Seasonal Variation in the Nodulation and Yield Parameters of Cowpea, (*Vigna Unguiculata* l. walp)

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Abstract

The study evaluated seven varieties of cowpea, Vigna unguiculata (L.) Walp., namely: Ife Brown, IT98K-128-3 , IT98K-506-1, IT93K-452-1 and IT95K-1072-57, IT06K-149-1, and IT06K136 that were laid out in a complete randomized block design (CRBD) with seven replications for the production of root nodules. It comprised of two field trials (during the wet season and the dry season of 2021 cropping season respectively) that were carried out at an experimental garden in Agbor, Delta State. The rainy season plants were rain-fed while the dry season experiment was watered manually when the rainy season finally ended. The study established that nodulation was a primary determinant of the agronomic and yield characteristics of legumes. Consequently, genotypes with large number of nodules per plant recorded more peduncles, pods per plant, longer pod lengths and consequently gave better grain yield and were recommended for inclusion in further cowpea hybridization programmes in the region

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Introduction

Cowpea, V*igna unguiculata* (L.) Walp is one of the most important legume crops found in Sub-Sahara Africa, being predominantly cultivated for forage, green pods, and grains. Legumes belong to the Fabaceae family, the 3rd largest land plant family with above 770 genera and 19,500 species (Lewis, 2013). It has been distinguished as an excellent source of protein for both humans and livestock. It is predominantly consumed for its rich protein content. The grains contain about 23% protein and 57% carbohydrates while the leaves contain between 23 to 34% protein (Pule-Meulenberg, *et al*. 2010). According to Danso (1992), nitrogen is a key element required for plant growth and the symptoms of its deficiency in soils range from poor yields to crop failures.

V. unguiculata derives its significant nitrogen needs from the nodules attached to its roots. Nodules are therefore important for nitrogen fixation. Nitrogen fixing root nodules are the selective symbiont between legumes and leguminous bacteria.

Nodulation: Root nodules occur on the roots of plants (primarily Fabaceae) that associate with symbiotic nitrogen fixing bacteria. Biological nitrogen fixation reduces the use of mineral nitrogen in agriculture (Novotny *et al.*2010). Furthermore, Pule-Meulenberg, *et al*. (2010) documented that cowpea is strongly dependent on nitrogen fixation for its nitrogen supply in plant hybridisation programmes. Doyle and Luckow (2003) noted that under nitrogen-restricting conditions, nodule-forming plants form a symbiotic relationship with a host-specific strain of bacteria called *rhizobia*. Cowpea freely forms root nodules with some members of the *Rhizobiaceae* such as *Rhizobium and Bradyrhizobium* (Mpepereke, *et al*., 1996). In place of this, high nitrogen content blocks nodule formation as there is no benefit for the plant of forming the symbiosis.

Nodulation of legumes by rhizobia involves two main processes, namely, bacterial infection and nodule formation. According to Ren (2018), legume nodules have been distinguished into determinate and indeterminate types. The determinate nodules are found on tropical (sub) legumes such as those of the genera *Glycine* (soybean), *Phaseolus* (common bean), *Lotus* and *Vigna.* Determinate type of nodules loses meristematic activity shortly after initiation thus growth is due to cell expansion resulting in mature spherical shaped nodules. On the other hands indeterminate nodules are found on temperate legumes like *Pisum* (pea), *Medicago* (alfalfa), *Trifolium* (clover) and *Vicia* (vetch). These nodules maintain an active apical meristem that produce new cells for growth over the life of the nodules, resulting in the nodules having a general cylindrical shape. Because they are actively growing, indeterminate nodules manifest zones which demarcate different stages of development / symbiosis (Foucher and Kondorosi, 2000, Monahan-Giovanelli *et al*., 2006).

Sprent (2001) indicated that papilionoideae has the highest value of nodulating species, and produce all known types of nodules. Doyle and Luckow (2003) recorded that within legume nodules, nitrogen gas from the atmosphere is converted into ammonia, which is then assimilated into amino acids (the building blocks of proteins), nucleotides (the building blocks of DNA and RNA as well as the important energy molecule ATP), and other cellular constituents such as vitamins, flavones and hormones. This ability to fix atmospheric nitrogen makes legumes an ideal agricultural organism whose requirement for nitrogen fertilizer is reduced. The writers reported that the energy for splitting the nitrogen gas in the nodule comes from the leaf after photosynthesis. This product of the breakdown of sucrose is malate which acts as the direct carbon source for the *rhizobia.* Nitrogen fixation in root nodules is very oxygen sensitive. Obadoni *et al.* (2003), added that the process is also highly influenced by phytohormones in V*igna*. Legume nodules have an iron-containing protein called leghaemoglobin, to facilitate the conversion of nitrogen gas to ammonia.

The first step of nodule formation begins with the leguminous bacteria receiving a signal from the host plant. The signal molecules are flavonoids and their glycosides. The signal molecules trigger the expression of the bacterial gene required for nodulation (Yoneyama and Natsume, 2010). When induced, the nodulation genes cause leguminous bacteria to trigger the production of lipochitooligosaccharide nod factors. Eckardt (2009), cited that Hirsch *et al.* (2009) had noted the interaction of two GRAS domain proteins Nodulation Signalling pathway (NSPI and NSP2 respectively) to form a complex that binds directly to form a specific promoter region of Nod factor inducible genes in *Medicago truncatula.* They demonstrated that the interaction between NSP1 and NSP2 is enhanced by Nod factor perception and is necessary for proper development of nodules. According to Monahan-Giovanelli *et al.,* (2006) when the Nod factor is sensed by the root, a number of biochemical and morphological changes occur: cell division is triggered in the root to create the nodule and the root hair growth is redirected to wind around the *rhizobia* several times until it completely encapsulates one or more *rhizobia*. The *rhizobia* encapsulated divide several times to form a micro colony. From this micro colony, the *rhizobia* enter the developing nodule through a structure called an infection thread, which grows through the root hair into the basal part of the epidermis cell, and onwards into the root cortex; they are then surrounded by a plant-derived membrane and differentiate into bacteria that fix nitrogen. Fernandez and Miller (1985), had shown that the number of nodules per plant was heritable and may be used as criteria for selection in crop improvement programmes.

Problems of the study: The conventional cowpea varieties which are cultivated for their high protein content in the savannas of Northern Nigeria are alien to the southern Nigerian rainforest climates where only some mottled land race varieties which are highly susceptible to pests, with less appeal to consumers is cultivated in intercrops with cereals, yams and cassava. Efforts are being made to utilize existing variability in genetic and biochemical compositions in different accessions of the crop plant to improve both their nutritional qualities as well as their economic yield potentials. Nodulation in plants is a determinant of both biological and economic yield.

The study intends to evaluate the agronomic importance of nodulation to biological and economic yield in cowpea varieties grown in Agbor, Delta State in order to create new opportunities and provide alternative crops for the local farmers, give the consumers access to new and novel home-grown foods, and increase the bio-diversity of crops grown by indigenous agriculturists in Delta State.

Objectives of the Study: The overall aim of the study is to determine the level of nodulation among selected cowpea varieties and use it as a basis for selection of genotypes that would exhibit high grain yield.

Field experiments: The experiments comprised of seven cowpea varieties of *Vigna unguiculata (L.) Walp.* namely: Ife Brown, IT98K-128-3 , IT98K-506-1, IT93K-452-1 and IT95K-1072-57, IT06K-149-1, and IT06K-136 (plate1) that were laid out in a complete randomized block design (CRBD) with seven replications. In the CRBD, the total number of plots was first determined by the product of the number of cowpea varieties and the number of replications. Thereafter a plot number was assigned to each experimental plot following which each cowpea variety was assigned at random. The rain-fed wet season experiment was planted in the month of April while the dry season experiment was planted in October, 2021, respectively.

The seven cowpea varieties were randomly assigned to the plots in each replicate by the lottery technique. The plot measured 2x2 meters per replicate, per variety. Cowpea was planted at intra-row spacing of 0.5 meters and between rows of 2 meters per replicate at a planting depth of 2-5cm. 3 seeds were planted per hole and was later thinned to 2 after emergence.

Data collection: The study collected data on the mean number of pods per peduncle, pods length, nodules per plant, and the yield parameters which include number of seeds per pod, seeds per plant, 100-seed weight and grain yield per plant.

Number of pods per peduncle: Five mature plants were randomly selected and the number of mature pods on each peduncle just before harvest were counted and recorded.

Pod length: The lengths of pods (cm) were determined by measuring selected pods on each plant with a ruler.

Nodules per plant: A plant was carefully uprooted from each replicate and the nodules on the roots were counted and recorded.

Number of seeds per pod: Five pods were randomly selected from each replicate and the pods were opened and the number of seeds per pod counted and expressed as a mean.

Number of seeds per plant: Five plants were randomly selected from each replicate and the mature dried pods collected. The pods were opened and the mean number of mature seeds was counted.

100 – seed weight:100 healthy, unblemished seeds per plant were obtained from each replicate and then weighed using an electronic weighing balance. The values were then recorded in grams.

Grain yield per plant: The grain yield per plant was calculated as the product of the number of pods per plant and seeds per pod, multiplied by 100- seed weight, divided by 100 (pod/plant x seed/pod x 100-seed weight /100). The values were expressed in grams. The values in grams were further converted to yield in kg/ha as follows: -

Size of each replicate = 2 m^2 x 2 m^2= 4m^2

4m^2 Yields (Y) = Y/ 1000 (to convert yield in gram to kilogram)

1 $m^{2\ will}$ yield Y/1000 x ¼ = Y/4000

10,000 m^2 = 1 hectares

Therefore, yield in hectares = Y/4000 x 10,000/1

= Y x 2.5 kg/ha.

Results and discussion

Table 1: Agronomic characteristics of seven cowpea genotype grown in the wet season

Genotypes	Nodules per plant	Pod per peduncle	Pod per plant	Pod length
Ife Brown	5.79	2.14	11.86	14.67
IT93K-452-1	7.71	2.07	14.36	13.57
IT98K-506-1	7.93	2.21	12.71	14.96
IT95K-1072-57	7.21	2.07	14.0	15.0
IT98K-128-3	8.21	2.21	15.29	13.99
IT06K-136	7.21	2.21	11.5	14.58
IT06K-149-1	5.71	2.21	15.79	15.86
Decision	NS	NS	NS	NS

Values are expressed as mean

Table2: Agronomic characteristics of seven cowpea genotype grown in the dry season

Genotypes	Nodules per plant	Pods per peduncle	Pods per plant	Pod length
Ife Brown	3.29	2.14	3.86[a]	10.84
IT93K-452-1	2.86	2.00	2.86[b]	11.98
IT98K-506-1	2.86	2.00	2.14[b]	11.43
IT95K-1072-57	3.86	2.00	2.86[b]	12.73
IT98K-128-3	4.00	2.14	2.71[b]	12.33
IT06K-136	3.14	2.00	2.43[b]	11.18
IT06K-149-1	3.43	2.00	2.57[b]	11.14
Decision	NS	NS	S	NS

Values are expressed as mean

Table 3: Seasonal variation in agronomic and yield parameters studied

	Wet Season		Dry Season		Total	
	Mean	SD	Mean	SD	Mean	SD
Nodules per plant	7.11	3.36	3.35	1.28	5.86	3.35
Pods per peduncle	2.16	0.37	2.04	0.20	2.12	0.33
Pods per plant	13.64	6.26	2.78	0.92	10.02	7.26
Pod length (cm)	14.66	2.12	11.66	2.18	13.66	2.56
Seeds per pod	11.09	2.35	4.71	1.95	8.96	3.74
Seeds per plant	243.98	860.96	12.95	6.48	166.97	710.24
100-Seed weight(g)	19.01	1.98	15.01	2.60	17.68	2.90
Grain yield per plant (kg/ha)	71.74	14.64	3.78	1.25	30.91	12.11

Values are expressed as mean $\pm$ SD

Nodules per plant; The number of nodules per plant varied from 5.71 to 8.21 across genotypes of the wet season (Table1) with a mean of 7.11$\pm$ **3.36** nodules per genotype (Table 3). Nodules among the dry season genotypes varied from 2.86 to 4.00 with a mean of 3.35$\pm$1.28 nodules per genotype (Table 3). Nodulation was not significant ($p>0.05$) among the cowpea varieties grown in both seasons (Tables 1 and 2). Nodules were more pronounced in IT98K-128-3 in both seasons with 8.21 and 4.00 nodules respectively (Tables 1 and 2). The least number of nodules were recorded in Ife Brown (5.79) and IT06K-149-1 (5.71) (wet season, Table 1) as well as in IT93K-506-1 (2.86) and IT93K.452-1 -2.86) (dry season, Table 2) respectively. Nodules are important for nitrogen fixation. According to Danso (1992), nitrogen is a key element required for plant growth and the symptoms of its deficiency in soils range from poor yields to crop failures. Nodules were more abundant among the rain-fed genotypes.

Pods per peduncle; The mean number of pods per peduncle for the wet season cowpea genotypes varied from 2.07 to 2.21 (Table 1), while the mean number of pods per peduncle for the dry season cowpea genotypes ranged from 2.00 to 2.14 (Table 2). Seasonal means for the wet season plants was 2.16 $\pm$ 0.37, while the mean number of pods per peduncle for dry season plants was 2.04 $\pm$ 0.20 (Table 3). Pods per peduncle was not significant across genotypes and seasons ($p>0.05$) (Table 1and 2). Highest mean number of pods per peduncle of 2.21 each was recorded in IT98K-506-1, IT98K-128-3, IT06K-136 and IT06K-149-1 among the wet season genotypes (Table 1). However, Ife Brown (2.14) and IT98K-128-3 (2.14) (Table 2) recorded the highest mean number of pods per peduncle among the dry season cowpea genotypes. The mean number of pods per peduncle recorded in this study was in agreement with Edeh and Igberi, (2012) who noted that the peduncles of cowpeas carry just one or two pods and sometimes none.

Number of pods per plant; The mean values of number of pods per plants for the wet seasons ranged from 11.50 to 15.79 (Table 1), while those of the dry season varied from 2.14 to 3.86 (Table 2). Seasonal means were 13.64$\pm$ 6.26 (wet season) and 2.78$\pm$ 0.92 (dry season) respectively (Table 3). Pods per plant were not significant among the wet season among cowpea genotypes ($p>0.05$) (Table 4) whereas significant differences were recorded among crops of the dry season ($p<0.05$) (Table 5). The highest number of pods was recorded for IT06K-149-1 (15.79), and IT98K-128-3 (15.29), followed by IT93K-452-1 (14.36), IT95K-1072-57 (14.00), (wet season, Table 2). On the other hand, Ife Brown (3.86) recorded the highest number of pods per plant in the dry season, followed by IT93K452-1 (2.86) and IT95K1072-57 (2.86) and IT98K-28-3 (2.71) respectively (Table 2). Seasonal mean was 13.64 $\pm$ 6.26 for the wet season plants compared to 2.78 $\pm$ 0.92 for the dry season plants (Table 3). The study noted that the cowpea genotype, IT06K-149-1 in the wet season produced the highest number of pods (15.79) (Table 1). Cowpea plants with good growth rate, shoot and root elongation and higher number of branches produced more pods than those with less branches. This was in agreement with Fawole (1986), who reported that higher number of branches are produced by the branching varieties of cowpea which consequently produce higher number of pods per plant than the non-branching varieties.

Pod length (cm)

The mean values of the pod length are represented in Tables 1 and 2 respectively .They vary from 13.99 to 15.86 cm (wet season crops, Table 1) and also ranged from 10.84 to 12.73 cm (dry season, Table 2). Seasonal mean was higher for the wet season plants (14.66 $\pm$ 2.12 cm) compared with 11.66 $\pm$ 2.18 cm recorded by the dry season plants (Table 3). Longest pods in the wet season were recorded in IT06K-149-1 (15.86 cm) and IT95K-1072-57 (15.00cm) respectively (Table 1). Among the dry season genotypes, pods were longest in IT95K-1072-57 (12.73 cm) and IT98K-128-3 (12.33 cm) respectively (Table 2). Pod length was not significant across the cowpea genotypes as well as in the seasons ($p>0.05$). In this study nodulation showed inverse correlation with pod length. For instance, IT06K-149-1 with the lowest number of nodules in the wet season produced the longest pods. Pod length is an important determinant of seeds per plant and grain yield. However pod length may fail to correlate with number of seeds per pod, as a longer pod may not necessarily mean more seeds. In this study the cowpea genotypes with the longest pod (IT06K-149-1, 15.86 cm) did not have the highest number of seeds.

100-Seed weight (grams)

The mean values for 100-seed weight varied from 17.10 to 21.56 g across cowpea genotypes grown in the wet season (Table 4). Also, the mean values for the dry season genotypes on 100-seed weight varied from 12.63 to 19.61 g (Table 5). Seasonal means were 19.01 ± 1.98 g (wet season) and 15.01 ± 2.60 g (dry season) respectively (Table 3). Among the wet season cowpea genotypes,

Ife Brown recorded the highest seed weight (21.56g), followed by IT06K-136 (20.04g), while the least was recorded in IT93K-452-1 (17.1g) (Table 4). Among the dry season genotypes, Ife Brown (19.61g) also recorded the highest seed weight, followed by IT98K-506-1 (15.33g), while the least value was recorded in IT98K-128-3 (12.63g) (Table 5). The cowpea genotypes showed significant differences ($p<0.05$) in 100- seed weight in both seasons (Tables 4 and 5). Comparatively, seeds from the wet season genotypes had higher seed weight than those of the dry season. Noggle and Fritz (2006) recorded that temperature and radiant energy fluctuations and moisture stress during flowering have been found to modify fruit and seed development. The cowpea genotypes of the dry season experienced varying degrees of these stressors which probably modified their pattern of fruit and seed development resulting to poor pod filling, low seeds per plant and low seed weight respectively.

Grain yield per plant (kg/ha): Seasonal means were 71.74 ± 14.64 kg/ha (wet season) and 3.78 ± 1.25kg/ha (dry season) (Table 3). Thus, grain yield was generally higher among genotypes of the wet season than those of the dry season. However, there was no significant differences among genotypes grown in the wet season, and those grown in the dry season respectively (Tables 4 & 5). This pattern of yield shown by cowpea genotypes of either season was in agreement with the citations of Fawole (1986), Noggle and Fritz (2006).

Table 4: Yield characteristics of seven cowpea genotypes grown in the wet season

Genotypes	Seeds per pod	Seeds per plant	100-seed weight	Grain yield per plant
Ife Brown	10.14	126.68	21.56^{a}	15.16
IT93K-452-1	9.95	144.53	17.1^{d}	9.16
IT98K-506-1	11.89	765.99	19.07^{bc}	9.18
IT95K-1072-57	11.8	173.53	18.08^{cd}	11.31
IT98K-128-3	11.49	176.3	17.81^{d}	15.7
IT06K-136	10.49	125.23	20.04^{b}	8.77
IT06K-149-1	11.85	195.58	19.41^{b}	11.84
Decision	NS	NS	S	NS

Table 5: Yield characteristics of seven cowpea genotypes grown in the dry season

Genotypes	Seeds per pod	Seeds per plant	100-seed weight	Grain yield/plant
Ife Brown	4.36^{bc}	16.33^{b}	19.61^{a}	42.84
IT93K-452-1	2.79^{d}	7.87^{d}	13.76^{bc}	32.11
IT98K-506-1	3.97^{c}	8.61^{d}	15.33^{b}	248.88
IT95K-1072-57	3.46^{cd}	9.93^{cd}	13.94^{bc}	42.39
IT98K-128-3	5.45^{b}	14.91^{bc}	12.63^{c}	32.50
IT06K-136	4.54^{bc}	11.18^{bcd}	14.79^{b}	34.11
IT06K-149-1	8.42^{a}	21.85^{a}	15.03^{b}	54.05
Decision	S	S	S	NS

Values are expressed mean.

Means followed by the same alphabets are not significantly different at 5% level of probability by the Duncan

Multiple Range Test.

Conclusion

The study recorded that nodules per plant, pods per plant, pods per peduncle, and pod length did not show significant differences among the cowpea varieties. Nodulation was a primary determinant of the agronomic characteristics of legumes. Consequently, some genotypes with large number of nodules per plant produced more peduncles, pods per plant, longer pod lengths and consequently gave better grain yield. An efficient system of nodulation in cowpea hybridization is therefore very strategic for sustainable biological and economic yield potentials.

Recommendations

From the study, high number of nodules 7.21 were recorded among IT06K-149-1(5.71), IT98K-128-3 (8.21,), IT93K-452-1 (7.71), IT95K-1072-57 (7.21,), with corresponding high mean number of pods per plant of 15.79, 15.29, 14.36, and 14.0 respectively. Furthermore, longest pods were recorded in IT06K-149-1(15.86 cm) and IT95K-1072-57 (15.00 cm). These agronomic characteristics are positive indicators of high productivity in cowpea. However, grain yield were highest among Ife Brown, IT98K-506-1, IT95K-1072-57 and IT06K-149-1. The above cowpea genotypes are therefore recommended for further hybridization due to their potential for quality biological and economic yield.

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