

Polyploidization and speciation: patterns of natural hybridization and gene flow in basil (*Ocimum* spp.)

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Abstract

The genus *Ocimum* maintains rich diversity of species through natural and artificial outcrossing. Africa is named as one of the epicentres of plant diversity including numerous *Ocimum* species. Cytological evidence has attributed such diversity to extensive genome augmentation through selection, speciation, polyploidy and hybridization. This study identified polyploidy as the major natural phenomenon that drives successful hybridization programs or otherwise, within and among four *Ocimum* species studied and showed rate of gene exchange among the concerned species. All *Ocimum* species and variants used were grown for two generations before hybridization experiments were carried out. Interspecific and intraspecific reciprocal crossing was done. The crossed flowers were monitored for flower abortion and fruit formation. The resulting progenies from successful crosses were raised along with their parents and evaluated for all identifiable agronomic character differences and similarities. All viable seeds resulted from crosses involving at least one polyploid parent (tetraploids *O. basilicum*, *O. canum* and *O. americanum*). The intra- and interspecific hybridization programs recorded success within *O. basilicum* and between *O. basilicum* and tetraploid *O. canum*. Hybridization within *O. canum* was difficult due to differences in ploidy levels of *O. canum* parents used. Hybrid progenies obtained within *O. basilicum* parents (b_1 and b_2) and between b_2 and tetraploid *O. canum* possessed morphological characters related to b_2 parent while offspring from crosses between b_1 and c_1 combined characters from both parents. Pollen fertility in both *O. basilicum* and *O. canum* parents and their reciprocal crosses was more than 50 percent. *O. basilicum* displayed higher gene exchange capacity than other species. *O. kilimandscharicum* and *O. americanum* parents exhibited allopatric behaviour and low crossability with other seemingly sympatric species used in the study. Genome imbalance and incompatibility were associated with some of the possible causes of sterility.

Keywords: hybrid; hybridization; *Ocimum*; polyploidy; speciation

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Introduction

Polyploidy, the duplication of either single (autopolyploid) or combinations of two or more different genomes (allopolyploids) is regarded as a dead end of evolution. Although, studies have shown that majority of available crops are polyploids with high diversity (Leitch and Leitch, 2008), however allopolyploids have been of more importance to plant breeders, evolutionists and systematists because they contribute more- to plant evolution, speciation and adaptation through interspecific hybridization (Gaebelein *et al.*, 2019). Despite the high rate of interspecific hybridization among polyploids, diversity is still minimal which might be contradicting the success of polyploidy in plant evolution and speciation. Members of *Ocimum* genus show wide variation in their ploidy levels and genome contents (Mukherjee and Datta, 2006; Matthew and Oziegbe, 2016). Polyploidy, selection, speciation and hybridization have been named as major causes of variability among *Ocimum* species (Rao *et al.*, 2011; Matthew and Oziegbe, 2016; Oziegbe *et al.*, 2016). Therefore, the use of a natural outcrosser like *Ocimum* in hybridization studies and determination compatibility levels in the genus is paramount. Also, the study will reveal pattern of gene flow which is critical to ascertain dominating factors contributing to evolution within the genus.

The genus *Ocimum* (family Lamiaceae) is one of the popular group of herbs in the world (Rewers and Jedrzejczyk, 2016). Many wild and domesticated, annual and perennial *Ocimum* species are distributed in tropical and subtropical regions of Asia, Africa, Central and South America (Sarwat *et al.*, 2016). In Nigeria, four *Ocimum* species have been reported which include, *O. basilicum* L., *O. canum* Sims., *O. gratissimum* L. and *O. suave* Willd (Burkill, 1995). The popularity of *Ocimum* species results from their nutritional values in addition to the industrial and medicinal applications of their biologically active components such as phenolic compounds which are of antioxidants, nematocidal, fungistatic and microbial activity (Patel *et al.*, 2015). More than 150 species, possessing great variation in morphology and biology, essential oil content and chemical composition have been identified (Pushpangadan and Bradu, 1995). Despite the variability within the genus, purple pigmentation has been recognised as an important phenotypic marker within the genus *Ocimum*.

Polymorphism has been reported within the genus *Ocimum* which was attributed to inter-specific hybridization. The presence of diverse basil species has been associated with their outcrossing capability and long-term cultivation, leading to their faster adaptability to new environments (Hasegawa *et al.*, 1997; Smitha and Tripathy, 2016). Two major groups have been identified in the genus; Basilicum and Sanctum groups (Khosla and Sobti, 1985). Members of the group Basilicum have greater outcrossing ability than members of Sanctum group, despite, individual species within the groups still maintain their identity which are both genetic and morphological. Genetic variation, a prerequisite for successful improvement in plant population can result to production of hybrids (Ene *et al.*, 2016). While important hybrids and polyploids which confer adaptive advantages can aid establishment in newer environment, the mode of gene exchange and role of reproductive isolation on polyploids and hybrids within the genus is not clear. The report of Peng *et al.* (2005) concluded that plant taxa, isolation mechanisms, genome homologies can be determined using experimental hybridization. In the light of this, it was then opined that experimental hybridization will shed more light on the mechanism of gene exchange and speciation within the genus using different allopatric and sympatric species with different ploidy levels.

This study was therefore carried out in order to understand the mechanism of gene exchange/flow and elucidate the evolutionary relationship that exists among *Ocimum* species using experimental hybridization as a tool. Moreover, this study was expected to answer some questions such as: (1) What is the success rate of hybridization between the sympatric and allopatric species i.e., species inhabit in the same and different geographical locations respectively? (2) what roles have ploidy levels played in *Ocimum* speciation? (3) Does the morphological analysis support the presence of natural hybrid?

Materials and Methods

Collection and cultivation of Ocimum species

The study was carried out in screen house and experimental garden of the Department of Botany, Obafemi Awolowo University, Ile-Ife to ensure there were no cross pollination and segregation respectively. The *Ocimum* variants used in this study were obtained from germplasm collections of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Six *Ocimum* variants of four species *O. canum* (c₁) 2n=4x=52, *O. canum* (c₂) 2n=2x=24, *O. basilicum* (b₁) 2n=4x=52, *O. basilicum* (b₂) 2n=6x=72, *O. americanum* 2n=4x=52 and *O. kilimandscharicum* (2n=6x=76) were used for the study (Matthew and Oziegbe, 2016; Oziegbe *et al.*, 2016).

Seeds of each *Ocimum* variants were germinated on moistened filter paper in petri dishes at room temperature. At 4-leaf stage, seedlings were transferred into small, perforated plastics cups filled with topsoil and were kept in the screen house till the seedlings were established. The seedlings were transplanted three weeks after germination into plastic buckets filled with topsoil at the rate of one plant per perforated bucket using complete randomized design (CRD).

Interspecific and intraspecific hybridization studies

Each *Ocimum* species and variants were grown for two generations before hybridization experiments were carried out on them to ensure that they were breeding true. Interspecific and intraspecific reciprocal crosses were carried out within and between the species studied. Physical emasculation of flower buds of each ovulate parent was carried out between 9.00 am - 1.00 pm before self-pollination occurs. Pollen grains from the desired pollen parents were transferred to the emasculated flowers on the ovulate parents between 1.30 pm - 2.30 pm when the stigmas were receptive.

Each crossed flower was bagged and labelled to prevent contamination by external pollens and for identification respectively. The crossed flowers were monitored for flower drop and fruit formation. The F₁ plants from successful crosses were raised along with their parents and evaluated for all identifiable agronomic character differences and similarities.

Morphological studies and statistical analysis

The characters studied include, a) Leaf-leaf length, leaf breadth and petiole length, b) Stem- plant height at first flowering, number of primary branches and plant hairiness, c) Flower- anther colour, filament colour, petal colour, stigma colour, style colour, sepal, colour, calyx colour, anther dehiscence and seed colour. d) Fruits-length of inflorescence, number of fruits per inflorescence and number of seeds per fruits, percentage seed set. e) Pollen fertility study- was determined by staining the pollen grains from just dehisced anthers in the parents and the F₁ hybrids with cotton blue in lactophenol for 48 hours. Full pollen grains with the cytoplasm contents stained uniformly with cotton blue in lactophenol were counted as viable pollens while those that do not stain or partially stained and with collapsed outline were counted as non-viable (Olorode and Baquar, 1976). Percentage pollen fertility was determined for each species, variants and F₁ hybrids using equation 1 as follows:

$$\text{Percentage pollen fertility} = \frac{\text{Total fertile}}{\text{Total pollen count}} \times 100$$

All quantitative parameters observed for the parents and their F₁ hybrids were subjected to data analysis. One-way Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) were used to separate the means.

Results

Interspecific and Intraspecific Hybridization Studies

Intraspecific crosses

Intraspecific crosses of *Ocimum basilicum* (b₁) x *O. basilicum* (b₂) produced viable seeds for both reciprocal crosses (Table 1). The F₁ plants generated were vigorous and floriferous in both reciprocal crosses (Table 1, 2 and 3; Figure 1; Plate1). The leaf length and breadth of *O. basilicum* (b₂) was significantly higher (5.46±0.08 cm and 2.76±0.071 cm) compared to the *O. basilicum* (b₁) and their F₁ hybrids. *Ocimum. basilicum* (b₂) and *O. basilicum* (b₁) x *O. basilicum* (b₂) had significantly longer petiole of 2.050.01 cm and 1.7000.05 cm respectively. The F₁ hybrids had significantly longer inflorescence compared to their parents. Only *O. basilicum* (b₁) had fruit set (79.83%) and seed set (54.25%) above 50% (Figure 1). However, the least pollen fertility (76.66%) observed was in *O. basilicum* (b₂) x *O. basilicum* (b₁) (Figure 1). The hybrids exhibited annual habit and morphological resemblance to the *O. basilicum* (b₂) parent in both reciprocal crosses (Table 3 and Plate 1). Intraspecific crosses between *O. canum* (c₁) x *O. canum* (c₂) failed and did not result in seed set (Table 3).

Interspecific crosses

Only reciprocal crosses between *Ocimum basilicum* species and *O. canum* (c₁) produced F₁ hybrids with viable seeds (Table 1). The leaf length of *O. canum* (c₁) was significantly longer (6.84±0.08 cm) compared to *O. basilicum* (b₁) and their F₁ hybrids. The least leaf breadth (1.85±0.03 cm) was observed in *O. basilicum* (b₁) compared to *O. canum* (c₁) and their F₁ hybrids. There were no significant differences in petiole and inflorescence lengths of *O. basilicum* (b₁), *O. canum* (c₁) and their F₁ hybrids. The least fruit set (38.37%) and seed set (26.64%) in the crosses between *O. basilicum* (b₁) and *O. canum* (c₁) was observed in the F₁ hybrid of *O. canum* (c₁) x *O. basilicum* (b₁) as showed in Figure 1. Percentage pollen fertility of the two parents and their reciprocal F₁ hybrids were far above 60% (Table 2, Figure 1 and Plate 1). The hybrids showed intermediate morphological characters between the two parents (Table 3 and Plate 1). The F₁ hybrid plants of *O. basilicum* (b₁) x *O. canum* (c₁) were vigorous and floriferous (Tables 3). The leaf length, leaf breadth and petiole length of 8.45±0.19 cm, 4.90±0.16 cm and 2.69±0.12 cm respectively were observed in *O. basilicum* (b₂) x *O. canum* (c₁) and these were significantly higher than that of the parents and the reciprocal F₁ hybrid while the significantly longest inflorescence (21.81±0.65 cm) was observed in the *O. canum* (c₁) x *O. basilicum* (b₂) (Table 2). The highest fruit set (69.45%) and seed set (49.12%) were observed in *O. canum* (c₁) while the least pollen fertility (59.00%) was observed in the F₁ hybrids (Table 2, Figure 1 and Plate 1). The F₁ hybrid plants of *O. canum* (c₁) x *O. basilicum* (b₂) exhibited annual habit and the hybrids showed morphological resemblance to *O. basilicum* (b₂) (Table 3 and Plate 1).

Some interspecific crosses produced F₁ hybrid plants which were sterile. These include *O. basilicum* (b₂) x *O. americanum*, *O. basilicum* (b₂) x *O. kilimandscharicum*, *O. canum* (c₁) x *O. americanum* and *O. canum* (c₂) x *O. americanum* (Table 1 and 2, Figure 1 and Plate 1).

Weak F₁ hybrid was produced in the crosses of *O. basilicum* (b₁) x *O. americanum* which germinated but died before maturity (Table 1) while non-viable F₁ hybrids seeds were formed in the crosses of *O. canum* (c₂) x *O. basilicum* (b₁), *O. americanum* x *O. basilicum* (b₁) and *O. americanum* x *O. canum* (c₁) which failed to germinate. All other crosses failed to produce F₁ hybrid seeds (Table 1).

Table 1. Summary of reciprocal intraspecific and interspecific crosses among the *Ocimum* species studied

♂ \ ♀	<i>O. basilicum</i> (b ₁) (2n=52)	<i>O. basilicum</i> (b ₂) (2n=72)	<i>O. canum</i> (c ₁) (2n=52)	<i>O. camum</i> (c ₂) (2n=24)	<i>O. americanum</i> (2n=52)	<i>O. kilimandscharicum</i> (2n=76)
<i>O. basilicum</i> (b ₁) (2n=52)	-	VF	VF	F	WK	F
<i>O. basilicum</i> (b ₂) (2n=72)	VF	-	VF	F	VIF	VIF
<i>O. canum</i> (c ₁) (2n=52)	VF	VF	-	F	VIF	F
<i>O. camum</i> (c ₂) (2n=24)	NV	F	F	-	VIF	F
<i>O. americanum</i> (2n=52)	NV	F	NV	F	-	F
<i>O. kilimandscharicum</i> (2n=76)	F	F	F	F	F	-

VF- F₁ hybrid flowered and produced abundant seed.

VIF- F₁ hybrid flowered but highly sterile.

WK - Weak F₁ hybrid (failed to flower).

NV - Hybrid seed formed but failed to germinate.

F - Hybridization failed to result in seed set.



Plate 1. *Ocimum* spp. and their F₁ hybrids. **A.)** *O. basilicum* (b₁); **B.)** *O. basilicum* (b₂); **C.)** *O. canum* (c₁); **D.)** *O. canum* (c₂); **E.)** *O. americanum*; **E.)** *O. kilimandscharicum*; **F.)** Reciprocal crosses between *O. basilicum* (b₁) x *O. basilicum* (b₂); **G.)** Reciprocal crosses between *O. basilicum* (b₁) x *O. canum* (c₁); **H.)** Reciprocal crosses between *O. canum* (c₁) x *O. basilicum* (b₁); **I.)** Reciprocal crosses between *O. canum* (c₁) x *O. basilicum* (b₂); **J.)** *O. basilicum* (b₂) x *O. americanum*; **K.)** *O. basilicum* (b₂) x *O. kilimandscharicum*; **L.)** *O. canum* (c₁) x *O. americanum*; **M.)** *O. canum* (c₂) x *O. americanum*.

Table 2. Quantitative characteristics of *Ocimum* spp. and their F₁ hybrids

Characters	Leaf length (cm)	Leaf breadth (cm)	Petiole length (cm)	Inflorescence length (cm)
<i>O. basilicum</i> (b ₁)	3.85±0.07a	1.85±0.03a	1.65±0.07ab	17.01±0.72cd
<i>O. basilicum</i> (b ₂)	5.46±0.08f	2.76±0.071e	2.05±0.01de	15.47±0.66cd
<i>O. canum</i> (c ₁)	6.84±0.08i	2.18±0.04cd	1.76±0.08abc	12.42±1.15b
<i>O. canum</i> (c ₂)	4.07±0.07ab	2.08±0.05bc	2.58±0.09f	9.58±0.51a
<i>O. americanum</i>	4.45±0.13c	1.87±0.05ab	1.69±0.07a	12.06±0.51b
<i>O. kilimandscharicum</i>	5.31±0.11ef	3.30±0.12f	2.49±0.12f	16.89±1.48efg
<i>O. basilicum</i> (b ₁) x <i>O. basilicum</i> (b ₂)	4.063±0.06ab	2.177±0.04cd	1.700±0.05cde	19.52±0.65ef
<i>O. basilicum</i> (b ₂) x <i>O. basilicum</i> (b ₁)	4.441±0.12c	2.359±0.07d	1.976±0.06b	21.00±0.80fgh
<i>O. basilicum</i> (b ₁) x <i>O. canum</i> (c ₁)	5.07±0.09de	2.13±0.05cd	1.6767±0.07ab	16.32±0.52cd
<i>O. canum</i> (c ₁) x <i>O. basilicum</i> (b ₁)	4.93±0.09d	2.14±0.05cd	1.68±0.07ab	17.65±0.79de
<i>O. basilicum</i> (b ₂) x <i>O. canum</i> (c ₁)	8.45±0.19j	4.90±0.16g	2.69±0.12f	20.05±1.12cd
<i>O. canum</i> (c ₁) x <i>O. basilicum</i> (b ₂)	5.55±0.08f	2.680±0.05e	1.815±0.09bcd	21.81±0.65gh
<i>O. basilicum</i> (b ₂) x <i>O. americanum</i>	4.26±0.080bc	2.12±0.06cd	1.50±0.05a	22.50±0.81h
<i>O. basilicum</i> (b ₂) x <i>O. kilimandscharicum</i>	5.90±0.10g	3.28±0.11f	2.56±0.13f	19.34±0.63ef
<i>O. canum</i> (c ₁) x <i>O. americanum</i>	7.15±0.12h	3.55±0.07f	2.67±0.05e	22.16±0.63h
<i>O. canum</i> (c ₂) x <i>O. americanum</i>	3.73±0.08a	1.70±0.05a	1.52±0.07a	14.81±0.27c

Table 3. Qualitative characteristics of *Ocimum* spp. and their F₁ hybrids

Characters	Anther colour	Petal colour	Style colour	Sepal colour	Calyx colour
<i>O. basilicum</i> (b ₁)	White	White	White	Green	Green
<i>O. basilicum</i> (b ₂)	White	White	Purple	Purple	Purple
<i>O. canum</i> (c ₁)	White	White	Light Purple	Green	Green
<i>O. canum</i> (c ₂)	White	White	Purple	Green	Green
<i>O. americanum</i>	White	White petal with purple tinges	Purple	Purple	Purple
<i>O. kilimandscharicum</i>	Orange	White	Purple	Purple	Purple
<i>O. basilicum</i> (b ₁) x <i>O. basilicum</i> (b ₂)	White	White	Purple	Purple	Purple
<i>O. basilicum</i> (b ₂) x <i>O. basilicum</i> (b ₁)	White	White	Purple	Purple	Purple
<i>O. basilicum</i> (b ₁) x <i>O. canum</i> (c ₁)	White	White	Purple	Green	Green
<i>O. canum</i> (c ₁) x <i>O. basilicum</i> (b ₁)	White	White	Purple	Green	Green
<i>O. basilicum</i> (b ₂) x <i>O. canum</i> (c ₁)	White	White	Purple	Purple	Purple
<i>O. canum</i> (c ₁) x <i>O. basilicum</i> (b ₂)	White	White	Purple	Purple	Purple
<i>O. basilicum</i> (b ₂) x <i>O. americanum</i>	White	white petal with purple tinges	Purple	Purple	Purple
<i>O. basilicum</i> (b ₂) x <i>O. kilimandscharicum</i>	Orange	White	Purple	Purple	Purple
<i>O. canum</i> (c ₁) x <i>O. americanum</i>	White	White petal with purple tinges	Purple	Purple	Purple
<i>O. canum</i> (c ₂) x <i>O. americanum</i>	White	White petal with purple tinges	Purple	Purple	Purple

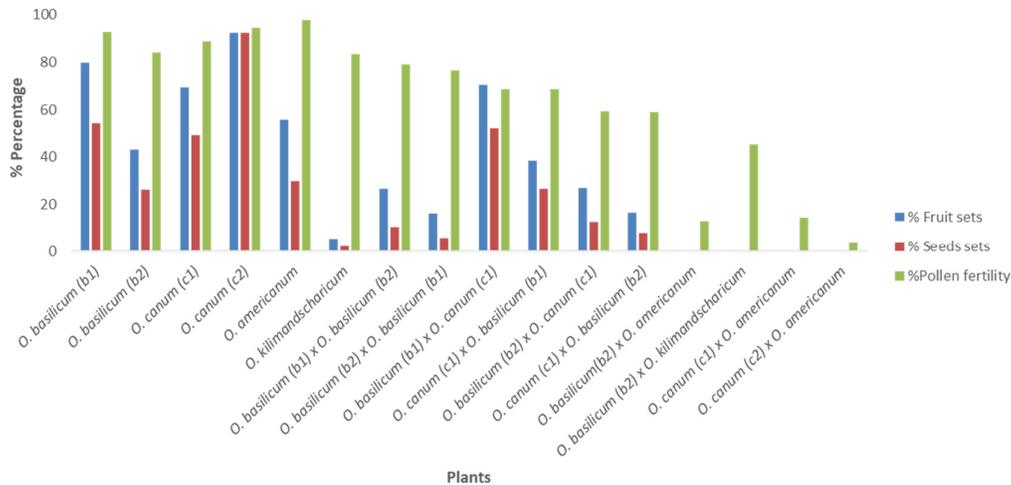


Figure 1. Percentage fruit sets, seed sets and pollen fertility of *Ocimum* spp. and their F₁ hybrids

Discussion

The hybridizations carried out in this study provided information on intraspecific and interspecific relationships among *Ocimum* group Basilicum. The genus *Ocimum* has long been acclaimed to be of high diversity (James *et al.*, 1999) with natural interspecific and intraspecific hybridization and polyploidy being common occurrences within this genus (Harley and Heywood, 1992). Pyne *et al.* (2018) reported high morphological diversity in the genus as a result of the outcrossing capability and longstanding cultivation of its members which was made possible by their floral structure that is suitable for pollination through insect visitations (Khosla, 1986).

The reciprocal intraspecific crosses between tetraploid *O. basilicum* (b₁) and hexaploid *O. basilicum* (b₂) produced hybrid seeds. The result showed compatibility in the genome of the species of *O. basilicum* studied. *O. basilicum* are not genetically isolated from one another, hence, interbreeds freely notwithstanding the genome content or variation (Paton and Putievsky 1996). Although, the fruit and seed set were low while the pollen fertility was high. This shows low hybrid vigour which might be due to considerable genome imbalance and incompatibility. Idowu and Oziegbe (2017) reported allopolyploidy in the genome of *O. basilicum*. This might create complication at meiotic cell division stages. Also, less stability had been reported in the hybrids allopolyploids, although, such hybrids drive speciation and evolutionary flux after meiotic stability (Coyne and Orr, 2004).

Intraspecific crosses in *O. canum* failed. Crosses between *O. canum* (c₂) x *O. canum* (c₁) formed non-viable seeds. This might be as a result of genome imbalance knowing that the c₂ is a diploid while c₁ is a polyploid relative (Pushpangadan and Sobti, 1982; Matthew and Oziegbe, 2016; Idowu and Oziegbe 2017). The results from the present study suggest that diversity at ploidy level in *Ocimum* indicates polyploidy or aneuploidy which can act as a partial or complete barrier to hybridization and gene flow (Levin 2002, Alix *et al.*, 2017; Lekhapan *et al.*, 2019). The evolutionary difference between the variants of *O. canum* might have deepened the reproductive isolation between them to the extent of making them genetically incompatible and uncrossable. Studies have shown that most diploids are often incompatible with their polyploid relatives (Schiessl *et al.*, 2018; Viruel *et al.*, 2019). Moreover, polyploidy has been reported to drive evolutionary process through speciation (Viruel *et al.*, 2019). *Ocimum canum* (c₁) might have arisen from genome duplication/polyploidization of *O. canum* (c₂) (Pushpangadan and Sobti, 1982). This study showed

incompatibility in the species *O. canum*. Idowu and Oziegbe (2017) reported F₁ hybrid seeds from the crosses of *O. canum* (c₂) x *O. canum* (c₁) whose plants were sterile. Mason and Batley (2015); Lloyd *et al.* (2014) reported that the adaptability of most new hybrid plants are gradual with low probability in genetic stability, meiotic stability and genome arrangements. These factors are very critical in plants and hybrid success in viability and fertility. Stebbins (1971) reported that hybrid sterility may be as a result of breakdown in the early stage of embryo development, which is often due to disharmony between the embryo and the endosperm which provides food material for its growth and the maternal tissue.

Only reciprocal interspecific crosses between *O. basilicum* and *O. canum* (c₁) produced viable F₁ hybrid seeds that grew to F₁ plants. Meiotic pairing of the genome of basil had revealed high allopolyploidy in the genus *Ocimum* for the polyploid plant (Pyne *et al.*, 2018). Despite allopolyploidy being reported to drive speciation and evolution (Alix *et al.*, 2017), stability of the hybrid seed produced is not guaranteed, and these were evident in the low seed and fruit sets according to this study from crosses between *O. basilicum* and *O. canum* (c₁). Although, the fruit and seed sets observed between *O. basilicum* (b₁) x *O. canum* (c₁) were above 50% which might have been as a result of genome balance at meiotic division. Furthermore, the result showed that *O. canum* is a better ovulate parent due to their crossbreeding habit with other species.

Ocimum kilimandscharicum and *O. americanum* displayed allopatric behaviour and failed to produce useful F₁ hybrid in most of their crosses with *O. basilicum* and *O. canum*. Marko (2008) stated that negligible gene exchange capacity from some specific members of a population to others make the concerned members evolutionary distant and impedes hybridization programs. Both *O. canum* species produced F₁ hybrid seed with *O. americanum*, nevertheless, the F₁ plants were sterile which might be as a result of post-zygotic isolation. *Ocimum canum* and *O. americanum* have often been referred to as siblings, hence, gene exchange between them although insignificant (Chowdhury *et al.*, 2017; Shanaida *et al.*, 2017). Also, one *O. basilicum* variant (b₁) crossed with *O. americanum*, produced a weak F₁ hybrid plant. *Ocimum basilicum* had often been reported to have high crossability, even though reproductive isolation often play a role in this ability (Paton and Putievsky, 1996). However, *O. basilicum* (b₂) is the only variant to produce F₁ hybrid plant with *O. kilimandscharicum* although F₁ hybrid plant was sterile. *Ocimum kilimandscharicum* is considered to have speciated considering the plant morphological characters and its gene exchange habit in this study with other variants (Vieira *et al.*, 2003). The high ploidy level of *O. basilicum* (b₂) and *O. kilimandscharicum* makes them more prone to cross ability, pairing abnormalities might have led to sterility of their progenies (Lekhapan *et al.*, 2019). This shows that polyploidy does not always result to successful hybrid production not until reproductive isolation is dealt with (Mayrose *et al.*, 2011; Alix *et al.*, 2017). However, polyploidy is still a major drive in speciation and evolution through hybridization (Madlung and Wendel, 2013; Palchetti *et al.*, 2020).

The contributory effect of polyploid in the drive of evolution and speciation cannot be over emphasised and this was also evident from the result of this study. Hybridization and exchange of genes was at the highest among *Ocimum* variants that has higher ploidy level. Figure 2 showed the probable origin of new species through polyploidisation and hybridization. Storme and Mason (2014) reported that speciation and diversity in plant is dependent on plant ploidy level and its individual genome level while hybridization (interspecific hybridization) drive the whole process (Mason and Batley, 2015; Kathe *et al.*, 2019). There were observable diversities among the F₁ hybrid plants reported in this study, although, high sterility was also observed among the hybrids which might be as a result of meiotic imbalance and hybrid instability. Anomaly at meiotic chromosome segregations also often result in sterility and non-viability in F₁ plants (Storme and Mason, 2014).

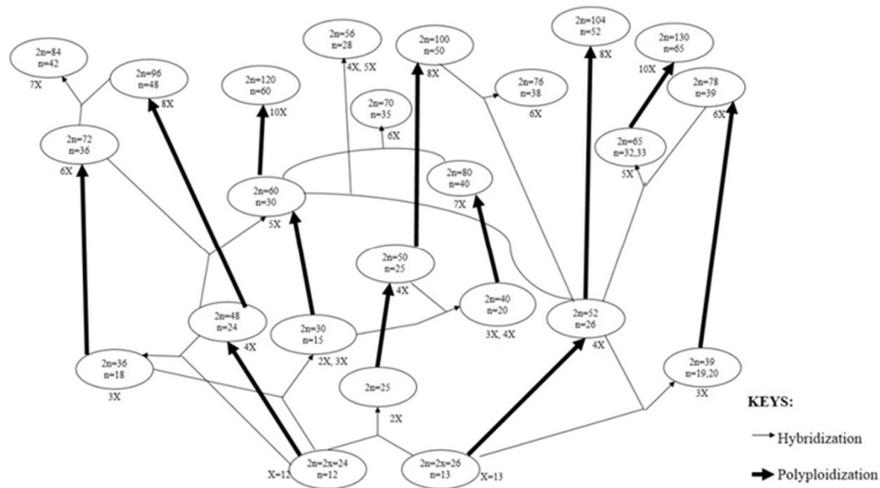


Figure 2. Possible relationship among *Ocimum* species studied based on present and previous findings

The exchange of genes that exist within *Ocimum* may be responsible for the wide adaptation and broad diversity found in the genus. Ehrendorfer (1959) and Rattenbury (1962) stated that speciation occurs most effectively and rapidly as a result of hybridization-isolation cycles, in which the gene pool is first enriched by hybridization among adaptively different populations and stable derivatives are then extracted from the resulting hybrid swarms by isolation and natural selection for particular adaptive properties. Stebbins (1968) stated that effectiveness of hybridization-isolation cycle is found in genera having highly specialized mechanisms for insect pollination.

Conclusions

From this study, the mechanisms of speciation among the *Ocimum* species studied are pre-zygotic and post-zygotic reproductive isolation. Polyploidy, diversity in basic chromosome numbers and hybridization have played significant roles in the evolution of species in the genus *Ocimum*. The study showed that polyploid species interbreed freely, although, with high level of post-zygotic mechanisms. The effect of pre-zygotic isolation mechanism (geographic reproductive isolation) was evident among allopatric and sympatric species study. However, the study showed the important role played by polyploid species in gene exchange which was evident in crossing abilities of *O. basilicum* (b_2) and *O. canum* (c_2) among both close and distant relative species. Stability of products of hybrids from these two species will be important in evolutionary drive and speciation. The gene exchange occurring among the *Ocimum* species might have created hybrid swarms which enrich the gene pool of the genus *Ocimum* leading to high adaptation and broad diversity within the genus *Ocimum*. This study shows that morphologically each species and hybrids still retain their characteristic identity. In order to broaden the knowledge of evolutionary relationship in the genus, further karyotypic studies to reveal karyological similarities and differences among *Ocimum* species is recommended.

Authors' Contributions

MO supervised the project; all the research were carried out by JOM. Data curation was done by JOM and MEO while JOM, SOA and TEA compiled the manuscript. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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