

AGRO-MORPHOLOGICAL EVALUATION AND ANALYSIS OF GENETIC PARAMETERS IN AFRICAN BASIL (*OCIMUM GRATISSIMUM* L.) IN SOUTHEASTERN NIGERIA

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Abstract

Six accessions of African basil (*Ocimum gratissimum* L.), a notable medicinal plant of the *Lamiaceae* family, were evaluated for genetic variability based on agro-morphological markers. Analysis of variance for eight quantitative traits revealed significant differences in the traits studied, indicating the existence of variability in the plant materials. High positive correlation coefficients were observed for most of the traits, suggesting the possibility of achieving simultaneous improvement for the traits, since selection for one of such traits should naturally result in progress for all positively correlated characters. Analysis of genetic parameters showed that virtually all the traits assessed had appreciable values of broad sense heritability, ranging from 47.37 % for raceme length to 97.08 % for dry matter yield, an indication of a high portion of heritable variation. Apart from raceme length and dry weight, whose values of heritability and genetic advance were moderate, all other characters investigated had high values for these genetic parameters. This ultimately depicts that these traits are under genetic control; therefore their phenotypic performance can be relied upon for effective selection.

Keywords: Genetic variability, quantitative traits, correlation, broad sense heritability, expected genetic advance

Introduction

There has been an increasing emphasis, over the years, on the need to develop and standardize traditional or herbal medicine as an alternative to synthetic drugs and antibiotics. An advocacy for the inclusion of traditional medicine in the curriculum of Nigerian Universities is currently gathering momentum, the justification of which may be anchored on the fact that about 70 – 80 % of the world's population depend on herbal medicines for their health care (Pei, 2001). Several species of *Ocimum* are known for their therapeutic potentials and medicinal properties. However, the supply of medicinal plants is hardly enough to satisfy the volume needed for traditional medicines, utilization by pharmaceutical industries and in some cases direct human consumption. This shortage arises from several factors, ranging from desert encroachment, lack of adequate incentives for researchers and deforestation, which destroys the natural habitats of important medicinal plants. The situation is worse for plant species which do not receive enough research attention, especially in the areas of germplasm conservation and improvement. This, unfortunately, is the fate of *Ocimum* species in Nigeria.

Ocimum gratissimum L., an aromatic plant of the *Lamiaceae* family, is found in the savanna and humid forest vegetation zones of Nigeria, where it is recognized by various names, such as “effirin” (Yoruba), “ahuji” (Igbo), “daidoya” (Hausa) and “ntong” (Efik and Ibibio). Commonly known as African or tree basil, it is cultivated in the tropics and subtropics, with greatest variability in India and Africa. The various utilization channels to which the plant can be put, including culinary, pharmacology, commercial and ethnomedicine have been extensively reported (Harjula, 1980; FAO, 1986; Cunningham, 1993; Nakamura *et al.*, 1999; Sulistiarini, 1999; Keita *et al.*, 2000; Orafidiya *et al.*, 2001; Holetz *et al.*, 2003; Pessoa *et al.*, 2003; Ezekwesili *et al.*, 2004; Adebolu & Oladimeji, 2005; Ijeh *et al.*, 2005; Lemos *et al.*, 2005; Cristiana *et al.*, 2006; Mbata & Saikia, 2007; Lexa *et al.*, 2008; Soforowa, 2008; Stewart & Gude, 2008; Kokwaro, 2009; Nweze & Eze, 2009; Prabhu *et al.*, 2009). *O. gratissimum* has been described as a variable polymorphic complex species, often subdivided into subspecies, varieties and forms, mainly based on the morphology of the fruiting calyx, different degree of hairiness and differences in chemical content (Orwa *et al.*, 2009). It is a perennial herb, woody at the base, reaching a height of 3 m, with broadly or narrowly ovate leaves, usually 5 cm – 13 cm long and 3 cm – 9cm wide, having petioles of 1 cm – 6 cm long; flowers arranged in a terminal simple or branched raceme, 5 cm – 30 cm long (Sulistiarini, 1999). Polyploidy and chromosome number variation characterize *Ocimum* species. Variable chromosome numbers such as $2n = 40$, 48 and 64 have been reported for *O. gratissimum*, while $2n = 48$, 52, 60, and 72 have been reported for *O. basilicum* (Sulistiarini, 1999; Panda, 2005; Mournita *et al.*, 2005; Edet & Aikpokpodion, 2014). However, two basic chromosome numbers, $x = 8$ and $x = 12$ have been reported for the genus, on the basis of which the various species have been classified into two broad groups: *basilicum* group ($x = 12$) and *sanctum* group ($x = 8$) (Darlington, 1974; Skaria, 2007).

Although found as a home garden crop in most cases, *O. gratissimum* is grown on a commercial scale in Vietnam (Sulistiarini, 1999). In Nigeria, the crop is found in small holdings, mainly utilized as a spice plant and flavouring ingredient, with far less than enough efforts made towards its genetic characterization, germplasm conservation and improvement. Considering the widely acclaimed potentials of this crop, especially in the aspect of alternative medicine, it is

imperative to conduct a careful genetic evaluation of the species in order to access the existing variability for further improvement.

This work was therefore, undertaken to characterize the accessions of African basil growing in the Southeastern region of Nigeria, with a view to assessing the genetic diversity in the species and the extent to which the variable traits are heritable.

Materials and Methods

Seeds of *Ocimum gratissimum* were collected from different locations in Cross River and Akwa Ibom States of Nigeria. The materials collected were tagged and the geographic information of the specific locations where collections were made, are documented in Table 1.

The seeds were raised in polybags between the months of February and March, 2010, maintained behind the Biological Sciences block of the University of Calabar. The six accession groups (MKE, IKN, KTA, MUN, ANA and AKP) were laid out in a Randomized Complete Block Design (RCBD) with five replicates for agro-morphological study. The experiment was located at the Experimental Farm of the Department of Genetics and Biotechnology, University of Calabar on lat 04.56⁰ N and long 08.52⁰ E. Transplanting was done at spacing of 1 m x 1 m and NPK fertilizer was applied at a recommended rate equivalent to 100 Kg/ha as a basal dose.

Data were collected on plant height, raceme length, leaf length, leaf width, leaf area, total shoot biomass (fresh and dry weights) and dry matter yield. Analysis of variance was applied to determine variability between accessions on the traits studied. Significantly different means were separated using Least Significant Difference (LSD) and linear correlation coefficients among the traits were computed. Components of variance were partitioned as described by Bliss *et al.* (1973) and the apportioned components used for the estimation of genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability (h^2B) and genetic advance (GA) at 5 % selection intensity ($k = 2.06$), reported as percentage of the mean. Genotypic and phenotypic coefficients of variation were computed as described by Singh and Chaudhury (1995), while broad sense heritability and genetic advance were estimated according to Allard (1960) and Johnson *et al.*, (1955), respectively.

Results and Discussion

The means and linear correlation coefficients of the eight quantitative traits evaluated are presented in Tables 2 and 3. Both pre- and post-harvest parameters showed significant differences between the accessions, indicating the existence of genetic variability among the accessions. However, separation of means revealed that ANA, KTA, MUN and IKN were not significantly different from each other in plant height; MKE and AKP were not significantly different from each other in the same trait, thereby producing two clusters for plant height. A similar trend was observed for other indices studied (Table 2), with remarkably lower dry matter content recorded in MUN as compared to all other accessions. This may suggest that plants in this accession group have relatively higher moisture content. Genetic variability in *O. gratissimum* accessions based on essential oil constituents, using randomly amplified polymorphic DNA (RAPD) markers and morphological markers were earlier studied by Vieira *et al.* (2001). Their report showed that some of the accessions were genetically distinct, grouping them into six chemotypes which were identical to the groups identified by morphological observations. Similar entries have also been made for other species of *Ocimum* (Lal *et al.*, 2012; Chen *et al.*, 2013). High positive correlation coefficients (Table 3) were recorded among most of the parameters studied, indicating of the possibility of achieving simultaneous improvement in the traits, since selection for one trait results in progress for all characters that are positively correlated and retrogress for traits that are negatively correlated. Therefore, selection for dry matter content which was negatively correlated with pre-harvest parameters and plant height which also showed high negative correlation with leaf width and leaf area is not likely to be beneficial.

The narrow differences observed between the genotypic coefficient of variation (GCV) and the phenotypic coefficient of variation (PCV) in plant height, leaf length, leaf width and dry matter content (Table 4) indicate the existence of sufficient genetic variability in the traits, which may facilitate effective selection. On the other hand, the moderately high differences between GCV and PCV in the remaining characters indicate relatively higher environmental influences on the traits. Virtually all the traits studied had high values of broad sense heritability, ranging from 47.37 % for raceme length to 97.08 % for dry matter content (Table 4), which suggests a high portion of heritable variation, which is ascribable to genotypic effects. It should be remembered that broad sense heritability is the relative magnitude of genotypic and phenotypic variances for the traits and plays a predictive role in selection procedures (Allard, 1960). Nevertheless, high heritability values combined with high genetic advance serves better in the prediction of the ultimate effect of selection (Ali *et al.*, 2002). Apart from raceme length and dry weight, whose values of heritability and genetic advance were moderate, all other

characters investigated had high values of heritability and genetic advance. This may ultimately depict that these traits are under genetic control. Hence, their phenotypic performance can be relied upon for effective selection.

Conclusion

With the significant differences recorded in the traits evaluated, the existence of genetic variability in the accessions studied is obvious. Thus, selection among the accession groups, with emphasis on traits with high heritability, genetic advance and positive correlation, is likely to be effective. The evidence of the occurrence of variability within the small sample utilized for this study indicates that there is the need for further assessment of genetic variation in this species with a wider scope and geographical space.

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Table 1: Geographic information of collection locations

State	Local Government Area	Accession group tag	Community	Lat (⁰ N)	Long (⁰ E)
Akwa Ibom	Mkpat Enin	MKE	- Ikot Obio Akwa	04.88	07.83
			- Ikot Abasi Ufon	04.76	07.79
			- Ukam	04.92	07.89
			- Mkpat Enin	04.83	07.80
Akwa Ibom	Ikono	IKN	- Ukpum Ikot Ntuen	04.75	07.78
			- Ukpum Ikot Ekwere	04.75	07.79
			- Ikot Anwana	04.82	07.80
Cross River	Akpabuyo	KTA	- Ekpene Tete	04.82	07.76
			- Ikot Ene	04.93	07.81
			- Ikot Ekpo Eyo	04.82	07.76
			- Ikot Nakanda	04.75	07.79
Cross River	Calabar Municipality	MUN	- Edim Otop	04.76	07.79
			- Nyakasang	04.89	07.83
			- Ikot Eneobong	04.83	07.80
Cross River	Calabar South	ANA	- Anantigha	04.64	07.68
			- Jebs	04.67	07.71
			- Uduak Orok	04.73	07.76
			- Ekpo Nsa	04.68	07.70
Cross River	Akamkpa	AKP	- Oban	04.61	07.32
			- Awi	04.63	07.74
			- Ako	04.60	07.31

Table 2: Means of eight quantitative traits observed in six accession groups of *Ocimum gratissimum*

Accession	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm ²)	Raceme length (cm)	Fresh weight (g)	Dry weight (g)	Dry matter content (%)
KTA	91.76	10.98	5.48	41.67	13.45	194.88	80.20	41.19
ANA	92.38	10.70	5.66	43.21	15.40	168.67	70.07	41.54
MUN	90.20	9.52	4.66	31.79	15.28	174.11	58.66	33.69
AKP	60.14	7.02	3.72	17.98	13.46	130.43	55.96	42.91
IKN	85.64	9.68	4.80	32.53	15.18	151.50	63.44	41.87
MKE	66.84	7.54	4.28	22.59	14.56	127.94	55.64	43.41
Grand mean	81.16	9.24	4.77	31.63	14.56	157.92	64.00	40.77
LSD (0.05)	8.63	0.94	0.51	6.91	1.09	26.37	10.66	0.81

Table 3: Linear correlation coefficients among eight quantitative traits in *Ocimum gratissimum*

Traits	Plant height	Leaf length	Leaf width	Leaf area	Raceme length	Fresh weight	Dry weight
Plant height	-	-	-	-	-	-	-
Leaf length	0.96	-	-	-	-	-	-
Leaf width	-0.89	0.96	-	-	-	-	-
Leaf area	-0.93	0.99	0.99	-	-	-	-
Raceme length	0.48	0.30	0.32	0.32	-	-	-
Fresh weight	0.89	0.91	0.81	0.87	0.05	-	-
Dry weight	0.71	0.86	0.86	0.86	-0.19	0.83	-
Dry matter content	-0.51	-0.31	-0.14	-0.22	-0.41	0.50	0.05

Table 4: Genetic parameters of variation for eight quantitative traits in *Ocimum gratissimum*

Traits	Mean square	δ^2_g	δ^2_e	δ^2_p	GCV (%)	PCV (%)	h^2_B (%)	GA
Plant height	986.94***	188.6	43.65	232.25	16.92	18.78	81.21	31.42
Leaf length	13.25***	2.55	0.52	3.07	17.28	18.96	83.06	32.45
Leaf width	2.65***	0.5	0.15	0.65	14.82	19.90	76.92	26.78
Leaf area	503.62***	95.13	27.98	123.11	30.84	35.08	77.27	55.84
Raceme length	3.86**	0.63	0.7	1.33	5.45	7.92	47.37	7.73
Fresh weight	3439.41***	678.24	408.19	1086.43	16.49	20.87	62.43	26.84
Dry weight	462.52***	79.18	66.63	145.81	13.9	18.87	54.30	21.10
DMC	63.63***	12.65	0.38	13.03	8.72	8.85	97.08	17.71

δ^2_g – genetic variance, δ^2_e – error variance, δ^2_p – phenotypic variance, GCV – genotypic coefficient of variation, PCV – phenotypic coefficient of variation, h^2_B – broad sense heritability, GA – genetic advance as a percentage of the mean, DMC – dry matter content, ** and *** - significant at 1% and 0.1% , respectively.