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Inheritance of Pigmentation in *Ocimum tenuiflorum* linn

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Abstract

This study investigated the mode of inheritance of purple pigmentation on the shoot of *Ocimum tenuiflorum* morphotypes found growing in South Western Nigeria. Intraspecific hybridization involving the three morphotypes (i.e. green, very light purple and deep purple morphotypes) were carried out. The F_1 plants obtained from each reciprocal crosses segregated into different shades of purple plants and a green plant at different ratios. A new morphotype with light purple pigmentation was observed at the F_1 generation from a cross between the green and the deep purple morphotype. The light purple morphotype obtained segregated back into the four morphotypes at the F_2 generation when selfed. It was concluded that the inheritance pattern of purple pigmentation in *O. tenuiflorum* followed the duplicate gene interaction with dosage effect.

Introduction

The genus *Ocimum* belongs to the family Lamiaceae (the mint family) which consists of 236 genera and about 7000 species, which makes it the largest family of the order Lamiales and the sixth largest family of flowering plants [1,2]. Members of the family are recognised by the 4-angled stems, opposite (very rarely alternate) leaves. The flowers are usually arranged in clusters and feature two-lipped, open-mouthed, tubular corollas (united petals) with five-lobed bell-like calyxes (united sepals), and having the fruits of 4 nutlets [3]. The genus *Ocimum* comprises of about 200 species of annual and perennial herbs and shrubs native to Tropical Asia (India and Pakistan), Tropical and Subtropical regions of Africa (Nigeria and Kenya), Tropical parts of America (Brazil), but widely distributed around the world and having numerous varieties cultivated under a multitude of ecological conditions [4-8]. Tropical Africa is considered to be the primary centre of origin of *Ocimum* species, because it is the area of maximum diversity. About six species of the genus *Ocimum* are currently recognized in West Africa and are generally distributed throughout the region. Five of the species which are *O. canum, O. basilicum, O. kilimandscharicum, O. gratisimum,* and *O. tenuiflorum* are found throughout Nigeria [9,10].

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Ocimum tenuiflorum is an aromatic plant with about 150 variants, one of the most famous perennial herbs which is native of India and widely spread as a cultivated plant and an escaped weed, covering entire Indian sub-continent ascending up to 1800 m in the Himalaya and the Andaman and Nicobar islands in the south [11]. It is largely distributed in Asia, Australia, West Africa and also in some Arabian countries mainly in drier sandy areas [12]. *Ocimum tenuiflorum* is also known as *Ocimum sanctum*. It is called Indian's holy basil in English, 'Kala-Tulsi' among the Hindi Speaking people of India, and 'Efinrin wewe' among the Yoruba speaking people of Southwestern Nigeria [13]. The stem and leaves of *O. tenuiflorum* contains a variety of constituents that may have biological activity, including saponins, flavonoids, triterpenoids and tannins [14]. The uses of *O. tenuiflorum* ranges from domestic to therapeutic and industrial uses.

Owing to the variability that exist both in morphological and chromosome number within *O. tenuiflorum*, different chromosome numbers of 2n=32, 2n=36, and 2n=64 have been reported by different authors [15]. The study of inheritance pattern of pigmented traits are required for their potential use as morphological markers in genetic studies and breeding work. This study investigated the inheritance of purple pigmentation observed on the shoot of *O. tenuiflorum* morphotypes.

Materials and methods

This study was carried out at the Department of Botany Obafemi Awolowo University Ile-Ife, (07^o 30' N, 04^o 40' E) Nigeria. The living plant materials of the *O. tenuiflorum* parental morphotypes were collected in the wild from different location within and outside Ile-Ife Osun State (Table 1).

The living plant materials used were transplanted into 7-litre buckets filled with top soil and raised to maturity and seeds were harvested. Each of the *Ocimum tenuiflorum* morphotypes were grown for five generations before hybridization experiments were carried out on them to ascertain their identity. After a pure line was achieved for all the morphotypes, intraspecific reciprocal crosses were carried out among the three identified morphotypes as indicated in Table 2. Physical emasculation of flower buds of each ovulate parent was carried out between 7.00 am - 9.00 am before self-pollination occurred. Pollen grains from the required pollen parents were transferred to the emasculated flowers on the ovulate parents between 11.00 am - 2.00 pm when the stigma were receptive. Each crossed flower was bagged and labelled to prevent contamination by external pollens and for identification. The crossed flowers were monitored for flower drop and fruit formations. F_1 hybrid seeds from the various reciprocal crosses were planted separately along with their parents in the screen house and characterized for qualitative morphological characters. The F_1 progenies were advanced by selfing. The F_2 seeds were planted and raised to maturity after which they were scored based on morphology. Data obtained were subjected to Chi-Square analysis using System Analysis Software (SAS).

Results

O. tenuiflorum (t_1) (green) x *O. tenuiflorum* (t_2) (very light purple) produced *O. tenuiflorum* (t_1) (green) and *O. tenuiflorum* (t_2) (very light purple) at the F_1 generation. *O. tenuiflorum* (t_1) green x *O. tenuiflorum* (t_3) (deep purple) produced *O. tenuiflorum* (t_4) (light purple) and *O. tenuiflorum* (t_2) (very light purple) at the F_1 generation. *O. tenuiflorum* (t_2) (very light purple) x *O. tenuiflorum* (t_3) (deep purple) produced *O. tenuiflorum* (t_3) (deep purple), *O. tenuiflorum* (t_4) (light purple) and *O. tenuiflorum* (t_2) (very light purple) at the F_1 generation. The F_1 *Ocimum tenuiflorum* (t_4) (very light purple) produced segregated to the green, very light purple, light purple and deep purple at the F_2 generation (Table 4). Some of the *O. tenuiflorum* (t4) (light purple) produced from *O. tenuiflorum* (t_2) (very light purple) x *O. tenuiflorum* (t_3) (deep purple) did not segregate at the F_2 generation. The hybrids from the various crosses segregated into different ratios at the F_1 and F_2 generations.

Discussion

From the data obtained in the F_1 and F_2 generation, the segregation pattern for purple pigmentation in *Ocimum tenuiflorum*, follow a model of duplicate gene interaction with dosage effect. This inheritance pattern is as described in Table 5. Based on the data obtained it was also observed that the *Ocimum tenuiflorum* (light purple morphotype) was produced from a cross of *Ocimum tenuiflorum* (green morphotype) and *Ocimum tenuiflorum* (deep purple morphotype) which are the dominant morphotypes in the wild. The Chi-square analysis for the segregation pattern showed that there is no significant difference in the observed and expected ratios of the segregation pattern and this led to the proposition of the duplicate gene interaction with dosage effect inheritance model that agrees with the chi square analysis.

The result from this study showed that the two duplicate factors (genes) tagged P_1 and P_2 are responsible for the purple pigment in *Ocimun tenuiflorum* while p_1 and p_2 are responsible for green pigmentation, which is in agreement with the findings of Owen [16] that reported the inheritance of cotyledon colour in soya bean and Harland [17] who worked on the inheritance of chlorophyll deficiency in cotton. The purple coloration is determined at two loci by a set of duplicate genes giving rise to four morphotypes; deep purple ($P_1P_1P_2p_2$), light purple ($P_1p_1P_2p_2$), very light purple ($P_1p_1p_2p_2$) and green ($p_1p_1p_2p_2$) with corresponding 3, 2, 1 and 0 dominant genes. This shows that only *O. tenuiflorum* (t_4) (light purple) can segregates into the four morphotypes as observed in this study. The genetic model proposed for the inheritance of the purple pigment in *O. tenuiflorum* agrees with the model proposed by Ayoola and Faluyi [18] in their study of the inheritance of caryopsis/ripened-hull colour in a selection from land races of *oryza sativa* in Nigeria.

The *O. tenuiflroum* (t_4) (light purple morphotype) produced from a cross between *O. tenuiflorum* (t_2) (very light purple morphotype) and *O. tenuiflorum* (t_3) (deep purple morphotype) might not segregates at the F_2 and F_3 generations because of the arrangement of the genes controlling the amount of purple colouration. (Table 5). Three genotypes are responsible for the phenotypic expression of the *O. tenuiflorum* (light purple morphotype) (i.e. $P_1p_1P_2p_2$, $P_1P_1p_2p_2$ and p1p1P_2P_2). It was revealed from this study that *O. tenuiflorum* (light purple morphotype) whose genotypes are $P_1P_1p_2p_2$ and $p_1p_1P_2P_2$ will not segregates at the F_2 and F_3 generations while the genotypes $P_1P_1P_2p_2$, P_1P_1-p_2P_2, $P_1p_1P_2P_2$ and $p_1P_1P_2P_2$ will segregates. (Table 5). This study revealed that the purple pigment on the leaf, petiole, stem and floral part in *Ocimum tenuiflorum* are inherited together. Varalakshmi[20] reported that purple pigmentation of leaf sheaths, leaf blades, internodes, bristles and glumes in pearl millet (*Pennisetun glaucum* L.) are inherited together. The result from the study revealed that the inheritance pattern of purple pigmentation in *Ocimum tenuiflorum* is due to the model of duplicate gene with dosage effect.

Figure 1: Inflorescence of *Ocimum tenuiflorum* morphotypes

A. Green Inflorescence of O. tenuiflorum (t1) morphotype
B. Greenish Purple Inflorescence of O. tenuiflorum (t2) morphotype
C. Purple Inflorescence of O. tenuiflorum (t3) morphotype
D. Purple Inflorescence of O. tenuiflorum (t4) morphotype

Table 1: Collection Sites of the Three Morphotypes of *Ocimum tenuiflorum* Studied.

***Ocimum tenuiflorum* Morphotypes**	**Collection Sites**	**Coordinates**
O. tenuiflorum (t_1) (green morphotype)	Oranfe, Ile-ife.	07° 30′ N, 04° 34′ E
O. tenuiflorum (t_2) (very light purple morphotype)	Oranfe, Ile-ife.	07° 30′ N, 04° 34′ E
O. tenuiflorum (t_3) (deep purple morphotype)	Oranfe, Ile-ife. Parakin, Ile-ife. Moro, Osun State. Osu, Osun State. Ilobu, Osun State.	07° 30′ N, 04° 34′ E 07° 29′ N, 04° 32′ E 07° 32′ N, 04° 27′ E 07° 28′ N, 04° 40′ E 07° 50′ N, 04° 29′ E

Table 2: Intraspecific Crosses Carried out on the three O. tenuiflorum Morphotypes Studied.

	***O. tenuiflorum* (t_1) (green morphotype)**	***O. tenuiflorum* (t_2) (very light purple)**	***O. tenuiflorum* (t_3) (deep purple)**
***O. tenuiflorum* (t_1) (green morphotype)**	-	green (t_1) x very light purple (t_2)	Green (t_1) x deep purple (t_3)
***O. tenuiflorum* (t_2) (very light purple morphotype)**	Very light purple (t_2) x green (t_1)	-	Very light purple (t_2) x deep purple (t_3)
***O. tenuiflorum* (t_3) (deep purple morphotype)**	Deep purple (t_3) x green(t_1)	Deep purple (t_3) x very light purple (t_2)	-

Table 3: Chi Square Analysis of Segregation Pattern of Morphotypes of *Ocimum tenuiflorum* at F_1 Generation.

Crosses	Generation	Morphotypes				Expected Ratio	Calculated X^2 values
		Green	Very Light Purple	Light Purple	Deep Purple		
Green x Very Light Purple	F_1	106	94	-	-	1:1	0.72
Green x Deep Purple	F_1	-	101	89	-	1:1	0.38
Very Light Purple x Deep Purple	F_1	-	51	94	47	1:2:1	0.25
Very Light Purple x Green	F_1	100	104	-	-	1:1	0.70
Deep Purple x Green	F_1	-	98	85	-	1:1	0.40
Deep Purple x Very Light Purple	F_1	-	63	90	50	1:2:1	0.27

Table 4: Chi Square Analysis of Segregation of purple pigmentation in *Ocimum tenuiflorum* **morphotypes at F_2 Generation**

Crosses	Generation	Morphotypes				Expected Ratio	Calculated X^2 values
		Green	Very Light Purple	Light Purple	Deep Purple		
Green x Very Light Purple (very Light Purple)	F_2	29	35	20	-	1:2:1	0.74
Deep Purple x Green (Very Light Purple)	F_2	7	14	3	-	1:2:1	0.66
Green x Deep Purple (Light Purple)	F_2	11	22	5	5	1:2:3:2	0.73
Green x Deep Purple (Light Purple)	F_2	32	31	9	2	1:2:3:2	0.47
Green x Deep Purple (Light Purple)	F_2	37	72	32	9	1:2:3:2	0.82
Deep Purple x Very Light Purple (Deep Purple)	F_2	-	-	60	108	1:2	0.96
Deep Purple x Very Light Purple (Light Purple	F_2	-	-	148	-	1	-
Deep Purple x Very Light Purple (Light Purple	F_2	79	55	32	6	1:2:3:2	0.45

Table 5: Assignment of Genotypes Based on the Duplicate Gene Hypothesis for Inheritance of Purple Pigment in *Ocimum tenuiflorum* morphotypes studied

Genotypes	Phenotypes	Morphotypes	Number of alleles Contributing to pigmentation
$P_1P_1P_2P_2$	Very Deep Purple	t_5	4
$P_1P_1P_2p_2$ $P_1P_1p_2P_2$ $P_1p_1P_2P_2$ $p_1P_1P_2P_2$	Deep Purple Deep Purple Deep Purple Deep Purple	t_3 t_3 t_3 t_3	3 3 3 3
$P_1p_1P_2p_2$ $P_1P_1p_2p_2$ $p_1p_1P_2P_2$	Light Purple Light Purple Light Purple	t_4 t_4 t_4	2 2 2
$P_1p_1p_2p_2$ $p_1p_1P_2p_2$	Very Light Purple Very Light Purple	t_2 t_2	1 1
$p_1p_1p_2p_2$	Green	(t_1)	0

Note: P- gene for purple colouration, p- gene for green colouration

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