture attempts at vegetatively propagating the plant using plant hormones like auxins and cytokinins are still at the experimental level (Ajayi *et al*., 2006; Balogun *et al.,* 2007; Sanusi *et al.,* 2008; Esuola and Akinyemi, 2011). The male to female plant ratio varies, ranging between 0.17-1.10 and 1.00-1.10 (Onwueme *et al*., 1986; Anyim and Akoroda, 1983). Female plants are reported to be more vigorous than male plants (Emebiri and Nwufo, 1996; Asiegbu, 1985).

Ajibade *et al.* (2005) compared protein, fat, fibre, ash, carbohydrate, tannin, phytate, oxalate, saponin and trypsin inhibitor contents of roots, stems and leaves in male and female plants of fluted pumpkin after flowering, using three genotypes of the crop species, and reported that female plants had significantly higher concentrations of nutrients, including protein and fats, while males had higher levels of dietary fibre, and anti-nutrients, including tannin, phytate, oxalate, saponin, and trypsin inhibitor. When they carried out a regression of sex on the biochemical contents on whole plant basis, they found that none of the variables they were working with was significant in distinguishing the sexes.

When the regression was done based on each plant part, however, they found that the concentration of trypsin inhibitor was a strong factor in differentiating between the sexes in specific plant parts, especially the roots and stems. For the leaf, which is the most important economic part of the plant, they observed that the fibre content was the most important factor as it explained about 94% of the differences between the leaves of the two sexes. They found out that the leaves of the male plants are significantly more fibrous than those of the female. They noted, however, that the use of this factor in sex identification should be specific to genotype, since their results indicated significant differences across genotypes in respect of this factor (fibre content of leaves).

Willie *et al.(*2018) carried out an evaluation of morphological and biochemical differences between male and female individual plants in fluted pumpkin and reported that the leaves of female plants had significantly higher moisture ($p<0.05$) and iron ($p<0.01$) contents than those of male plants. They also observed that crude fibre content was higher ($p<0.05$) in leaves of male plants than in leaves of female plants, and that there was no significant difference between the leaves of male plants and those of female plants with respect to their fat, crude protein, ash and carbohydrate contents. Male plants had significantly higher ($p<0.05$) saponin content than females, and there was no significant difference between males and females with respect to tannin, oxalate and trypsin inhibitor in the leaves.

This study was set up to examine the possibility and accuracy of early sex prediction in fluted pumpkin by use of some biochemical predictors. Specifically, the research was aimed at examining the possibility and accuracy of predicting sex in fluted pumpkin 8 weeks after planting, using levels of leaf protein, fat, fibre, moisture content, ash, carbohydrate, iron, tannin, oxalate, phytate and trypsin inhibitor, as prediction indices.

MATERIALS AND METHODS

The field experiment was conducted in the Eastern Farm of the Michael Okpara University of Agriculture, Umudike, southeastern Nigeria. Umudike is located at an altitude of 122m above sea level. It is found between latitude 05^0 29'N and longitude 07° 32'E, and has a mean soil temperature of 12.1°C It has a mean annual rainfall range of 1512 – 2200 mm, which is distributed over nine to ten months in bimodal rainfall pattern. The monthly minimum air temperature ranges from 28 to 35°C. The relative humidity varies from 51 to 87%. The average number of sunshine hours varies from 3 to 7 and appears always lowest in the months of July and August (NRCRI, Umudike Meteorological Station, 2016). The fluted pumpkin seeds used were obtained from pods sourced from a local farmer in Olokoro, Umuahaia South Local Government Area of Abia State, southeastern Nigeria. The pods were opened and a total of ninety-six (96) seeds extracted from them were planted in manually prepared beds at a planting depth of about 3cm and a planting space of about 1 m. The field was kept weed-free throughout the duration of the project by manual weeding. From the 96 seedlings that emerged, thirty individual plants were randomly selected, 8 weeks after planting and tagged with ribbons having identification numbers written with indelible ink. From each of these thirty plants, three leaves, one from the lower, one from the central, and one from the upper part of the vine, were sampled and taken to the laboratory where they were analyzed for nutrient and anti-nutrient and mineral contents. The nutrient content of interest included moisture, fat, protein, fibre, ash, carbohydrate contents, while the ant-nutrient content included the tannin, oxalate, phytate, trypsin inhibitor. For mineral content, the samples were analyzed for iron (Fe). Moisture content was determined by the gravimetric method described by Bradley Jnr (2003). Fat content of the sample was determined by the continuous solvent extraction method using a soxhlex apparatus. The method was described by Min and Bolt, (2003). Protein content was determined by the Kjeldahl digestion method.

Crude fibre content was determined by the method described by Bemiller, (2003). Iron content was determined by Atomic absorption spectrophotometer following dry ash acid extraction method (Carpenter and Hendricks 2003). Carbohydrate was calculated by difference as described by Bemiller, (2003) using the formula below.

$$\%CHO = 100 - \% [protein + fat + fibre + ash + moisture\ content]$$

Tanin content was determined using the Folin–Denis spectrophotometric method as described by Kirk and Sawyer, (1991). Oxalate, phytate, and trypsin inhibitor contents were determined as described by Onwuka, (2005).

Method of prediction

For each parameter, two replications were generated from each plant sample. Values obtained for the two replicates were added together and the sum divided by two to obtain a mean. Using the ranges of the mean values for each parameter, the mean values were divided into two groups, - one, high and the other, low. Individual plants with high moisture, protein, fat, and carbohydrate contents were predicted to be females while those with low levels of these components were predicted to be males. Plants with high levels of fibre, ash, tannin, oxalate, phytate, and trypsin inhibitor were predicted to be males, and those low in these parameters predicted to be females. The plants were tagged. The predictions were recorded as Predicted Sex and the plants left to grow to sexual maturity, i.e., to flower.

At sexual maturity when flowers appeared, the sampled plants, the sex of which had been predicted based on their biochemical composition, were visually observed and males and females noted, this time, based on the type of flowers (male or female) that each individual plant bore. These observations were noted as the Observed Sex and recorded against the Predicted Sex for each given plant of the thirty plants sampled. The records were cross-checked. When Predicted Sex corresponded with Observed Sex, the prediction was taken to be a Success, whereas when the two did not correspond, the prediction was considered a Failure. This procedure was carried out for each of the parameters studied. Accuracy of prediction, recorded in percentage, was computed as the number of successful predictions (successes) divided by the total number of predictions, multiplied by one hundred.

$$\%\text{ Accuracy of prediction} = \frac{\text{Number of success} \times 100}{\text{Total number of predictions}}$$

RESULTS AND DISCUSSION

Of the 30 plants sampled for early sex prediction, one died, and one did not flower, i.e., manifested neither maleness nor femaleness. The percentages of accuracy of computations were therefore based on 30 – 1 = 29 plants. Table 1 shows the percentage accuracy (%) of early sex prediction based on nutrient, and Table 2, shows the percentage accuracy (%) of early sex prediction based on anti-nutrient, composition of fluted pumpkin leaves 8 weeks after planting. As Table 1 shows, for the nutrients, the highest accuracy percentage (65.52%) was recorded for protein content. This was followed by moisture content (44.83%), fat content (41.38%), ash and carbohydrate contents (34.48%, each), and finally by iron content (37.93%). Similarly, for the anti-nutrients, the higher accuracy percentage (37.93%) was recorded for tannin and saponin contents. This was followed by 34.48%, recorded for oxalate and trypsin inhibitor contents. From these observations, it could be deduced that protein content of the leaves (high protein level held as an index of femaleness, and low protein content, as an index of maleness) was the most reliable index for predicting sex during the vegetative phase of growth (8 weeks after planting) in fluted pumpkin. All the other parameters had low percentages of accuracy (less than 50%), and so, were unreliable in predicting sex, 8 weeks after planting, in fluted pumpkin.

Ajibade *et al.* (2005) compared protein, fat, fibre, ash, carbohydrate, tannin, phytate, oxalate, saponin and trypsin inhibitor contents of roots, stems and leaves in male and female plants of fluted pumpkin, using three genotypes of the crop species, after the plant had flowered, indicating which individuals were males, and which were females, and reported that female plants had significantly higher concentrations of nutrients, including protein and fats, while males had higher levels of dietary fibre, and anti-nutrients, including tannin, phytate, oxalate, saponin, and trypsin inhibitor. When the regression was done based on each plant part, however, they found that the concentration of trypsin inhibitor was a strong factor in differentiating between the sexes in specific plant parts, especially the roots and stems. For the leaf, they observed that the fibre content was the most important factor as it explained about 94% of the differences between the leaves of the two sexes. They found out that the leaves of the male plants were significantly more fibrous than those of the female. Also, Willie *et al.* (2018) who studied the morphological and biochemical differences between male and female individual plants in fluted pumpkin reported that the leaves of female plants had significantly higher moisture ($p<0.05$) and iron ($p<0.01$) contents than those of male plants, and that crude fibre content was higher ($p<0.05$) in leaves of male plants than in leaves of female plants. The results of the present study seem to corroborate with the findings of Ajibade *et al.* (2005) with respect to leaf protein content, but differs from the earlier reports in leaf fibre content. Also, the results obtained here do not contradict their finding that the concentration of trypsin inhibitor was a strong factor in differentiating between the sexes in the roots and stems, but not leaves, of the plant because roots and stems were not analyzed in this study, and for the leaves that were analyzed, trypsin inhibitor with a low accuracy percentage of 34.48 has also shown itself to be a poor index for predicting sex in the crop. It must be pointed out that both Ajibade *et al.* (2005) as well as Willie *et al.* (2018) carried out their studies on the plant after it had flowered, while in the present study, analysis was done several weeks before flowering. In this crop, males flower weeks before females, and for the males, signs of senescence such as a yellowing of the leaves follows flowering closely, unlike in the female, which remains

Table 1. Percentage accuracy (%) of early sex prediction based on nutrient composition of fluted pumpkin leaves 8 weeks after planting.

Nutrients	Success	Failure	Accuracy (%)
Protein	19	10	65.52
Fat	12	17	41.38
Fibre	10	19	34.48
Moisture content	13	16	44.83
Ash	10	19	34.48
Carbohydrate	10	19	34.48
Iron	11	18	37.93

Table 2. Percentage accuracy (%) of early sex prediction based on anti-nutrient composition in fluted pumpkin leaves 8 weeks after planting.

Anti-nutrients	Success	Failure	Accuracy (%)
Tannin	11	18	37.93
Oxalate	10	19	34.48
Phytate	11	18	37.93
Trypsin inhibitor	10	19	34.48

green and vigorous for a much longer time. Generally, senescence in plants also comes with increased leaf fibre and decreased moisture and iron contents. This is why it is not surprising that both Ajibade *et al.* (2005) as well as Willie et al. (2018) reported that leaves of male plants had significantly more fibre than those of females in their studies, contrary to the present observation that the use of leaf fibre content (high fibre content used to predict maleness, and low fibre content used to predict femaleness) in the plant had a very low accuracy percentage (34.48%). The earlier workers cited here investigated differences between the sexes when the males and females were at different physiological phases (even though they were of the same chronological age), whereas in the present study, prediction was made following analysis carried out on young, yet-to-flower plants that later manifested different sexes. This distinction is important.

Conclusion

The results showed that of the eleven physiological indices upon which prediction was based, leaf protein content was the most reliable index for early fluted pumpkin sex identification, with an accuracy value of 65.52%. It may be concluded that for any given young plant at the vegetative phase of growth, the higher the probability of the plant manifesting femaleness at maturity.

Authors' Declaration

We declare that this study is an original research by our research team and we agree to publish it in the journal.

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