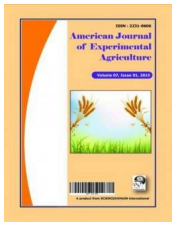

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Identification of Facultative Apomixis in Fluted Pumpkin (*Telfairia occidentalis* Hook F.) through Emasculation Method

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Authors' contributions

This work was carried out in collaboration between all authors. Author LSF designed the study, wrote the protocol and wrote the first draft of the manuscript and performed the statistical analysis. Authors DKO, ACO, AMA and LAH reviewed the experimental design and all drafts of the manuscript. Author AOO managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Apomixis is seed and embryo formation without fertilization. A study was carried out from August, 2012 to May, 2014 in order to investigate the possibility of occurrence of apomixis in fluted pumpkin (*Telfairia occidentalis* Hook. F.) using emasculation method. Five genotypes of fluted pumpkin were used. Ten plants from each genotype were planted out in a screened environment at the

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department of Crop, Soil and Pest Management, Federal University of Technology, Akure. The crop being dioecious, all the male plants were emasculated (by total removal from the site) as soon as they were noticed, leaving only the female plants. Seeds obtained from the fruits of isolated female plants were later subjected to viability test. After several weeks of flowering, flowers and developing ovaries abortion, they eventually set fruits. All the genotypes produced fruits (one or two) though at different times. Both apomictic and parthenocarpic fruits were produced. The seeds obtained from the apomictic fruits of all the genotypes had high seedling emergence percentage and good seedling vigour. Apomictic fruits, seeds and seedlings were similar to those of sexually produced fruits. The observed facultative apomixis could be as a result of natural selection. This study confirms that apomictic form of reproduction occurs naturally in fluted pumpkin. Facultative apomixis in the fluted pumpkin is being reported for the first time and its implication in the genetic improvement of the crop is discussed.

Keywords: Telfairia occidentalis; apomixis; dioecious; genotypes.

1. INTRODUCTION

Fluted pumpkin is among the important indigenous leaf and seed vegetables in West Africa. Its nutritional, medicinal, multi-utility, perennial and industrial potential earned it prominence among indigenous vegetables. Fluted pumpkin is a dioecious plant, having separate male (staminate) plant and female (pistillate) plant. There are wide variations in sex ratio but there tends to be more males than female plants [1-4]. Male plants start flowering earlier (mean of 129 days compared with 150 days after planting for female plants) and over a longer period (59 days versus 17 days for female plants) [5]. The inability to distinguish sex at early stage and the preponderance of males in natural populations hinder its productivity [6]. The female plants are known to produce large succulent leaves and fruits that attract high prices in the markets. The male plants, on the other hand, produce small less attractive leaves and no fruit [7]. Thus, there is high discrimination against the male plants because they are of no economic importance to farmers.

Apomixis is defined as the asexual formation of a seed from the maternal tissues of the ovule, avoiding the processes of meiosis and fertilization, leading to embryo development [8]. Apomixis may be obligate or facultative, resulting in either only apomictic produced embryos and seeds, or a combination of apomictic and sexually-derived embryos and seeds, respectively [9,10]. Apomixis has the potential to make a significant contribution toward meeting food production demand throughout the developing world in the 21st century [11]. Apomixiscan be beneficial to farmers by lowering seed prices and enable farmers to perpetuate improved apomictic seeds for many generations without altering their genetic makeups.

In spite of these benefits of apomixis, there is a valid concern about its possible effect on genetic diversity. This might lead to genetic vulnerability because of absence of the gene recombination through sexual reproduction. However, most apomicts, in addition to their clonal offspring, also produce offspring via sex (facultative apomicts) [12]. Facultative apomixis can increase considerably the cytotype diversity within apomictic populations. These facultative apomicts enjoy dual benefits of maintaining variation through sex and advantages of the asexuality provided by apomixis. In fact, facultative apomicts maintain variability almost, if not equally as well as exclusively sexually reproductive plants [13].

There are different methods of identifying apomictic plants [14,15]. Most of these methods are expensive, require high technical know-how (e.g. molecular marker, auxin test, cytological and flow cytometry) and labour intensive. Emasculation method is an ancient and simpler method of detecting apomixis which involves careful emasculation of stamen and isolation of the maternal parents. In this case, if fertile seeds are produced in the absence of pollen, apomixis is likely present [16,17,14,15]. Emasculation method could be useful for identification of apomixis in dioecious plant, since the male plant can easily be emasculated by total removal.

Often, apomixis is associated with some processes in plant such as polyembrony, polyploidy and perennial life cycle [18-20]. For instance, in perennial crops such as hawkweed (*Hieracium spp*.), Kentucky bluegrass

(*Poapratensis* L.), citrus and mango, apomixis, polyploidy and polyembryony occur simultaneously [21-25]. Fluted pumpkin, apart from being dioecious, it is perennial, polyembryonic and polyploidy [26-28]. An understanding of the reproductive biology of fluted pumpkin is necessary because these processes in the crop might imply the possibility of apomixis. Apomixis could help in genetic improvement of the crop and could be a remedy to sex discrimination in fluted pumpkin because it would be expected that apomictic seed of fluted pumpkin would be replica of the maternal plant which is the desire of the farmers. The exploitation of apomixis would greatly facilitate the ability of plant breeders to fixate and propagate genetic heterozygosity and associated hybrid vigour in crop plants [29]. Therefore, this study was initiated to investigate possible occurrence of apomixis in fluted pumpkin using emasculation method.

2. MATERIALS AND METHODS

This experiment was carried out from August, 2012 to May, 2014 beside the screen house of the Department of Crop, Soil and Pest Management Federal University of Technology, Akure(7°16' N, 5°12' E),located in the rainforest area of South-western Nigeria. The location is characterized by a bimodal pattern of rainfall with an annual mean of about 1300 mm with a mean temperature of 27°C.The experimental site was an isolated environment which was far from nearby farms. Five fluted pumpkin genotypes obtained from two states of Nigeria were used for the trial. Since the aim of the experiment was to investigate possibility of fruits and seeds production in the absence pollen, no experimental design was used. The experimental area measured 11.25 m^2 and was divided into five plots each plot for each genotypes. Ten seeds from each genotype were planted on flat on plot allocated to it. Regular weeding and watering were carried out when necessary and the plants were staked. The crop being dioecious and the fact that the male plants start flowering earlier [5], all male plants were emasculated (by total removal from the site) as soon as they were noticed. Among the remaining female plants, one female plant per genotype was tagged and monitored for fruits set. Fine mesh bags were used cover the female flowers to protect them from any external pollinators as shown in Plate 1. Putative apomicticfruits harvested from isolated female plants were counted and the number of seeds per fruit recorded. Average fruit weight was taken and measured in kilogram likewise average seed weight and average fruit length were measured in gram and centimetre respectively using appropriate equipment.One putative apomictic fruit from each genotype was selected for viability test. All filled seeds from each genotype were divided into three, one for each replicate. In March, 2014, the seeds were planted in nursery boxes arranged in completely randomised design with three replications in order to test the viability of the seeds and compare them morphologically. Saw dust was used as growth medium to hasten the germination as suggested by [5]. Data collection started at seven days after sowing (7DAS) on ten seedlings per box. Seedlings that emerged every day from 7DAS till 14DAS were counted and recorded and were used to compute days to 50% emergence. At 14DAS data were collected emergence percentage, number of leaves, vine length (cm). Emergence percentage (E%) was calculated as

$$E\% = \frac{\text{Number of seedlings emerged at 14DAS}}{\text{No of seeds sown}} \text{ x } 100$$

Seedling vigour (SV) was computed as

$$SV = \frac{\text{vine length x emergence percentage}}{100}$$

The data obtained were subjected to analysis of variance using SPSS (Statistical Package for Social Sciences) Version 15 software and significant means were separated using Tukey test.

3. RESULTS

There were more male plants among the seeds (across the genotypes) planted than the female, thus very few female plants were left after emasculation (total removal) of the male plants. All the isolated female plants left in the screen environment flowered, though at different times. They produced numerous flowers (>100 per plant). Flowers and developing ovaries abortion were very high as shown in Plate 2.In the case of flower abortion, after petal drop the ovary withered while in the case of developing ovary abortion, the ovary showed an initial increment in size for few days after petal drop but later shrivelled. Apart from few flowers that developed into fruits other flowers and developing ovaries were aborted. Abnormal flowering was also observed in some of the female plant during this study for example tendrils produced flower buds (Plates 3a and 3b) and twin flowers (Plate 3c).It

took about 8 weeks of continuous flowering before there were successful fruits set. As soon as fruits set were successful in each of the female plants, flower production ceased. All the genotypes evaluated produced fruits though at different times. Observation of fruit developmental traits indicated that fluted pumpkin comprises both traits of apomixis and parthenocarpy (Plates 4a and 4b). Two of the genotypes (Ftm11 and Ftm12) produced parthenocarpic fruits and they were seedless when opened. Each female plant produced one or two fruits (Table 1). Variation was observed in fruit and seed characteristics among the genotypes. Genotype Ftn64 produced 1 fruit, its fruit was biggest in terms of fruit weight and fruit length and had the highest number of seeds. Whereas, Ftm11 produced 2 fruits had the lowest average fruit weight and least number of seeds/fruit. Result obtained from the viability test showed that all the apomictic seeds from all the genotypes were viable (Table 2). This was evidenced by high emergence percentage observed in the seedlings which ranged from 68.18 (Ftm12) to 96.97% (Ftm11). Significant differences ($P<0.01$) were observed among the five genotypes for the five seedling characters evaluated. The general performance of the seedlings showed they had good vigour as seedling vigour index ranged from 14.40 (Ftn63) to 49.40 (Ftn62). It took about 10 to 14 days after sowing to obtain more than 50% emergence. Genotype Ftn62 that emerged earliest, had high emergence % of 95% and had the highest values for vine length (52.00 cm), number of leaves (9) and seedling vigour (49.40).

Plate 1. Isolation of female flower with fine mesh

Table 1. Fruit and seed characteristics of apomictic fruits obtained from the five genotypes

Genotypes	Number of apomictic fruits	Number of parthenocarpic fruits	Average Fruit weight(kg)	Average fruit length (cm)	Number of seeds/fruit	Average seed weight(g)
Ftm12	2	2	4.90	52.00	66	17.16
Ftn62	1	0	5.20	50.00	80	15.30
Ftn64	1	0	5.90	58.00	81	11.28
Ftn63	2	0	3.00	36.00	50	9.70
Ftm11	2	1	1.70	43.00	33	11.40

Plate 2. Flowers and developing ovaries abortion

Plate 3a. Flower bud on tendrils

Plate 3b. Flower bud on tendrils

Plate 3c. Twins flower bud

Plate 3. Abnormality in flowering of female plants

4. DISCUSSION

The numerous flowers produced by the female plants were unusual. Female plants have been reported to produce less than 20 flowers [2]. Unusual numerous flowers, abnormality in flowering and high abortion of flower and developing ovary might be attributed to response of the plants to physiological stress. This stress might have been caused by delay in fruit set because of absence of pollen. Hence, more flowers were produced over a long period. The interruption in the flower production when there was sign of successful fruit set might be attributed to physiological shift in the allocation and partitioning of photosynthate from flower production to fruit development. According to [30], altering either the size or the activities of the sink results in changes in translocation patterns.

Plate 4a. Apomictic fruits **Plate 4b. Pathenocarpic fruit**

Plate 4. Apomictic fruits and parthenocarpic fruit of fluted pumpkin obtained

Table 2. Means performance of seedlings of apomictic seeds obtained from the five genotypes

Genotypes	Days to 50% emergence	Emergence %	Vine length (cm)	Number of leaves	Seedling vigourindex
Ftm12	14.00a	68.18d	23.00c	8.00b	15.68c
Ftn62	10.00c	95.00b	52.00a	9.00a	49.40a
Ftn64	14.00a	80.25c	20.10d	9.00a	16.13c
Ftn63	14.00a	80.00c	18.00e	7.00c	14.40d
Ftm11	12.00b	96.97a	33.50b	8.00b	32.48b

Different letters show statistical differences at P<0.05 by Tukey's test

The result from this study revealed that asexual reproduction in form apomixis occurs in fluted pumpkin as all isolated female plants from different genotypes set fruits successfully in absence of pollination. Parthnocarpic trait in some members of the family *Cucurbitaceae* like water melon and some edible fruits is desirable but in fluted pumpkin seedlessness is undesirable because the fruits are not edible. The seed is economically important. Therefore, the parthenocarpic trait in this crop cannot be improved upon. The differences observed in fruit and seed characteristics among the genotypes might be due to differences in genetic makeup of the genotypes. The viability test revealed all the apomictic seeds were viable and had good vigour. The result obtained on fruit, seed and seedling characteristics of the apomictic fruits were consistent with other earlier works on the crop where non-apomictic seeds were used [31-33]. Hence, apomictic fruits, seeds and seedlings obtained were morphologically similar to sexually produced fruits. Therefore, the breeding system of fluted pumpkin could be said to be facultative apomixis. Apomixis has been reported in more than 300 species and at least 35 different families, and commonly may be identified in the *Gramineae, Compositeae, Rosaceae, Euphorbiaceae Rutaceae* and *Musaceae* [34,9,35,36,10,37] but has not been reported in fluted pumpkin. Although, [2] reported that fluted pumpkin grown in isolation produced a fruit with four seeds which the authors opined to be either monoecious plant or it was exhibiting parthenocarpy. Contrary to this opinion, it could not have been parthenocarpy because there were seeds and the crop is not monoecious. The result from this study revealed that all the fruits produced were through apomixis. Occurrence of facultative apomixis in fluted pumpkin can be

supported by the fact that the crop being dioecious, it is an outcrossing species and apomixis is much more prevalent among outcrossing species than inbreeding species [38]. Fluted pumpkin exhibits polyploidy, polyembryony and it are perennial. Also, strong correlation between apomixis and polyploidy, polyembryony, perenniallife cycle have been reported [39,38,19]. Apomicts are often found in highly disturbed areas or where individuals are widely dispersed [38], so it is possible that isolation of the female plants may be an influencing factor for apomixis in fluted pumpkin. Therefore, occurrence of apomixis may be as a result of sexual evolution through natural selection. This is the first time apomixis will be reported in this crop. Hence, this discovery of the occurrence of apomixis in fluted pumpkin is a great opportunity for the improvement of the crop. This will lead to improved seed yield per unit area and issue associated with poor yield from the male plant would be eliminated.

5. CONCLUSION

This study confirms that sexual and apomitic forms of reproduction occur naturally in fluted pumpkin. Both produce morphologically similar fruits and viable seeds. The observed facultative apomixis could be as a result of natural selection. Apomixis in the fluted pumpkin is reported for the first time. This has great implication in the genetic improvement of the crop especially in enhancing its leaf and seed yield which can only be obtained from the female plant. It can be concluded that among methods of identifying apomixis emasculation method is workable and especially when used for dioecious species. However, more advanced techniques such as auxintest and molecular analysis can be used to confirm any result obtain from emasculation method.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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