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Phenotypic Variation in Reproductive Traits of Forty Six Clones of *Gongronema latifolia* Benth. from Southeastern Nigeria

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ABSTRACT

This study was carried out to identify phenotypic variations in the reproductive traits of forty six clones of *Gongronema latifolia* obtained from different localities in southeastern Nigeria. Significant phenotypic variations were observed in the reproductive traits of the species. Clones obtained from close habitats showed distinct variation in the expression of their reproductive characters. For example, EBS 015 and EBS 016 were collected from Ebonyi State and they were directly opposite each other with respect to floral traits and seed fill capability of the follicles. Days to flowering in the species was observed as the most important discriminating trait as it influenced the quantity and quality of seeds produced by clones in a follicle. Clones ENS 001, ENS 008, EBS 015 and IMS 020, which initiated flowering earlier, had a higher number of filled seeds in their follicles and a higher seed weight. Clones IMS 025 and IMS 026 flowered early and also retained highest number of filled follicles for the two years of follicle harvest. On the contrary, clones ABS 043, ABS 042 and ABS 041 had longer days to flowering and retained only one or two filled follicle(s) per inflorescence for the same period. Three floret colors that have not been reported in literature including yellowish green, all yellow and purple were identified in the species. Our data clearly supports that *G. latifolia* could be differentiated into different genetic subspecies based on reproductive traits of flower color, flowering habit (early or late), seed filling and seed weight and that these are reproductive traits of interest to a breeder.

Keywords: environment, flowers, follicles, phenotype, seeds

INTRODUCTION

Gongronema latifolia Benth. (Asclepiadaceae) is native to West Africa and is found as far South as Northern Zimbabwe (Nielsen 1965). It is called *utazi* in southeastern Nigeria where it is available in virgin forests and used as a leafy vegetable. It is a good source of protein, vitamins, iron and minerals (Okafor 2005). The leaves can be harvested and eaten green (unprocessed) by diabetic and hypertensive patients to treat themselves (Etukudo 2003). The plant is also useful in the treatment of cough, malaria, constipation, catarrh/cold and typhoid (Okafor 1979; Agbo *et al.* 2005).

G. latifolia propagates by means of runner stems even though it is a climber. Runner stems can develop new vines that can be cut and propagated because it develops roots at the point of growth of a new vine. The plant can also be propagated vegetatively by stem cuttings (Agbo and Obi 2006). The plant produces flowers between 14 to 24 months after establishment. The flowers develop on the axils of the growing stems in the form of an inflorescence. Seeds developed from the flowers are a good source of commercial propagation of the plant (Agbo and Obi 2007). Seeds from the follicles can show high (80–100%) germination percentages. In the natural habitat (forest), the plants shade off their leaves as well as some vines during the dry season (December-January). They grow new leaves and shoots by February and flower between February and May every year.

It has been observed by local farmers (pers. comm.) that *G. latifolia* species exhibit phenotypic variations in floral and follicle coloration shapes and sizes. Variations that can be observed within individuals of the same population are based on environmental modifications, genetic recombination and nutrition (Stebbins 1950). Ellison *et al.* (2004) attributed morphological variation in characters of plants of the same species in different areas to be a result of phenotypic reactions to local environmental conditions, genetic variation and evolution.

Little is known in the literature about the genetic variability of *G. latifolia* clones in virgin forests in Nigeria and is therefore, unclassified. Knowledge of the genetic variation in floral and follicle shapes and colors of *G. latifolia* clones is important for use in breeding programmes and for conservation purposes. Phenotypic evaluation of the clones could be used to group them and for subsequent selection of progenitors that might constitute a new breeding population. The objectives of the present study were to identify the phenotypic variations in the flowers and follicles of 46 clones and to identify the quantitative and qualitative characters of interest to plant breeders.

MATERIALS AND METHODS

Stem cuttings of 46 clones of *G. latifolia* were sampled from 17 localities in different states of southeastern Nigeria in February, 2004 (**Fig. 1, Table 1**). The sampled stem cuttings were used to establish a germplasm garden at the Department of Crop Science Research Farm, University of Nigeria, Nsukka, in April, 2004 following the method for vegetative propagation of the species as described by Agbo and Obi (2006). The plants were transplanted to the field on a clone-to-row basis at a spacing of 1 m × 0.5 m. Three rows of 10 plants each were planted in each plot. The field layout was randomized complete block design with three replications. The plants were staked with bamboo of about 2 m high, weeded periodically and manured with poultry droppings at a rate of 10 tons/ha. By February, 2005, the established field started to flower. Data were collected on flower color, size and shape as follows: the length of the inflorescence stalk, the inflorescence length, number of branches of the inflorescence, number of florets per inflorescence, petal length, number of stamens per floret, color of inflorescence stalk and color of florets. Data on the follicle characteristics included number of matured follicles per inflorescence, length and thickness of the follicles, number of filled and non-

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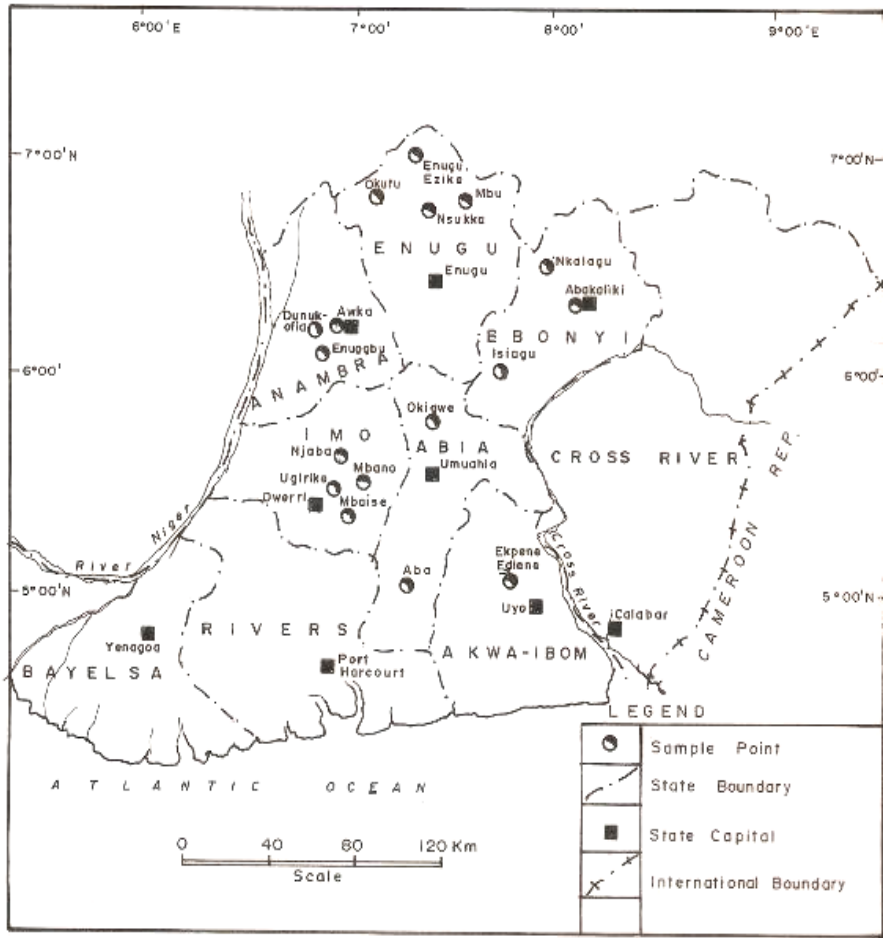

Fig. 1 Map of old South Eastern Nigeria showing sample points.

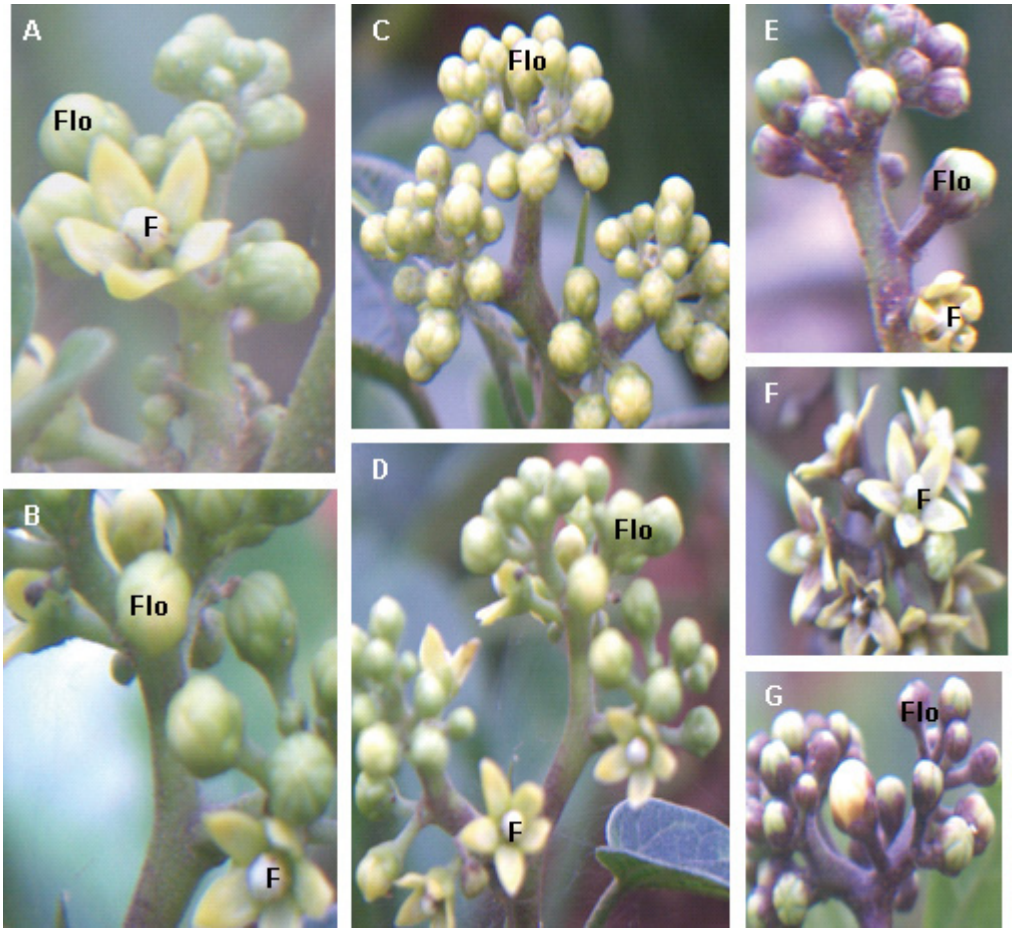

Fig. 2 Inflorescence stalks of different clones showing different colors as follows: (**A**) green with purple spots; (**B**) greenish purple; (**C**) green with purple patches; (**E**) purple with spots of green; (**G**) all purple. Florets of different clones showing different colors as: (**B**) greenish yellow; (**D**) yellowish green; (**A**) all yellow; (**F**) yellowish purple. Flo = inflorescent stalk; F = floret.

Table 1 Some collection data on clones of *G. latifolia* from 17 localities in southeastern Nigeria.

Clones code number	Locality name	State of locality
ENS 001	Nsukka	Enugu
ENS 002	Nsukka	"
ENS 003	Nsukka	"
ENS 004	Mbu	"
ENS 005	Mbu	"
ENS 006	Mbu	"
ENS 007	Mbu	"
ENS 008	Mbu	"
ENS 009	Enugu Ezike	"
ENS 010	Enugu Ezike	"
ENS 011	Okutu	"
ENS 012	Okutu	"
EBS 013	Nkalagu	Ebonyi
EBS 014	Nkalagu	"
EBS 015	Nkalagu	"
EBS 016	Abakiliki	"
EBS 017	Isi-agu	"
EBS 018	Isi-agu	"
EBS 019	Isi-agu	"
IMS 020	Njiaba	Imo
IMS 021	Njiaba	"
IMS 022	Njiaba	"
IMS 023	Ogirike	"
IMS 024	Ogirike	"
IMS 025	Mbaise	"
IMS 026	Mbaise	"
IMS 027	Mbaise	"
IMS 028	Mbano	"
IMS 029	Mbano	"
AIS 030	Ekpene Ediene	Akwaibom
AIS 031	Ekpene Ediene	"
AIS 032	Ekpene Ediene	"
AIS 033	Ekpene Ediene	"
ANS 034	Enuogbu	Anambra
ANS 035	Enuogbu	"
ANS 036	Enuogbu	"
ANS 037	Awka	"
ANS 038	Awka	"
ANS 039	Dunukofia	"
ANS 040	Dunukofia	"
ABS 041	Aba	Abia
ABS 042	Aba	"
ABS 043	Aba	"
ABS 044	Okigwe	"
ABS 045	Okigwe	"
ABS 046	Okigwe	"

filled seeds per follicle and 1000-seed weight. Days from transplanting to start of flowering and setting of follicle to maturity were recorded. Data on flowers were collected in 2005 and 2006 while data on follicles were collected in 2006 and 2007. Data on all the attributes were collected on six plants marked randomly within each clone in the middle of each plot for the periods 2005 to 2007. To show the level of phenotypic variations in the floral and follicle characters of the different clones, we used one-way-analysis of variance (ANOVA) to test the extent of variance of the clones' reproductive morphology. We performed principal component analysis (PCA) to identify important determinant traits for classifying the clones based on the reproductive morphology because of high correlations among the variables. We used genotype + genotype-by-environment interaction (GGE) biplot as put forth by Kang and Yan (2003) to identify high or low discriminating clones and traits using standardized data of the clones. The data were analyzed using computer software, Genstat Discovery edition 2.0 (2005). Least significant difference as outlined by Obi (2002) was used to separate the means.

RESULTS

The reproductive characters of the 46 clones of *G. latifolia* are presented in **Tables 2** and **3**. The levels of variation in the reproductive characters displayed wide diversity among the clones. Clone IMS 027 had a significantly ($P<0.05$) longer inflorescence and inflorescence stalk than any other clone in both years. The highest number of florets (340) per inflorescence was observed in clone EBS 015 and was significantly different from other clones with the exception of EBS 013 and AIS 033. There was no significant difference in the petal length and number of stamens per floret in all the clones for the two years of growth of the clones. The clones could be grouped into six distinct groups on the basis of the color of the inflorescence stalk and into four groups on the basis of color of the florets (**Table 4**). The representation of the clones into different flowering colors of the inflorescence stalk showed that 32, 8, 3, 1, 1 and 1 clones had green with spots of purple, greenish purple, green with patches of purple, all greenish, purple with spots of green and all purple colors, respectively (**Fig. 2**). On the other hand, the representation of the clones with respect to colors of florets revealed that 29, 11, 5 and 1 clone had greenish yellow, yellowish green, all yellow and yellowish purple color, respectively (**Fig. 2**). Clones 1MS 025 and EBS 018 had significantly ($P<0.05$) higher number of matured follicles per inflorescence (10 or 11) respectively in the two years. On the other hand, clones ANS 037 and ANS 039 had only one follicle per inflorescence for the two years. Clones EBS 017 and AIS 030 had significantly ($P<0.05$) longer follicles. Similarly, clone ENS 008 had thicker follicles. Four clones (ENS 009, EBS 018, IMS 028 and ABS 045) had seeds that did not fill. Clones EBS 017 and EBS 015 had significantly higher number of filled seeds per follicle. Furthermore, clone ENS 001 had a significantly higher seed weight for both years.

The correlation matrix of the reproductive traits highlights the levels of associations between some pairs of traits (**Table 5**). Positive and significant correlation existed between number of branches of an inflorescence and number of florets per inflorescence ($r = 0.576$; $n = 46$). Length of the inflorescence also had a positive and significant relationship with the length of the inflorescence stalk, the number of branches per inflorescence and the number of florets per inflorescence. In contrast, days to flower initiation of the clones had a negative and significant correlation with the number of matured follicles per inflorescence, follicle length, thickness and days to maturity of follicles. The highest positive correlation existed between seed weight and the number of viable seeds per follicle ($r = 0.707$: $n = 46$).

The four component axes retained in the principal component analysis (PCA) showed delineating reproductive characters in the clones (**Table 6**). PRIN1, which accounted for about 24% of the total variability in the reproductive characters, showed that days to flowering and maturity of follicles as well as number of viable seeds in a follicle had the highest eigen vector values on the axis. Similarly, the length of the inflorescence, seed weight and the number of non-viable seeds per follicle weighed most on PRIN 2, which accounted for 18% variability. Furthermore, PRIN 3 revealed that the number of matured follicles and follicle length could explain 14% variability in the reproductive traits of *G. latifolia* species. PRIN 4 showed that flower characteristics, petal length and the length of the inflorescence stalk accounted for 10% of variability in the species. The pattern of clonal distribution with respect to the reproductive traits is shown in **Fig. 3A**. The distribution of the clones is similar in both years and it depicts wide diversity of the clones. Clones IMS 027, EBS 013, and EBS 015, with longer vector lengths from the discriminating clones had high values in the flowering traits of the species. On the other hand, ANS 039, ENS 005, ANS 036 and ENS 003, with shorter vector length, had low values in the flowering traits of the species. However, clones EBS 016 and AIS 032 with relatively long vector lengths from the biplot origin but on the opposite side of high discriminating clones are discriminating clones with respect to low values in the flowering traits. About 50% of the population had an average

Table 2a Quantitative and qualitative characters of the inflorescence of the 46 *G latifolia* clones collected from southeastern Nigeria in 2005.

Clones	Length of inflorescence stalk (cm)	Length of inflorescence (cm)	№ of inflorescence branches	№ of florets per inflorescence	Petal length	№ of stamens per floret	Color of inflorescence stalk	Color of florets
ENS 001	1.10	6.93	4.00	185.30	0.35	4.00	GPP	GYY
ENS 002	1.00	7.90	4.00	166.60	0.35	4.00	GPP	GYY
ENS 003	1.06	6.90	4.00	136.00	0.35	4.00	GPP	GYY
ENS 004	0.80	9.00	4.30	183.30	0.35	4.00	GSP	GYY
ENS 005	1.00	6.86	4.00	107.60	0.35	4.00	GSP	GYY
ENS 006	2.05	12.21	4.60	201.30	0.35	4.00	GPP	YGG
ENS 007	1.13	7.16	5.30	208.60	0.36	4.00	GSP	GYY
ENS 008	0.40	8.43	6.30	192.50	0.35	4.00	GPP	GYY
ENS 009	1.10	9.93	4.30	201.30	0.35	4.00	GSP	YGG
ENS 010	1.00	8.90	5.30	208.00	0.34	4.00	GWP	GYY
ENS 011	1.56	9.63	5.00	160.30	0.34	4.00	GWP	GYY
ENS 012	1.56	9.33	5.00	150.60	0.35	4.00	GWP	GYY
EBS 013	2.36	13.36	6.00	310.00	0.35	4.00	GSP	GYY
EBS 014	2.06	12.24	5.40	160.80	0.35	4.00	GSP	GYY
EBS 015	2.06	14.83	6.00	340.60	0.36	4.00	GPP	GYY
EBS 016	1.33	9.42	4.00	161.60	0.33	4.00	GSP	GYY
EBS 017	1.33	10.46	4.00	170.00	0.37	4.00	GSP	YYY
EBS 018	0.46	9.50	5.00	192.50	0.36	4.00	GSP	YGG
EBS 019	0.33	8.53	4.60	162.00	0.35	4.00	PSG	YGG
IMS 020	1.10	10.93	3.60	201.00	0.35	4.00	PPP	YPP
IMS 021	0.53	10.30	4.30	194.60	0.35	4.00	GSP	GYY
IMS 022	1.13	13.21	4.00	196.00	0.35	4.00	GSP	GYY
IMS 023	0.80	12.50	4.60	207.00	0.34	4.00	GSP	GYY
IMS 024	1.33	10.43	3.60	194.60	0.36	4.00	GSP	GYY
IMS 025	0.86	13.63	4.30	240.20	0.35	4.00	GSP	GYY
IMS 026	0.43	13.23	5.60	271.60	0.35	4.00	GSP	GYY
IMS 027	3.46	16.20	5.60	222.00	0.35	4.00	GGG	YGG
IMS 028	1.10	10.93	5.30	230.00	0.35	4.00	GSP	GYY
IMS 029	0.83	9.42	5.00	206.30	0.35	4.00	GSP	GYY
AIS 030	2.00	10.36	4.00	208.60	0.35	4.00	GSP	YYY
AIS 031	2.10	16.06	4.60	220.00	0.35	4.00	GSP	YYY
AIS 032	1.60	9.30	4.30	172.30	0.33	4.00	GSP	YYY
AIS 033	1.20	10.50	5.00	289.60	0.36	4.00	GSP	YYY
ANS 034	1.60	9.23	4.60	166.60	0.35	4.00	GSP	YGG
ANS 035	1.20	10.30	5.00	274.30	0.35	4.00	GSP	YGG
ANS 036	0.80	7.16	4.00	107.60	0.35	4.00	GSP	YGG
ANS 037	0.50	8.43	4.30	192.50	0.35	4.00	GSP	YGG
ANS 038	1.50	8.00	4.30	186.00	0.34	4.00	GSP	YGG
ANS 039	1.11	6.93	4.00	172.30	0.36	4.00	GSP	GYY
ANS 040	1.10	8.73	5.30	264.00	0.35	4.00	GSP	YGG
ABS 041	1.00	8.00	4.30	193.40	0.35	4.00	GSP	GYY
ABS 042	0.86	10.30	5.30	260.20	0.35	4.00	GSP	GYY
ABS 043	0.80	9.30	4.00	170.80	0.35	4.00	GSP	GYY
ABS 044	0.43	12.36	4.60	252.40	0.35	4.00	GSP	GYY
ABS 045	0.46	10.46	4.60	230.00	0.35	4.00	GSP	GYY
ABS 046	1.06	10.93	4.60	210.00	0.35	4.00	GSP	GYY
F-LSD $_{(P<0.05)}$	0.17	3.22	0.70	59.93	-	-		

GGG = all green; GWP = green with patches of purple; GSP = greenish with spots of purple; GPP = greenish purple; GYY = greenish yellow; PSG = purple with spots of green; PPP = all purple; YGG = yellowish green; YPP = yellowish purple; YYY = all yellow. n = 138 for each trait (46 clones × 3)

value of the flowering traits level and were thus concentrated at the central portion of the biplot. However, there was a closer concentration of the clones in 2006 which could be attributed to further acclimatization of the clones to the environment. Similarly, **Fig. 3B** depicts the distribution of the clones with respect to the follicle traits. Clones IMS 025, AIS 032, IMS 026, EBS 015, ENS 008, IMS 020 and ENS 001 are distinctly high in most of the characters with the exception of days to flowering and the number of non-viable seeds. On the other hand, three clones, including ABS 043, ABS 042, and ABS 041, are distinctly high in days to flowering and low in most other traits. Furthermore, clones ABS 045, ENS 009 and EBS 018 had a high number of unfilled seeds and a low value of other traits. The majority of clones had average follicle traits. The clones had a similar distribution of their follicle traits in the biplot in 2007 with a relatively high concentration of average clones due to acclimatization to the environment.

DISCUSSION

The result of this study illustrates that morphological variation in reproductive traits of the species is primarily associated with clonal genetic constitution and the environment. The negligible change in form and color of traits from their primary habitats and Nsukka, where they were grown, and in the three years of growth indicated the low level of different environments (primary habitat and place of research) to the performance of the clones. However, flowering in the species that fell specifically within February to May during

Table 2b Quantitative and qualitative characters of the inflorescence of the 46 *G latifolia* clones collected from southeastern Nigeria in 2006.

Clones	Length of inflorescence stalk (cm)	Length of inflorescence (cm)	№ of inflorescence branches	№ of florets per inflorescence	Petal length	№ of stamens per floret	Color of inflorescence stalk	Color of florets
ENS 001	1.05	6.90	4.00	180.20	0.35	4.00	GPP	GYY
ENS 002	1.00	7.92	4.00	169.20	0.35	4.00	GPP	GYY
ENS 003	1.02	6.91	4.30	139.00	0.35	4.00	GPP	GYY
ENS 004	0.83	9.02	4.30	186.30	0.35	4.00	GSP	GYY
ENS 005	1.00	6.90	4.00	110.30	0.35	4.00	GSP	GYY
ENS 006	2.03	12.10	4.60	210.60	0.35	4.00	GPP	YGG
ENS 007	1.14	7.76	5.00	221.60	0.36	4.00	GSP	GYY
ENS 008	0.60	9.00	6.00	200.30	0.35	4.00	GPP	GYY
ENS 009	1.11	9.96	4.30	206.30	0.35	4.00	GSP	YGG
ENS 010	1.00	9.01	5.30	210.00	0.34	4.00	GWP	GYY
ENS 011	1.33	10.00	5.00	170.30	0.35	4.00	GWP	GYY
ENS 012	1.56	9.63	5.00	160.60	0.35	4.00	GWP	GYY
EBS 013	2.30	13.06	6.00	308.00	0.35	4.00	GSP	GYY
EBS 014	2.03	12.20	5.40	160.30	0.35	4.00	GSP	GYY
EBS 015	2.16	14.80	6.00	336.50	0.36	4.00	GPP	GYY
EBS 016	1.33	9.45	4.00	162.30	0.33	4.00	GSP	GYY
EBS 017	1.32	10.42	4.00	170.00	0.36	4.00	GSP	YYY
EBS 018	0.50	9.52	5.00	196.30	0.35	4.00	GSP	YGG
EBS 019	0.36	8.60	4.60	162.00	0.35	4.00	PSG	YGG
IMS 020	1.05	10.96	3.60	206.30	0.35	4.00	PPP	YPP
IMS 021	0.60	10.33	4.30	196.80	0.35	4.00	GSP	GYY
IMS 022	1.03	13.20	4.00	196.00	0.35	4.00	GSP	GYY
IMS 023	0.82	12.48	4.60	208.00	0.34	4.00	GSP	GYY
IMS 024	1.32	10.45	3.60	196.20	0.35	4.00	GSP	GYY
IMS 025	0.88	13.60	4.30	236.30	0.35	4.00	GSP	GYY
IMS 026	0.46	13.20	5.60	273.30	0.35	4.00	GSP	GYY
IMS 027	3.42	16.32	5.60	232.00	0.35	4.00	GGG	YGG
IMS 028	1.05	10.92	5.30	226.00	0.35	4.00	GSP	GYY
IMS 029	0.90	9.40	5.00	208.60	0.35	4.00	GSP	GYY
AIS 030	1.18	10.35	4.00	205.60	0.35	4.00	GSP	YYY
AIS 031	2.12	16.02	4.60	228.00	0.35	4.00	GSP	YYY
AIS 032	1.56	9.36	4.30	180.30	0.35	4.00	GSP	YYY
AIS 033	1.30	12.50	5.00	293.60	0.36	4.00	GSP	YYY
ANS 034	1.60	9.30	4.60	170.30	0.35	4.00	GSP	YGG
ANS 035	1.16	10.33	5.00	282.30	0.35	4.00	GSP	YGG
ANS 036	0.80	7.17	4.00	108.36	0.35	4.00	GSP	YGG
ANS 037	0.60	8.62	4.30	199.30	0.35	4.00	GSP	YGG
ANS 038	1.42	8.12	4.30	190.00	0.34	4.0	GSP	YGG
ANS 039	1.00	6.89	4.00	168.60	0.36	4.00	GSP	GYY
ANS 040	1.10	8.70	5.30	258.00	0.35	4.00	GSP	YGG
ABS 041	1.00	8.20	4.30	196.20	0.35	4.00	GSP	GYY
ABS 042	0.90	10.30	5.30	264.30	0.35	4.00	GSP	GYY
ABS 043	0.82	9.36	4.00	172.30	0.35	4.00	GSP	GYY
ABS 044	0.46	12.32	4.60	250.30	0.35	4.00	GSP	GYY
ABS 045	0.46	10.42	4.60	233.00	0.35	4.00	GSP	GYY
ABS 046	1.03	10.96	4.60	214.00	0.35	4.00	GSP	GYY
F-LSD $_{(P<0.05)}$	0.21	3.53	0.70	60.02	-	-		

GGG = all green; GWP =green with patches of purple; GSP = Greenish with spots of purple; GPP = greenish purple; GYY = greenish yellow; PSG = purple with spots of green; PPP = all purple; YGG = yellowish green; YPP = yellowish purple; YYY = all yellow. n = 138 for each trait (46 clones × 3)

2005 through 2007 suggest an influence by the environment on flower initiation, duration and performance in the species wherever they exist. Clones that initiated flowering earlier, had a better fill of their seeds. They also performed better in other follicle traits that increased seed yield per follicle such as length and thickness. The negative correlation between days to flowering and the number of matured follicles per inflorescence, follicle length and thickness illustrates that clones that flower later on in the year do not set or set and abort most of their follicles before maturity. The different clones flowered between the months of February and May over the two years and those that started to flower earlier had a higher number of filled, thicker and matured follicles. For example clones IMS 025 and IMS 026 from original, close environment flowered early and had the highest number of follicles filled with seeds over both years. Also clones ENS 008, EBS 015 and IMS 020 flowered early and had either a thick follicle or higher number of viable seeds per follicle, which determines the level of success in pollination. Our result is in agreement with reports of high seed yield in early flowering cowpea species (Singh *et al.* 1997; IITA 1998; Ezeaku *et al*. 2008). It could be deduced from this result that the species flowers within specific months in a year (February to May) and that clones that flower from April set only few follicles. The species could also be judged to be poor in follicle setting considering an average of 203 florets per inflorescence out of which only 3 follicles are retained to maturity. Clones ENS 008 and EBS 017 flowered early and ENS 008 had the thickest follicle of 15 cm while EBS 017 had the longest follicle of 11 cm.

The importance of the associations in the reproductive

Table 3a Days to flowering, maturity and follicle traits of the clones used for study in 2006.

Clones	NMF	FL	FTT	DMF	DTF	NNS	NVS	SWT
ENS 001	3.00	7.00	14.33	359.0	275.67	1.33	57.67	9.40
ENS 002	4.33	6.00	12.33	313.7	292.67	1.67	52.00	6.63
ENS 003	4.00	6.67	11.67	315.7	304.00	2.00	56.67	5.07
ENS 004	2.67	7.33	14.00	344.0	344.67	3.00	58.00	6.20
ENS 005	2.33	9.00	12.67	307.0	344.33	9.00	58.00	1.50
ENS 006	1.67	6.00	13.33	343.7	322.67	21.67	4.33	1.10
ENS 007	2.67	7.00	13.00	410.3	273.33	3.00	67.00	5.00
ENS 008	3.67	9.00	15.00	333.7	271.67	6.67	83.33	7.87
ENS 009	2.33	9.33	13.00	321.3	345.33	61.67	0.00	2.37
ENS 010	4.00	9.00	13.33	313.0	345.00	3.33	42.67	5.00
ENS 011	2.00	6.67	11.67	310.0	342.33	3.33	45.33	4.90
ENS 012	2.00	7.33	10.67	381.7	276.00	1.67	41.33	5.00
EBS 013	2.00	6.00	9.67	375.0	275.67	1.33	34.67	5.00
EBS 014	3.33	8.00	10.33	315.3	342.33	1.33	38.00	4.76
EBS 015	4.00	8.00	13.00	447.3	274.67	2.67	87.67	6.00
EBS 016	1.67	6.33	13.00	326.0	348.67	1.33	54.00	4.73
EBS 017	4.33	11.33	9.00	361.0	273.67	0.00	87.33	3.93
EBS 018	9.67	8.33	10.00	360.0	275.67	36.67	0.00	1.33
EBS 019	4.33	7.33	11.00	360.3	276.33	1.33	54.00	5.00
IMS 020	3.67	8.33	14.00	408.0	272.00	0.00	63.67	8.63
IMS 021	3.67	7.67	10.67	365.7	271.67	0.00	34.00	4.30
IMS 022	1.67	6.00	11.00	325.0	283.00	2.33	48.00	5.00
IMS 023	3.33	9.67	13.67	354.7	281.67	2.33	63.00	5.50
IMS 024	1.67	5.33	13.33	310.67	284.00	1.33	37.33	4.93
IMS 025	10.67	9.67	11.67	359.3	281.67	7.00	39.33	5.20
IMS 026	8.67	7.33	14.67	353.7	276.00	4.00	63.33	5.73
IMS 027	2.33	8.67	10.33	365.3	285.67	2.67	47.67	4.53
IMS 028	2.33	9.33	11.00	353.3	341.00	30.67	0.00	1.90
IMS 029	3.33	9.67	11.33	418.0	346.00	1.67	56.00	6.10
AIS 030	4.00	10.33	11.67	359.0	279.00	2.67	69.33	6.63
AIS 031	3.33	8.00	13.00	377.00	279.33	0.00	46.33	5.90
AIS 032	8.33	9.67	14.00	350.00	287.33	1.33	59.67	6.30
AIS 033	4.00	9.67	10.33	362.33	277.33	0.00	65.00	6.70
ANS 034	3.33	10.00	8.00	369.67	290.00	1.67	51.33	5.16
ANS 035	1.67	7.67	9.33	356.00	302.67	1.00	40.00	4.77
ANS 036	1.67	7.67	8.67	343.33	295.67	1.33	41.00	5.60
ANS 037	1.00	9.33	9.00	413.67	342.33	1.67	46.33	5.43
ANS 038	2.00	9.33	8.33	306.67	351.67	0.00	32.00	5.70
ANS 039	1.00	5.00	10.00	346.00	353.33	1.33	38.67	5.50
ANS 040	1.33	6.67	8.33	334.33	407.33	1.33	36.67	5.20
ABS 041	1.67	4.67	8.00	307.00	411.00	1.33	41.33	4.70
ABS 042	1.33	4.33	7.33	322.67	371.67	1.67	49.33	5.87
ABS 043	1.33	5.67	10.33	324.67	391.67	1.33	59.33	5.63
ABS 044	2.33	5.00	7.00	347.33	404.00	1.67	39.00	4.70
ABS 045	2.67	8.00	7.33	310.67	333.67	39.67	0.00	1.63
sABS 046	2.00	6.33	9.67	320.00	272.33	2.00	36.67	5.10
F-LSD$_{(P<0.05)}$	1.69	1.66	1.66	9.99	15.99	2.96	8.48	0.53

DMF = days to maturity of follicle; DTF = days to flowering; FL = follicle length; FTT = follicle thickness, NMF = number of matured follicles per inflorescence; NNS = number of non-viable/filled seeds; NVS = number of viable/filled seeds; SWT = 1000-seed weight. n = 138 for each trait (46 clones × 3)

traits is further explained by the results of PCA. Days to flowering which influences the quantity and quality of seeds produced in the species as well as the number of viable seeds per follicle had the highest eigen vector values and was thus a high delineating trait in the species. **Fig. 3B**, which shows clones ENS 001, EBS 015, ENS 008 and IMS 020 with lower days to flowering with more filled seeds in their follicles and a greater seed weight, further supports the importance of early flowering in the species. Meanwhile, the level of filling, which determines viability and size of the seeds (data not shown) determines seed weight. This is reflected in the seed weights of clones ENS 009, EBS 017 and EBS 018. While clones ENS 009 and EBS 018 had no filled seeds, EBS 017 had filled but smaller sized seeds and both factors (level of filling and size of seeds) resulted in lower seed weight even if EBS 017 had a high number of viable seeds per follicle. Some of our results on floral colors are contrary to the finding of Nielsen (1965) who noted only greenish-yellow flowers in the species. However, out of our 46 clones 29 (which formed a majority) had similar greenish yellow color, five clones had only yellowish color and one of the clones had yellowish purple color. The two colors (only yellowish and yellowish violet were distinctly different from the rest of the studied clones including the ones described by Nielsen (1965). We identified clones that had follicles with equal length with the ones described by Nielsen (1965). For example, Nielsen identified clones whose follicles were 7.4 cm long whereas clones ANS 039 and EBS 017 had follicles that measured 5 and 11.33 cm, respectively. The grand mean length of all the follicles measured was 7.34 cm which is similar to the 7.4 cm described by Nielsen.

Clones IMS 025, IMS 026 and AIS 032 distinctly positioned in **Fig. 3B** illustrate that they had a high number of matured follicles per inflorescence as well as many viable seeds in the follicles. The three clones suggest genetic

Table 3b Days to flowering, maturity and follicle traits of the clones used for study in 2007.

Clones	NMF	FL	FTT	DMF	NNS	NVS	SWT
ENS 001	1.00	6.67	14.00	360.00	2.67	58.67	8.77
ENS 002	1.33	5.33	11.00	302.00	3.67	56.00	6.07
ENS 003	1.67	6.00	11.00	309.67	3.67	57.33	4.96
ENS 004	1.33	7.00	13.00	332.00	2.33	58.67	5.93
ENS 005	1.67	8.67	12.00	302.33	2.00	57.67	1.53
ENS 006	2.33	5.67	13.00	329.67	1.33	1.33	0.93
ENS 007	1.67	6.33	12.67	401.33	2.00	65.33	5.00
ENS 008	2.67	8.67	14.33	324.67	3.33	69.33	7.06
ENS 009	2.60	8.67	11.67	312.67	1.67	0.00	2.00
ENS 010	2.00	8.00	13.00	306.33	3.00	41.33	5.16
ENS 011	1.67	6.33	10.33	300.33	1.67	43.33	4.83
ENS 012	1.67	7.00	10.00	361.33	1.67	41.33	4.90
EBS 013	1.00	5.67	9.00	352.33	2.00	36.00	5.03
EBS 014	1.33	7.67	10.00	306.67	3.00	36.67	4.76
EBS 015	1.33	8.00	12.33	424.00	4.33	78.00	5.60
EBS 016	1.33	5.67	11.67	311.00	1.33	48.00	4.70
EBS 017	3.86	10.67	8.00	352.67	3.67	82.67	4.83
EBS 018	10.00	7.67	9.00	338.00	9.67	0.00	1.33
EBS 019	0.67	6.67	10.00	346.67	4.00	53.33	4.96
IMS 020	0.67	7.00	13.00	390.67	4.00	62.67	8.03
IMS 021	3.43	7.33	9.67	357.33	2.67	36.67	4.10
IMS 022	1.00	5.67	10.67	320.00	2.00	47.33	4.80
IMS 023	1.67	9.00	12.33	343.33	3.00	60.00	5.16
IMS 024	1.00	5.67	9.00	303.33	1.67	37.67	4.36
IMS 025	2.33	8.33	10.67	335.33	10.33	39.33	4.86
IMS 026	1.67	7.00	13.67	345.67	8.33	59.33	5.06
IMS 027	2.00	8.33	10.00	349.33	2.33	0.00	1.53
IMS 028	2.67	8.67	10.67	345.00	2.00	46.00	4.40
IMS 029	1.33	9.00	10.33	402.00	3.00	54.00	5.77
AIS 030	2.00	9.67	9.67	342.67	3.67	62.67	6.23
AIS 031	3.63	8.00	12.67	370.00	3.00	68.00	5.67
AIS 032	1.00	9.00	13.67	339.33	7.33	59.67	6.00
AIS 033	3.67	10.00	10.67	353.67	3.33	79.33	6.67
ANS 034	1.33	9.00	8.33	354.67	3.00	41.33	5.87
ANS 035	1.00	8.00	9.00	349.67	1.33	41.33	4.83
ANS 036	1.33	7.00	7.67	338.67	1.33	41.00	5.73
ANS 037	1.00	9.00	8.67	406.00	1.00	51.33	5.33
ANS 038	0.00	9.33	8.67	330.67	2.00	52.67	6.27
ANS 039	1.00	4.67	9.67	338.67	1.00	43.33	5.60
ANS 040	1.33	6.33	7.67	318.33	1.33	38.67	5.17
ABS 041	1.33	5.00	8.33	298.33	1.67	45.33	5.03
ABS 042	1.67	6.00	7.67	313.33	1.67	49.67	6.06
ABS 043	1.33	6.00	10.00	314.33	1.33	59.33	5.87
ABS 044	1.67	5.33	7.33	330.67	2.67	45.00	5.13
ABS 045	2.67	8.33	6.67	302.00	2.67	0.00	1.50
ABS 046	1.67	6.00	9.33	303.33	2.00	38.67	5.77
F-LSD $_{(P<0.05)}$	2.13	1.33	1.39	16.45	1.34	7.12	0.55

DMF = days to maturity of follicle; DTF = days to flowering; FL = follicle length; FTT = follicle thickness, NMF = number of matured follicles per inflorescence; NNS = number of non-viable/filled seeds; NVS = number of viable/filled seeds and SWT = 1000-seed weight. n = 138 for each trait (46 clones × 3)

similarity in pollen production and follicle setting among them. Conversely, four clones (ENS 009, EBS 018, IMS 028 and EBS 045) that produced seeds that did not fill in both years suggest genetic sterility inherent in the clones which calls for further studies. Such sterility could not be attributed to late flowering or an environmental factor. This is because the unfilled clones started flowering in March when the clones that filled their follicles also came to flowering and both were equally exposed to the same amount of rainfall, Sunshine, temperature and other environmental influences. The morphological variation observed in the reproductive traits of the clones of *G. latifolia* suggest the contributions of genetic differentiation and the environment which have been reported by other researchers to cause wider variation within plant species (Stebbins 1950; Ellison *et al*. 2004). Phenotypic variation in the reproductive traits among plant species, which is a prerequisite to the formation of subspecies and species, has been reported in other plants by different researchers (Sakai *et al.* 1999; de Carvalho *et al.* 2004; Ellison *et al.* 2004). Our data clearly supports the notion that *G. latifolia* could be differentiated into different genetic subspecies based on reproductive traits of flower color, flowering habit (early and late), seed filling and seed weight and that these could be traits of interest to a breeder working on this species. The greater phenotypic variance observed in the delineating traits will facilitate the accurate assessment of clonal value based on phenotype and hence increase the efficiency of clonal selections for wider genetic diversity for making crosses.

Our study has shown significant phenotypic variations in the reproductive traits of 46 *G. latifolia* clones. Significant variations and similarities occurred in clones obtained from close habitats. For example, EBS 015 and EBS 016, both collected from Ebonyi State are directly opposite with respect to floral traits and seed fill capability of the follicles. Also, clones IMS 025 and IMS 026, both obtained from

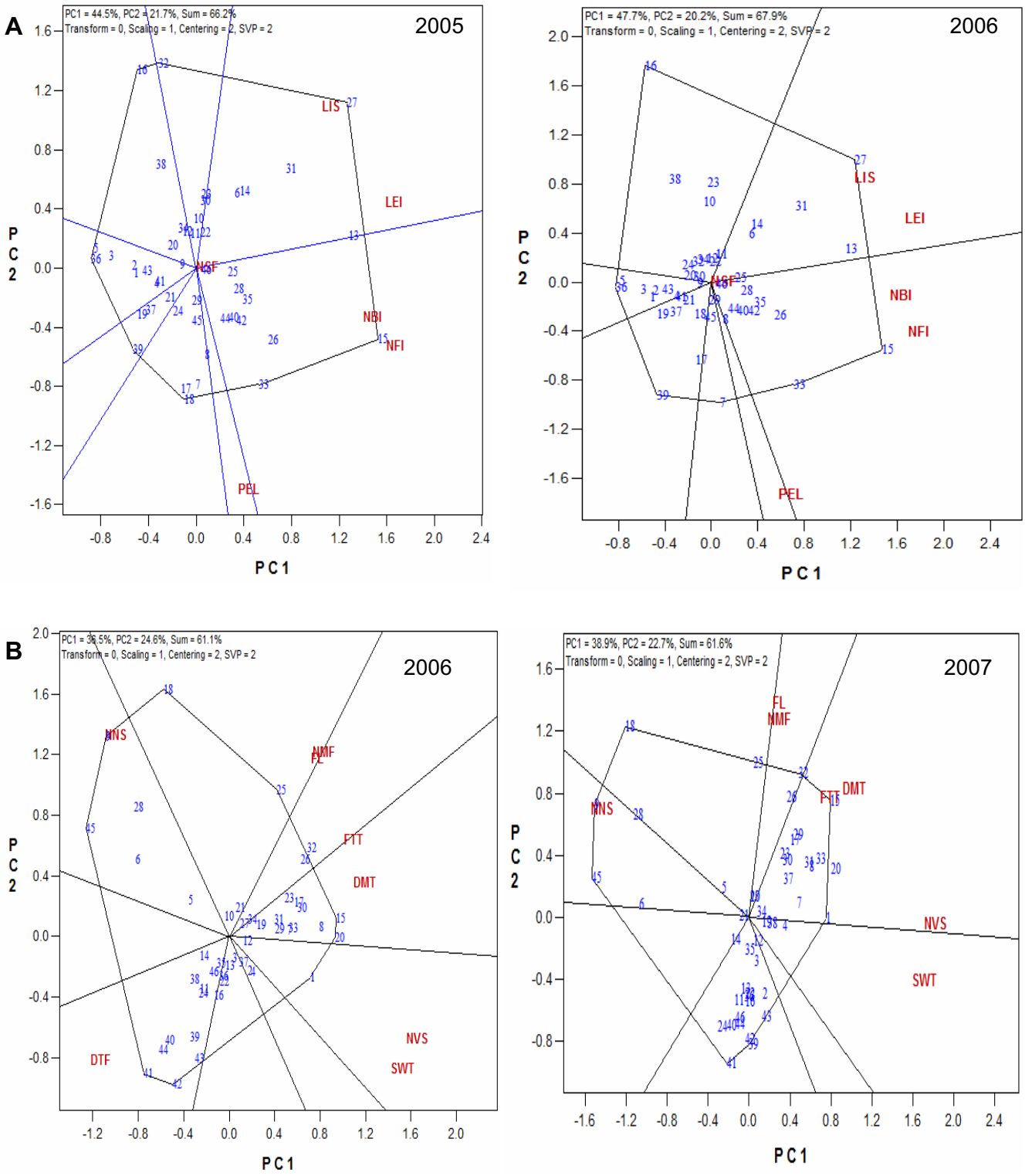

Fig. 3 (**A**) Biplot distribution of different clones in 2005 and 2006 in respect to flowering traits. Where: LIS = length of inflorescence stalk; NBI = number of branches per inflorescence; NFI = number of follicles per inflorescence; LEI = length of inflorescence; PEL = petal length. Numbers 1 to 46 represent the Clones used for study as outlined in **Table 1**. (**B**) Biplot distribution of follicle traits of the different clones in 2006 and 2007. Where: NMF = number of matured follicles per inflorescence; FL = follicle length; FTT = follicle thickness, DTF = days to flowering; DMF = days to maturity of follicle; NNS = number of non-viable/filled seeds; NVS = number of viable/filled seeds; SWT = 1000-seed weight. Numbers 1 to 46 represent the Clones used for the study as outlined in **Table 1**.

Imo State, are similar with respect to follicle traits. The morphological variations observed in the reproductive traits suggest the contribution of genetic differentiation and the environment. Days to flowering in the species was observed as the most important delineating trait as it influenced the quantity and quality of seeds produced by clones in a follicle. Clones ENS 001, ENS 008, EBS 015 and IMS 020, which initiated flowering earlier, had more filled seeds in their follicles and a greater seed weight. Clones IMS 025 and IMS 026 flowered early and also had the highest number of filled follicles over both years of follicle harvest. On the contrary, clones ABS 043, ABS 042 and ABS 041 had longer days to flowering and low values for follicle traits. Three floret colors that have not yet been reported in literature including yellowish green, all yellow and yellowish purple (**Fig. 2**) were identified in the species. It could be argued that the observed distinct variations in the reproductive traits of the clones call for differentiation of *G. latifolia*

Table 4 Representation of the forty six clones based on color of the inflorescence stalk and florets.

Groups	Color	№ of clones with the color on inflorescence stalk
1.	Greenish with spots of purple	32
2.	Greenish purple	8
3.	Greenish with patches of purple	3
4.	All greenish	1
5.	Purple with spots of green	1
6.	All purple	1
	Total	46
		Number of clones with the color on florets
1.	Greenish yellow	29
2.	Yellowish green	11
3.	All yellow	5
4.	Yellowish purple	1
	Total	46

Table 5 Correlation matrix of the flower and follicle units measured.

	LIS	NBI	NFI	LEI	DTF	NMF	FL	FTT	NVS	NNS	DMT	SWT
LIS	-											
NBI	0.209	-										
NFI	0.154	0.576**	-									
LEI	0.484**	0.51*	0.581**	-								
DTF	-0.181	-0.070	-0.057	-0.215	-							
NMF	-0.171	0.093	0.106	-0.013	-0.440**	-						
FL	0.088	0.064	-0.051	0.084	-0.359*	0.404**	-					
FTT	0.041	0.045	-0.085	0.069	-0.395**	0.337*	0.205	-				
NVS	0.048	0.035	0.003	-0.033	-0.289	0.094	0.174	0.335*	-			
NNS	-0.150	0.050	0.037	0.019	0.102	0.118	0.174	0.030	-0.695**	-		
DMT	0.141	0.258	0.376**	0.278	-0.410**	0.164	0.317*	0.171	0.325*	-0.168	-	
SWT	0.004	-0.052	0.081	-0.081	0.244	0.036	0.006	0.267	0.707**	-0.661**	0.270	-

* = Significant at 5% probability level

** = significant at 1% probability level

LIS = length of inflorescence stalk; NBI = number of branches per inflorescence; NFI = number of follicle per inflorescence; LEI = length of inflorescence; DTF = days to flowering; NMF = number of matured follicles per inflorescence; FL = follicle length; FTT = follicle thickness; NVS = number of viable/filled seeds; NNS = number of non-viable/unfilled seeds; DMT = days to maturity of follicle and SWT = 1000-seed weight.

Table 6 Eigen vector values for principal components of the reproductive traits of the different *G. latifolia* clones.

Reproductive traits	PRIN 1	PRIN 2	PRIN 3	PRIN 4
FL	0.22578	-0.08274	-0.42817	0.02020
FTT	0.26137	0.11402	-0.35292	-0.33274
DMF	0.39479	-0.10675	0.03638	0.28630
DTF	-0.38676	0.02980	0.27476	-0.10065
LEI	0.26143	-0.40155	0.15135	-0.26624
LIS	0.17888	-0.18104	0.23877	-0.42675
NBI	0.20527	-0.34899	0.19686	-0.07131
NFI	0.23454	-0.38487	0.30588	0.06302
NMF	0.23588	-0.10397	-0.46543	0.04239
NNS	-0.24350	-0.40650	-0.36472	0.05883
NSF	0.00000	0.00000	0.00000	0.00000
NVS	0.38354	0.37846	0.11034	0.06721
PEL	0.08327	-0.14074	0.11321	0.72434
SWT	0.32986	0.40600	0.16862	-0.01674

FL = follicle length; FTT = follicle thickness; DMF = days to maturity of follicle; DTF = days to flowering; LEI = length of inflorescence; LIS = length of inflorescence stalk; NBI = number of branches per inflorescence; NFI = number of follicle per inflorescence; NMF = number of matured follicles per inflorescence; NSF = number of stamens per floret; NVS = number of viable/filled seeds; NNS = number of non-viable/unfilled seeds; PEL = petal length; SWT = 1000-seed weight.

into different genetic subspecies.

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