Floral Biology and Pollination Ecology of Cowpea (*Vigna Unguiculata* L. Walp)

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Abstract

Studies on the floral biology and pollination ecology of three varieties of cowpea (*Vigna unguiculata* L. Walp) were carried out. The varieties studied were Var. Oloyin, Var. Sokoto, and Var. Drum. Cowpea is a self pollinated crop which is encouraged by the arrangements of the floral parts. However, Insects visiting cowpea flowers have been implicated in the movement of pollen from one cowpea plant to another. Flower opening of cowpea begins between 6:00am and 6:30am and closes between 11:30am and 12:00pm. Pollen analysis showed similarities in the pollen morphology of the cowpea varieties. Moreover Var. Drum produced the highest number of pollen grains per flower in terms of pollen productivity.

Keywords: Flora biology, Pollination, *Vigna unguiculata*, Fabaccae, Nigeria

1. Introduction

Cowpea (V*igna unguiculata* L.Walp) belongs to the family Fabaceae. It is a grain legume grown in savanna regions of the tropics and subtropics with some 150-190 species. The cultivated cowpea is based on five so-called–groups (Cultigroups) namely; *unguiculata*, *sesquipedalis*, *textiles*, *melanophtalmus*, and *biflora*. (Westphall, 1974; Marechal *et.al*., 1978, Ng and Marechal, 1985). Its value lies in its high protein content (23-29%) with potentials for perhaps 35%, and its ability to fix atmospheric nitrogen, which allows it to grow on and improve poor soil (Steele, 1972). At present, this species is the second most important pulse crop (Marechal, *et al* 1978) and in Nigeria, it is generally cultivated for its seed (shelled green or dried), Pods and leaves which are consumed in fresh firm as green vegetables while snacks and mail meal dishes are prepared from the dried grain. All the plant parts used for food are nutritious, making it extremely valuable where many people cannot afford protein foods such as meat and fish. The seed is valued as a nutritional supplement to cereals and an extender of animal proteins (Kay, 1976; Coetzee, 1995). It is very palatable, highly nutritious and relatively free of metabolites or other toxins (Kay 1976, Quass, 1995). The rest of the cowpea plant, after pods are harvested, is also used as a nutritious livestock fodder. Medicinally, cowpeas are sacred to Hausa and Yoruba tribes; they are used in folk medicinal practices and are prescribed for sacrifices to abate evil and to pacify the spirits of sickly children. Hausa and Edo tribes use cowpea medicinally; one or two seeds are ground and mixed with soil or oil to treat stubborn bowel. Its diversity of uses, nutritive content and storage qualities has made cowpeas an integral part of the farming system in the West Africa region. Although cowpea plays a key role as food/food supplement in Nigeria where more than 90% of the country's 120 million people depend on this genus daily in one way or another, studies on its floral biology and pollination ecology are lacking. This study describes the floral biology and pollination ecology of three varieties of cowpea that are widely cultivated in Nigeria.

2. Materials and Methods

2.1 Source of Study Materials

The seeds of the three local varieties of cowpea (*Vigna unguiculata* L.Walp) used for this study were sourced from Agricultural Development Programme, Ministry of Agriculture, Ikare-Akoko. Ondo State, Nigeria. The seeds of the three local varieties used are namely; Var. Oloyin, Var. Drum, Var. Sokoto. The varieties are of different sizes, shapes and colour. The Var. Oloyin is slightly brown in colour Plate (a), Var. Sokoto is white with black spot around the helium Plate (b) while Var. Drum is deep brown in colour and bigger than the other two varieties Plate (c). Best seeds from the three varieties were sorted out for planting.

2.2 Planting materials and methods

The planting materials used were plastic pots, top soils, watering cans. Holes were made at the bottom of each pot to avoid flooding and maintain proper drainage system.

The pot evaluation method was used for planting. Plastic pots (with diameter and depth of about 10cm and 20cm respectively) were used. This method was reported to be reliable (Singh *et. al.* 1999). Fifteen plastic pots were filled with the top soils and arranged in an open field. The pots were arranged in rows, each row consisted of five pots representing each variety. Spacing between each row was about (25-30) cm and about (10-15) cm within row spacing. The cowpea seeds were planted with about 2-3 seeds in each pot in a depth of about 5cm and were

watered moderately and regularly. The field experiment was conducted at the Experimental farm, Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

2.3 Germination and growth rate

The planting date of the varieties were recorded, and as they began to germinate, the growth rate of each variety in each row were observed, measured and recorded as well. Seedlings from each pot were thinned to about 1-2 plants per pot after two weeks. The plants were watered regularly and data on the plant height were taken each day and later at weekly intervals for five weeks.

2.4 Data analysis

Data on the growth rate were statistically analyzed using Tukey's Honestly Significant Test. Means were compared at 5% probability and ANOVA tables provided.

2.5 Flower morphology and anthesis

The flower morphology was described using 15 inflorescences of each variety. The morphology of the floral parts were assessed and noted using Gill(1988) as a guide. Anthesis of the inflorescences of the varieties was monitored. Observations of the phenology of the different varieties commenced right from the beginning of the flowering until the development of pods. The time of anthesis initiation and termination in the flower of each variety, the time of flower opening and closing daily were also observed and documented.

2.6 Flower visitors

Flower visitors were monitored by direct observation in the field from 6.00 hrs to 15.00hrs throughout the period of study and voucher specimens were collected with an insect net for identification at the Department of Environmental Biology and Fisheries laboratory, Adekunle Ajasin University, Akungba-Akoko. They were thereafter classified either as pollinators or robbers (Dafni 1992, Inouye 1980). Different insects encountered on flowers were registered.

2.7 Collection of fresh flower samples

Mature flower heads of each variety of cowpea were collected. The flowers were stored in plastics vials containing glycerine to prevent desiccation and growth on samples.The floral parts were observed and the numbers of anthers produced per flower were recorded.

2.8 Pollen slide Preparation

Acetolysis method (Erdtman 1969) was used in preparing the pollen for analysis. The anthers collected were transferred from the vials to calibrated centrifuge tubes. This method involves the introduction of acetolysis mixture comprising of acetic anhydride mixed with concentrated sulphuric acid (H_2SO_4) in the ratio of 9: 1 to the samples in the plastic test tubes. The anthers were crushed using glass rods, with one glass rod for each sample to avoid contamination of one sample by the other.

Volume of the samples was determined and read and drops of glycerine was added and the samples (mostly pollen grains) were then transferred into storage vials from where they were mounted on slide.

2.9 Mounting and Counting

10µl of the sample was placed on a slide using micropipette and a cover slip was placed over it. Three slides were prepared for each variety. The slides were observed using X40 objectives lens and counting of the pollen grains was done with both X10 and X40 objectives lens depending on the size of the grain. The pollen in each of the three slides was counted and an average count determined.

Furthermore, pollen measurement for each variety was noted and a total of thirteen grains were measured for each variety.

3. Results

3.1 Habit and Floral Morphology

The species is an annual herb reaching a height of up to 80cm with a strong tap root and many lateral roots in the soil surface. Leaves are alternate and trifoliate. The first pair of leaves is simple and opposite. Leaves exhibit considerable variation in size (6-16 x 4-11 cm) and shape (lanceolate to ovate) and they are usually dark green in colour .The leave petiole is 5-25 cm long. The stems are striate, smooth or slightly hairy.

The flowers are arranged in racemose or intermediate inflorescence at the distal ends of 5-60 cm long peduncles. Flowers are borne in alternate pairs, with usually only two flowers per inflorescence. Flowers are conspicuous, self- pollinating, borne on short pedicels and the corollas may be white, dirty yellow pink, pale blue or purple in colour (Plate 2d). Flowers open in the early day and close at approximately midday. After blooming (opening once) they wilt and collapse.

4. Discussion

From germination through plant establishment to flowering, the varieties showed some morphological differences and similarities as well.

There were significant differences between the growth rates among the varieties with Var. Oloyin showing the fastest growth with gradual reduction in the second week (Table I). The study also showed that there was no significant difference between Var. Oloyin and Var. Drum from the second to the fifth week.

Table 2 showed the cumulative emergence rate of the three varieties. With equal planting depth of about 5cm, the earliest leaf emergence was observed in Var. Oloyin 3 days after planting with an average length of 1.5cm followed by Var. Drum with the same length a day after. There was no significant difference, $P \geq 0.05$ in the growth rate between Var. Oloyin and Var. Drum within the period of study as shown in Table 2 and Fig 1.

The growth rate in var. Drum as shown in Table 2 was more progressive than the other two varieties as it ranged between 2.0±0.5 and 33.6±1.5 giving a significant difference in the fifth week compared to the other varieties.

The growth forms of the three varieties vary from each other. Right from germination to flowering and fruiting period, Var. Oloyin maintained an erect growth form while the other two varieties were either trailing or crawling and climbing.

4.1 Anthesis

There was also variation in the flowering period of the three varieties. Flowering started thirty-nine days after planting with Var. Oloyin emerging as the first variety to flower, Var. Sokoto started to flower one hundred and forty seven days after planting and Var. Drum, one hundred and fifty five days after planting. (Table 3) showed details of the flowering date of each variety in each row.

4.2 Floral Morphology

The morphological characteristics of the inflorescences of the 3 varieties are the same and each inflorescence bears about 4-5 flowers on each variety and each flower produce nectar and pollen. The flowers are borne in multiple racemes; the flower stalks that arise from leaf axil have about two to three pods per peduncle and about four in some case.

The flower head samples collected from each variety showed that there are similarities in the colour, shape, and size of the flowers. The flowers have variable colours which range from white, cream, yellow or light purple to dark purple and the colours can be in different combinations. In all the varieties, flower opening is initiated between the hours of 6:00am - 6:30am and are closed between 11:30am-12:00pm. When the weather is hot and dry, the flowers close earlier compared to when the weather is cold and humid. After about one or two days of flower opening and closing, the flower wilts and fall off from the cowpea plant. This signifies that cowpea flowers does better or bloom under a cool and humid condition but the onset of hot and dry weather initiates flower closing. The stigmas are receptive over a short period of time and unfertilized flowers drop off within 24 hours after anthesis and the fertilized ovary may remain attached for 48 hours after anthesis.

4.3 Pollination Mechanism

Cowpea is self pollinating crop and usually pollination occurs in cowpea flower before they open. The enclosure of the pistil and stamen within the keel enhances self pollination. Pollen grains are transferred from the anther to the stigma and thus pollination occurs. However, Cowpea flowers produce nectars which attract insects to them. Various insects such as Butterflies, Houseflies (*Musca domestica),* Honeybees (*Apis mellifera*) were seen around the cowpea flower. Honeybee was observed to be the most prominent out of the various insects observed.

Apis mellifera was observed to start visitation to the flower immediately after flower opening was initiated. The bee lands on the wing of the flower thereby depressing it and allowing the stamens and stigma to be pushed out of the corolla. The bee then adopts a supine feeding posture with the head positioned towards the nectar chamber and collects nectar. After nectar feeding, it gradually withdraws from the chamber to its landing position and then flies away on to the next flower or away from the vicinity.

However, during this process of feeding, the hairs on the bee brush the anthers and pollen adheres to them and these pollen grains are transferred to another flower. Pollen grains of cowpea are heavy and sticky and could not be readily transferred by wind, therefore insects are responsible for transfer of pollen grains and consequently cross pollination in cowpea plant. *Apis mellifera* has been previously reported as good pollen collectors and as such; effective pollinators (Nepi and Pacini, 1993, Luo and Li, 1999).

4.4 Pollen Productivity

Pollen productivity refers to the quantity of pollen produced per anther as per flower or per inflorescence. Table 4 showed the pollen grain productivity per anther with Var. Drum producing the highest pollen grain per anther (10400) followed by Var. Sokoto (436) and Var. Oloyin (361). The high number of pollen grain production per anther per flower of Var. Drum may however be responsible for the high number of seed production as seen in the pod.

The average measurement of pollen from polar to equator showed that Var. Drum has the largest pollen size than the other two varieties (Table 5). The pollen morphology showed some similarities in the exine structure (i.e. pattern) with all the varieties showing recticuloid pattern (Plate 3). The pollen grains are circular in nature and

this can be related to a structural adaptation for effective pollination by insects (Gemines, 1991; Edeoga *et.al*., 1996).

5. Conclusion and Recommendation

The study showed the differences and similarities between the three *Vigna* varieties right from the period of germination to the flowering period. Var. Oloyin was the first to germinate, produced flower and subsequently pods while Var. Drum recorded the highest pollen productivity. Consequently Farmers are advised to plant these varieties for commercial purpose due to the ease of production and fast maturity rate and for large seed production.

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Table 1. Growth rate in terms of Height at three times in each Week

WEEK	Oloyin	Sokoto	Drum
1	1.5±0.2a	0±0.0b	0±0.0b
	2.4±0.3a	0±0.0c	1.5±0.3b
	4.5±0.7a	0±0.0b	4.2±0.3a
2	7.1±1.0a	1.6±0.2b	7.6±0.6a
	16.9±1.0a	11.3±0.5b	15.3±1.8a
	19.7±1.7a	13.3±0.6b	16.9±2.2a
3	20.9±1.7a	14.3±0.5b	17.9±2.2a
	21.5±1.7a	15.0±0.6b	18.8±2.2a
	23.0±1.6a	16.5±0.6b	21.1±2.3a
4	24.5±1.8a	18.5±0.7b	23.3±2.3a
	26.4±1.7a	20.5±1.0b	26.3±2.9a
	29.0±1.8a	23.2±1.2b	29.9±3.1a
5	29.9±2.0a	25.6±1.3b	32.0±3.0a
	31.1±1.9a	28.0±1.7b	33,8±2.8a
	31.9±1.9a	30.1±1.7b	36.4±3.2a

Means in each row bearing the same letter are not significantly different at the 5 % level of probability by Tukey's Honestly Significant Test.

Table 2. Cummulative Height of the three Varieties Studied

WEEK	Oloyin	Sokoto	Drum
1	2.8±0.4a	0±0.0c	2.0±0.5b
2	15.1±1.5a	9.2±1.4b	13.4±1.3a
3	21.8±0.8a	15.3±0.4c	18.9±1.1b
4	26.6±0.9a	20.7±0.7b	26.1±1.4a
5	30.7±0.9b	27.8±0.9c	33.6±1.5a

Means in each row bearing the same letter are not significantly different at the 5 % level of probability by Tukey's Honestly Significant Test.

Table 3. Number of days of Flowering of each Variety

	Var.Oloyin	Var.Sokoto	Var.Drum
1st Plant	47	148	158
2nd Plant	45	149	156
3rd Plant	42	151	157
4th Plant	39	147	156
5th Plant	41	147	155
Average	42.8	148.4	156.4

Table 4. Number of Pollen grains per Anther per Flower

Vigna Varieties	Amount of glycerine mixture and residue	Pollen average in 10μl	Quantity of Pollen grains in 0.5 ml	Number of anthers used	Number of pollen grains per anther	Number of anthers per flower	Total number of pollen grains per flower
Var.Oloyin	0.5ml	361	18050	50	361	10	3610
Var.Sokoto	0.5ml	764	30560	70	436	10	4360
Var. Drum	0.5ml	1665	83250	80	1040	10	10400

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Table 5. Pollen Measurement and Morphology

Vigna Varieties	average measurement P/E	Exine pattern
Var. Oloyin	51.05µm / 50.22µm (Average) 40-62µm/42.5-62.5µm (Range)	Reticuloid
Var. Sokoto	57.25µm / 56.25µm (Average) 50-67.5µ / 47.5- 65µm (Range)	Reticuloid
Var. Drum	61.14µm / 59.16µm (Average) 37.5-75µm/32.5-60µm (Range)	Reticuloid

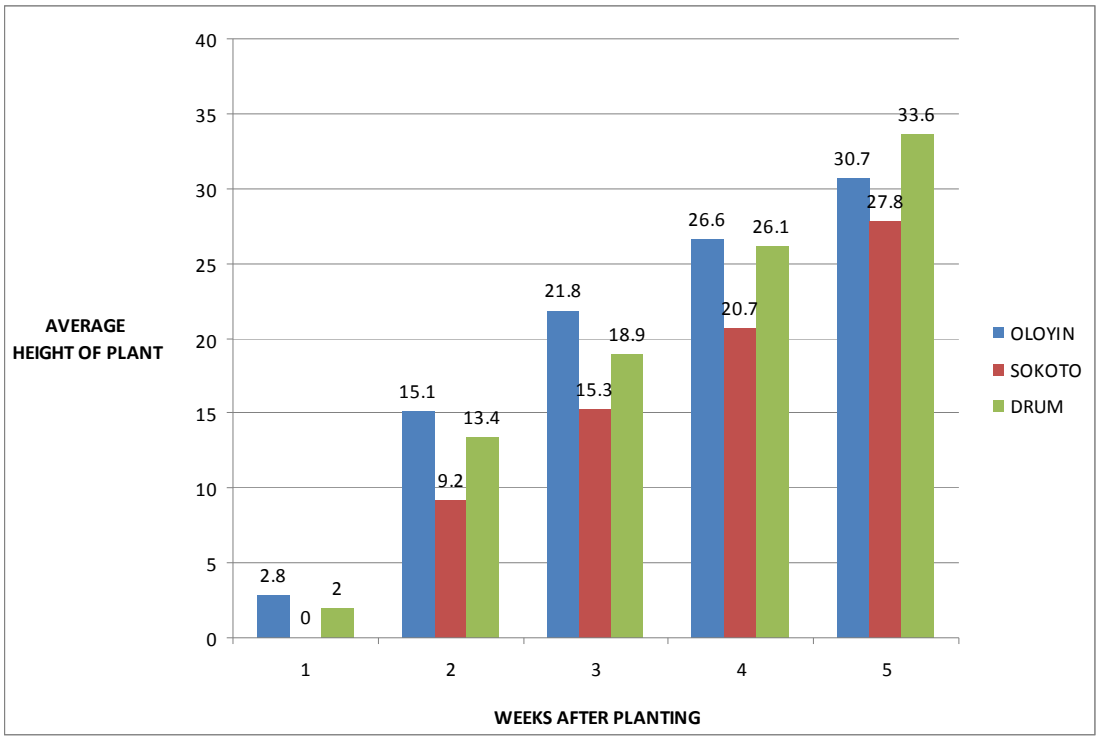

Figure 1. Comparative growth rate of cowpea varieties studied

Appendix I: Anova for cumulative height of the three Varieties studied

WEEK 1

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Sample	22.19267	2	11.09633	127.3002	1.67E-13	3.402826
Columns	26.69633	1	26.69633	306.2677	3.62E-15	4.259677
Interaction	22.19267	2	11.09633	127.3002	1.67E-13	3.402826
Within	2.092	24	0.087167			
Total	73.17367	29				

WEEK2

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	618.5327	2	309.2663	66.20043	1.7E-10	3.402826
Columns	141.267	1	141.267	30.2391	1.18E-05	4.259677
Interaction	10.514	2	5.257	1.125294	0.341088	3.402826
Within	112.12	24	4.671667			
Total	882.4337	29				

WEEK 3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	36.062	2	18.031	2.0738	0.147652	3.402826
Columns	88.752	1	88.752	10.20764	0.003888	4.259677
Interaction	1.194	2	0.597	0.068663	0.933824	3.402826
Within	208.672	24	8.694667			
Total	334.68	29				

WEEK 4

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	152.9927	2	76.49633	5.17479	0.013536	3.402826
Columns	203.8413	1	203.8413	13.78937	0.001083	4.259677
Interaction	3.492667	2	1.746333	0.118135	0.889089	3.402826
Within	354.78	24	14.7825			
Total	715.1067	29				

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ANOVA WEEK 5

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	100.026	2	50.013	2.550404	0.099009	3.402826
Columns	241.968	1	241.968	12.33912	0.001785	4.259677
Interaction	0.422	2	0.211	0.01076	0.989303	3.402826
Within	470.636	24	19.60983			
Total	813.052	29				

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Sample	2864.425	2	1432.213	8.211132	0.000379	3.042963
Columns	34690.85	15	2312.724	13.25926	1.590320	1.718757
Interaction	4982.671	30	166.089	0.952218	0.542614	1.518742
Within	33489.27	192	174.4233			

a b c

Plate 1. Samples of the three cowpea varieties

Plate 2. Parts of Cowpea plant showing (a) pod, (b) Leaf, (c) *Apis mellifera* collecting nectar on (d) flower.

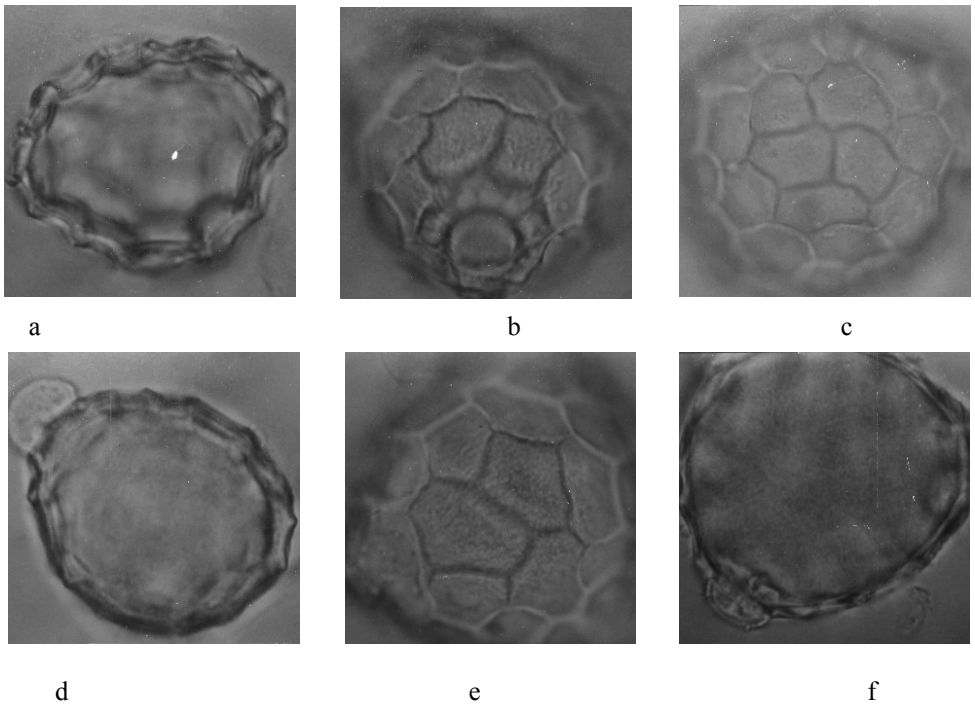

Plate 3. Photomicrographs of pollen of some selected varieties of cowpea, *Vigna unguiculata* L. (Walp)
a and b: Var. Oloyin showing exine and reticuloid structures,
c and d: Var. Sokoto showing reticuloid and exine structures,
e and f: Var. Drum showing reticuloid and exine structures

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