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Original Article

Floral Biology of Fluted Pumpkin (*Telfairia occidentalis* Hook. F.)

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Abstract

Knowledge of floral biology is essential to crop improvement. Ten genotypes of fluted pumpkin (*Telfairia occidentalis*) were observed for floral morphology, phenology and insect visitation for two consecutive years. Functional dioecy of fluted pumpkin was confirmed, whereas none of the studied genotypes was monoecious. Floral structures differed significantly among the genotypes. Both male and female flowers were symmetrical, pentasepalous, fimbriate and non-bright pentapetalous, but male flowers were more numerous. Male inflorescences emerged from 11 to 14 weeks after planting and the female flower buds appeared about 4 weeks later. The flowering period of the male flowers was longer than that of female flowers and both gender flowering periods coincided for a specific interval. It took between 11 to 14 days from bud initiation to anthesis and flowering ceased when there were occurrences of successful fruits set. In both gender flowers petals started unfurling at around 6.00 pm and full bloom was achieved by dawn, while petal shrivelled at sun set (between 6.30 pm to 7.30 pm). The anthers dehisced at anthesis of the male flowers; the pollen grains were whitish and sticky. Only the male flowers have nectar and pollen and this may explain infrequent female flowers visitation. Hover flies (*Cheilosia* species) were the major floral visitors observed.

Keywords: dioecious, floral biology, fluted pumpkin, hover flies, phenology

Introduction

The understanding of flower morphology and flowering characteristics is important in angiosperms' reproduction and breeding. This is because flowering initiates the sexual reproductive cycle of all spermatophytes (Elliot *et al*., 1982). Reproductive biology provides information on the nature of species, adaptation, speciation, systematics and hybridization (Anderson *et al*., 2002; Neal and Anderson, 2005).

Sexual and asexual forms of reproduction are known in angiosperm, whereas the latter does not entail formation of zygotes by sexual organs and genetic recombination, while the former does. Sexual reproduction of plants is determined by two important factors, the intrinsic characteristics of plant breeding systems and the extrinsic interactions between plants and their pollen vectors (Dafni *et al*., 2005). These factors differentiated sexual plants as self-pollinated or cross-pollinated. Self-pollinated species accept pollen primarily from the anthers of the same flower, while cross-pollinated species accept pollen from different sources (Acquaah, 2007). Self-pollinated flowers may be cleistogamous (pollination before anthesis), or chasmogamous (polloination after anthesis). Rarely, both co-exist in plants, for instance in *Cotnmelina benghalensis* (Kaul *et al*., 2002; Kaul and Koul, 2009).

Cross pollination requires agents which can either be abiotic (wind, water or gravity) or biotic (animals, mainly insects). Cucurbits' flowers need insect-mediated pollination for successful seed and fruit set. Bees play an essential role in the pollination of most *Cucurbitaceae* (Robinson *et al*., 1989; Nepi and Pacini, 1993; Agbagwa *et al*., 2007), while other insects like thrips and hover flies have also been reported to successfully pollinate cucurbits (Larson *et al*., 2001; Agbagwa *et al*., 2007; Sajjad and Saeed, 2010). This is because flowers of cucurbits are not

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known to be hermaphrodite and most of them have bright coloured flowers (usually yellow) which attract insects. According to Schaefer and Renner (2011) more than 75% of the species in cucurbits are monoecious, while there are also dioecious species and a few species have been reported as androdioecious (separate male and hermaphrodite individuals). In addition, cucurbits have attractive scent, nectar and sticky pollen grains.

Fluted pumpkin (*Telfaria occidentalis*) is an indigenous West African cucurbit, important as nutritious leaf and seed vegetable. Fluted pumpkin is believed to be dioecious, with separate male and female plants. The male plants flowers' earlier than the female ones. This crop exhibits polyembryony (Esiaba, 1982; Odiyi, 2003; Onovo *et al*., 2009) and facultative apomixis (Fayeun *et al*., 2016). These phenomena have been suggested to improve the crop, but the limitation of these is the absence of genetic recombination that is only possible through meiosis and syngamy. Currently, farmers cultivate landraces due to unavailability of improved varieties and this resulted in unpredictable and low yield, poor quality and low tolerance to diseases and pests (Fayeun and Odiyi, 2015). One of the reasons for this is the inability of plant breeders to develop varieties through hybridization. Knowledge of reproductive biology, which entails floral biology, pollination mechanisms and breeding systems, is a prerequisite for plant breeding and without this progress crop improvement through hybridization will be limited. The available studies on pollination biology and breeding systems of this crop are insufficient.

The documented reports on reproductive biology of fluted pumpkin stated that there are more than 800 open male flowers to a single opened female flower and male flowers open in the evening till the next morning, while female flowers open in the morning till late in the evening (Odiaka and Schippers, 2004). According to Akoroda *et al*. (1990), about 10-15% of a given female population do not flower in the first year of planting and abortion of fruits is high. According to Odiaka and Schippers (2004), both male and female flowers are similar structurally except the presence of inferior cylindrical ovary and 3 large, heart-shaped stigma in the female flower.

This study was initiated to describe floral morphology and pollination mechanism of fluted pumpkin in ten genotypes; observations were made on phenology and floral visitors. To this step, significant floral structures functional dioecy was established, the female flower lacks nectar and pollen thus receive less visitors than the male flower and hover flies (*Cheilosia* species) are among the suspected pollinators of fluted pumpkin in the study area.

Materials and Methods

Ten fluted pumpkin genotypes sourced from four states in southern Nigeria were used for the experiment. The experiment was carried out from September 2012 to December 2013 and March to July 2014 at the Teaching and Research Farm of Federal University of Technology, Akure, latitude 7°16'N, longitude 05°12'E and the ecology of the zone is classified as rainforest with sandy clay loam soil.

The land was prepared manually and the experiment was laid out in a randomized complete block design (RCBD) with three replications. The experimental area measured 160 m^2. Each replication had 10 plots of 2 × 2 m raised bed, with 1 m inter-plot spacing. Seeds were first raised in the nursery using sawdust as growth medium before transplanting at two weeks after planting. One seedling was transplanted per hole on beds at a spacing of 1 by 1 m, resulting in nine plant stands per plot. Weeding was done manually at three weekly intervals.

Flower morphology

Twenty five anthesized (fully open) male and female flowers were collected from each of the ten genotypes. Measurements taken on the floral parts of the female flowers were: petal length, petal width, ovary length, ovary width, ovary diameter, style length, stigma diameter, pedicel length, pedicel width, sepal length and sepal width. The measurements taken on the floral parts of the male flowers were: petal length, petal width and filament length. The means of the floral structures were computed for each of the genotypes according to Gomez and Gomez (1984).

Flower phenology

The flower biology of fluted pumpkin was further studied on a single flower basis following the protocol proposed by Dafni (1992). Ten developing female flower buds whose corollas were about 5 mm long were tagged on 5 plants of the same genotype, giving a total of 50 flower buds as the sampling units. This was carried out on four genotypes. The measurement of the flowering progress of the tagged flower buds was carried out daily till anthesis and petal drop as shown in Fig. 1. Records were taken on the following parameters: daily bud length, inflorescence length, days to anthesis and days to flower wilting. The lengths were measured in centimetres using a ruler. The regression analysis of the progressive female flower buds development in the four genotypes was analysed using Microsoft Excel.

Floral visitors

Observations of the floral visitors were carried out on the field, from 7:30 a.m. to 6.00 p.m. for 14 days. Foraging behaviour of the visitors for floral resources (nectar and pollen) were observed and recorded, as well as the time, frequency and duration of visits.

Witness specimens were collected and their taxonomic determination was done by specialists of the Entomological Unit of the Department of Crop, Soil and Pest Management of the Federal University of Technology, Akure, Nigeria.

Results

Morphology of the male flower

Fluted pumpkin was observed to be dioecious, with two types (staminate and pistilate) of imperfect flowers bore on different plants. Flowers on staminate plants appear earlier and are more numerous than the flowers of pistilate plants. The male flowers are arranged on a simple pedunculate raceme inflorescence with pedicelate

Fig. 1. The measurement of the progressive development of female flower from bud till maturity

a- flower bud of about 0.5 cm length; b- flower bud of about 1.0 cm length; c- flower bud of about 1.5 cm length; d- flower bud of about 2.0 cm; e- flower bud of about 2.5 cm length; f- flower bud of about 3 cm; g- flower bud of about 3.5 cm length; h- flower bud of about 4.0 cm; i- flower bud of about 6.0 cm length; j- flower bud of about 7.0 cm length

flowers (Fig. 2). Attached to the base of the inflorescence is one long-pedicellate flower which opens before others. Single raceme can bear about 30 to 100 flower buds, each with a cup shaped receptacle (thalamus) at the end of the pedicel. The thalamus is perigynous with the androecium situated in the centre and other parts of flower are located on the rim of the thalamus, almost at the same level (Fig. 3). The flower is polysepalous, with five sepals, triangular, green, deeply 5-lobed, of about 0.6-1.2 cm long. The 5 petals are symmetrical, deeply 5-lobed and have pilose inner surface. The length and width of the male petals ranged from 2.75 cm to 3.90 cm and 1.2 cm and 1.85 cm, respectively. The colour of the claw of the petal ranges from white to light purple on both surfaces and the limb is purple on the inside, while the outside is white with purple stripes (Figs. 2 and 3). The petals are fimbriate, caryophyllaceous, ascending imbricate and valvate. There are 3 dorsifixed, syngenesious stamens each with dithecous introrse. The stamens are inserted and epipetalous. The anther opens longitudinally; the

Fig. 2. Male fluted pumpkin raceme of staminate flowers showing variation in color

Fig. 3. Structure of fluted pumpkin male flowers

A = Petals, B = Stamen, C = Dehisced anther showing whitish and sticky pollen grains, D = Nectar chamber (Base of stamen where nectar is stored)

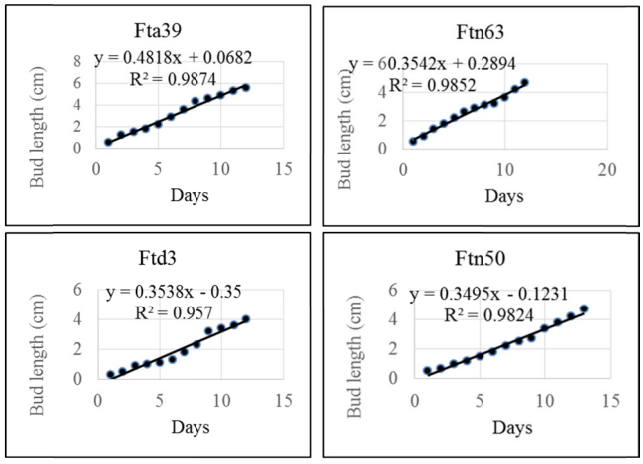

Fig. 5. Variation in progressive female flower bud development in four different genotypes of fluted pumpkin

pollen grains are whitish and sticky. The slightly scented and colourless nectar is stored at the base of the stamen in a cup formed by the thalamus (Fig. 3).

Fig. 4. Fluted pumpkin female flowers showing petal colour, sepal, ovary, stigma and ovules

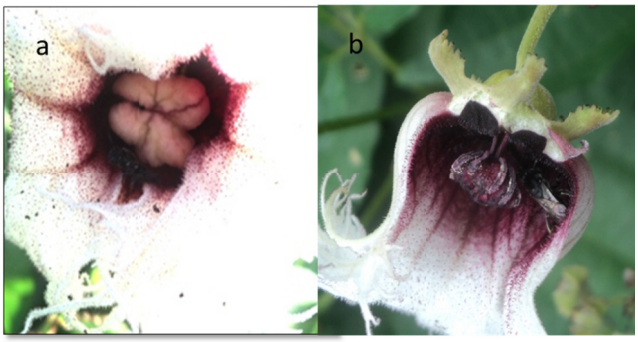

Fig. 6. Hoverfly *Cheilosia* sp. on female (a) and male flower (b) of fluted pumpkin

a- Hoverfly *Cheilosia* sp. on female flower; b- Hoverfly *Cheilosia* sp. on male flower

Morphology of female flower

The female flower is always solitary in leaf axils, with pedicle from 1.5 cm to 8.20 cm in length. Unlike in the case of male flowers, the thalamus is epigynous (Fig. 4). The sepals and petals are similar to males' except that the female sepals are deciduous and petals are larger. The female flower is syncarpous with 3 large, cordate stigmas on the style. The colour of stigma ranges from cream to red. The style is inserted. The ovary is inferior, cylindrical, with 10 prominent ridges, unilocular and parietal, with the placenta intruding deeply into the ovary, thus making the chamber multilocular. Carpels are completely fused (Fig. 4).

Floral phenology

Emergence of inflorescences begins at between 11-14 weeks after planting, with the male flowers emerging first

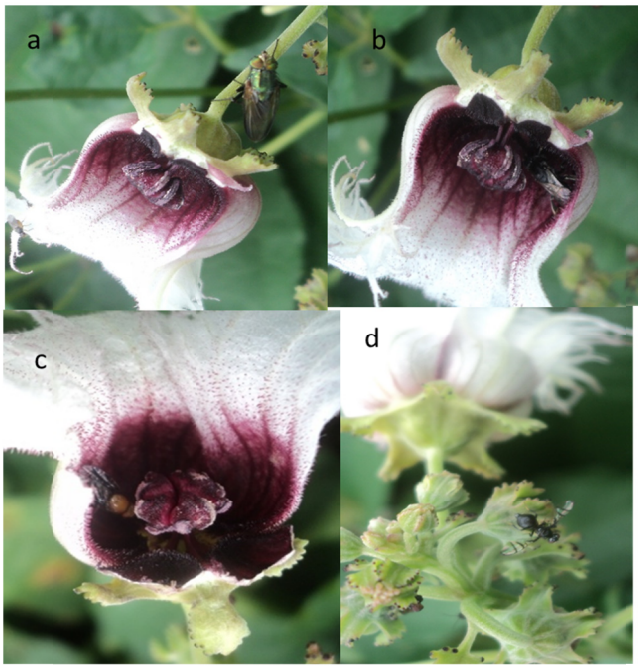

Fig. 7. Registered flower visitors to male flowers of fluted pumpkin at the study site. (a) Hover fly *Cheilosia* sp.; (b) Green fly (*Musca* sp.); (c) Fruit fly (*Drosophila* sp.); (d) Thick-headed fly (*Conopidae* sp.)

Fig. 8. Flower constancy exhibited by honey bee foraging flower of water leaf and neglecting fluted pumpkin flowers

and predominating in number all through the flowering period. The female flower buds appear about 4 weeks later. The ratio of male to female flowers per plant throughout the flowering period was about 1,000:1. The overall flowering period in the male inflorescence was 12 to 20 weeks and about 4 weeks in the female ones, depending on when fruits set. Fig. 5 depicts the progressive development of female flower from 2 days after bud initiation (0.5 cm) to about 12 days after bud initiation, when the flower opens (anthesis) (7 cm). It took between 11 to 14 days from bud initiation to anthesis. When fruits were set successfully in female plants, flowering ceased. In both types of flowers, opening was initiated before dawn.

Fig. 9. Hover fly (*Cheilosia* sp.) the suspected major pollinator of fluted pumpkin

During the study, petals of both flowers started unfurling by 6.00 pm and this continued through the night and full bloom was achieved by dawn. The anthers dehisced after the male flowers have opened. Flowers in both genders opened by outward stretching of the corolla (Figs. 3 and 4) and closed by wilting at sun set (6.30 pm to 7.30 pm).

Pollinators and pollination ecology

Among the various insects on the flowers of fluted pumpkin, the hover flies or fruit flies or syrphids (*Cheilosia* species) were regular floral visitors (Figs. 6, 7 and 9). Table 1 show that the male flowers received more visits from hover flies than the female flowers. On each observation day, the hover flies arrived at the flowers about the time of full flower anthesis, between 7.30 am and 8.30 am. They moved from the tip of the petals to nectar chamber under the stamen (Fig. 7). At each time of observation, most often one hover fly was seen foraging on a flower. The hover flies are very swift in their foraging habit. Movement from the tip of the petals to the anthers and then to the nectar chamber for nectar, thus feeding lasts between 1 and 3 minutes. Hover flies are brisk, spending few minutes (1-3 minutes) on a male flower; after spending this time feeding, they fly and land on the petals of another flower most often on another males' flower. Rarely do they forage on female plants. Hover flies keep on doing this foraging till about 5.00 pm. They seem to be the potential pollinators.

Table 1. Average number and percentage of visits of different species of flies to male and female flowers of fluted pumpkin

Flower visitors	Male flowers		Female flowers	
	Number of visits	Percentage (%)	Number of visits	Percentage (%)
Hover fly (*Cheilosia* sp.)	25	86.20	0.2	100.00
Thick-headed fly (*Conopidae* sp.)	1	3.45	0	0
Fruit fly (*Drosophila* sp.)	1	3.45	0	0
Green fly (*Musca* sp.)	2	6.90	0	0
Total observation	29		0.2	

Ants were other visitors. Ants were always on studied plants, they moved round the whole plant. During the observations, they were seen on flowers of both male and female plants, but not seen feeding on nectar.

Honey bees (*Apis mellifera*) were seen on the field foraging on the flowers of other plants (Fig. 7). Throughout the period of observation, honey bees were not seen foraging either on male or female flowers of fluted pumpkin.

Discussion

The results of floral morphology from the current study confirmed that fluted pumpkin is a dioecious plant, with two imperfect flowers borne, on different plants. Contrary to previous reported rare occurrence of monoecious forms of the plant (Akoroda *et al*., 1990), no monoecious plant was observed in the hereby study, as well as in previous ones (Fayeun *et al*., 2012; Fayeun and Odiyi, 2015; Fayeun *et al*., 2016). Similarly, Ojeifo and Ajekenrenbiaghan (2006) disagreed with this report of Akoroda *et al*. (1990). Within the *Cucurbitaceae*, monoecy is common and dioecy is comparatively rare (Schaefer and Renner, 2011). Gender dimorphism is characteristic of genus *Telfairia*. The two main species (*T. pedata* and *T. occidentalis*) of this genus are dioecious (Odiaka and Schippers, 2004; Okoli, 2007). Both male and female flowers are symmetrical, pentasepalous, fimbriate and pentapetalous, but there are conspicuous differences between them. Flowers on staminate plants appear earlier and are more numerous than the flowers of pistilate plants. The male flowers are arranged on a simple pedunculate raceme inflorescence, with one long-pedicellate flower at the base of the inflorescence, which opens before others, while the female flowers are solitary. This is similar to the report of Odiaka and Schippers (2004). Each male flower has a cup shaped receptacle (thalamus) at the end of the pedicel. Inside this cup nectar is stored. This is lacking in pistilate flower.

Flowering in fluted pumpkin begins with the appearance of the male inflorescence, which opened in between 3 to 5 weeks after the appearance of the inflorescence, depending on genotype. The male flowers do dominate the flowering period with a ratio of about 1000:1 male to female flowers per plant. This observation

is in line with the findings published by Akoroda *et al*. (1990), where over 800:1 male to female were reported. Although flowering in male and female inflorescences began at different times, the peaks of flowering in both sexes almost overlapped, favouring female receptivity (Nepi and Pacini, 1993; Agbagwa *et al*., 2007). Fluted pumpkin male flowers that bloomed when inflorescences were 4 weeks old were usually bigger and produced more pollen grains and nectar, thus attracting more insect visitors. This is similar to *Cucurbita moschata* flowers where 2 weeks old flowers on inflorescences are bigger (Agbagwa *et al*., 2007). Anthesis occurs at dawn in both flowers. Flowers in male plants and petals in female plants drop at dusk. The anthers dehisced at anthesis in the male flowers.

Compared to the flowers of other cucurbits that have mostly bright yellow petals, the white to cream and deep purple colour of the petals of the fluted pumpkin flowers are less attractive. This might account for observed lack of visitation by butterfly, honey bee and other insects that are enticed by attractive colours. The colour of the fluted pumpkin flowers seems to favour hover flies than other pollinating insects. White and yellow flowers have been reported to attract hover flies (Sajjad and Saeed, 2010). Nevertheless, the slightly scented nectar and the caryophyllaceous shape of the flowers favour insect pollination. The caryophyllaceous throat provides shade for hover flies on sunny days. It seems the slightly scented nectar is the major attractant of visitors. This justified the abundance of visitors on the male flowers than on the female flowers. Since the pistilate flower lack nectar and pollen, it has no reward to offer its visitors. This may account for the high unsuccessful fruit set in female plants. Poor fruits set were reported in the crop also by Akoroda *et al*. (1990). According to Faegri and Pijl (1979), nectar and pollen are the major rewards in insect pollinated plants and are presented only at certain times. The major pollinator insects observed in this study on fluted pumpkin were hover flies; they have been previously reported as effective pollinators (Larson *et al*., 2001; Sajjad and Saeed, 2010).

Flower constancy is a very important aspect of pollinator effectiveness; it refers to the behaviour of pollinators focusing their attention on a certain type of flowers, regardless of others being available for foraging (Waser, 1986; Waser and Ollerton, 2006). Flower constancy is beneficial to pollination because the more constant a bee is to a given flower type, the higher are the chances that it will transfer appropriate pollen for successful fertilization (Adamson, 2011). From the current study it was obvious that honey bee might not be a pollinator of this crop in the study area. This observation is contrary to the report of Odiaka and Schippers (2004) saying that crop is pollinated by mostly bees of the genus *Trigona*. In the hereby experiment, bees were seen on the field foraging other flowers, but never landed on fluted pumpkin flowers. Bees have been known to be selective and constant in their forage. Honeybee *Apis mellifera* is known for its characteristic floral constancy (Cakmak *et al*., 2000).

Conclusions

In conclusion, the current study confirmed that fluted pumpkin is dioecious and floral structures differed significantly among the genotypes studied. Both male and female flowers are symmetrical, pentasepalous, fimbriate and non-bright (white to cream and deep purple) pentapetalous. Flowers in both gender flowers opened before dawn and closed by wilting at sun set. Anthers dehisced at anthesis of the male flower. The female flower lacks nectar and pollen, thus received fewer visitors than the male flower. Hover flies (*Cheilosia* sp.) are among the suspected pollinators of fluted pumpkin.

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