American Journal of Plant Sciences, **2014, 5, 126-131**
Published Online January 2014 (http://www.scirp.org/journal/ajps)
http://dx.doi.org/10.4236/ajps.2014.51016

Karyotype Analysis of *Ocimum basilicum* in South-Eastern Nigeria

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Received October 15th, 2013; revised December 18th, 2013; accepted January 3rd, 2014

ABSTRACT

***Ocimum basilicum* is widely distributed in the tropical and subtropical regions of the world, with greatest variability in Africa and India. It is valued in many countries for its culinary, medicinal, industrial and religious importance. Although cytogenetic entries on the plant have been made in other geographical locations of the world, in Nigeria, such entries, prior to this report, have been limited if not completely unavailable. In this analysis, axillary buds, obtained from growing plants, were used to conduct mitotic study. Results from this study showed chromosome counts of 2n = 48 and 60, thus bringing to light the existence of chromosome number variation and the possibility of polyploidy at different levels in the plant species in this agro-ecological zone. This research has, therefore, established that at least there are two cytotypes in the population of *Ocimum basilicum* growing in the humid forest vegetation zone of Nigeria. Analysis of the two cytotypes revealed asymmetrical karyotypes, indicative of advancement in the evolutionary trend of the plant species.**

KEYWORDS

Karyotype; Cytogenetics; Cytotype; Ideogram; Mitotic; Polyploidy

1. Introduction

Ocimum basilicum, known commonly as sweet basil, is an important aromatic plant of the *Lamiaceae* family, which has about 252 genera and 6700 species, most of which are medicinal [1,2]. The genus, *Ocimum*, is cosmopolitan with its distribution spanning from the Mediterranean to Central Asia, with greatest variability in Africa and India. Among more than 150 species of *Ocimum*, sweet basil is the major essential oil crop commercially cultivated in many countries [3]. It is found in tropical Asia, Africa, America and subtropical regions of the world, from sea level to an altitude of about 1500 m [4,5].

Ocimum basilicum thrives in full sunshine, requiring 0.6 m - 4.2 m rainfall, soil pH of 4.3 to 8.2 and well drained sandy loam soils [6,7]. Clayey soils and waterlogged areas are unsuitable for the cultivation of the plant. It is susceptible to frost and the crop growth can be adversely affected in areas which experience heavy and continuous rainfall [7]. Sweet basil is an herbaceous plant with plant height ranging between 0.3 m and 1 m. The leaves are ovate, often puckered; flower pink or white, with fruits having four small nutlets which are mucilaginous when wet [4].

Elaborate scientific investigations, ranging from phytochemistry, pathogenic and insecticidal activities, utilization as a spice and flavouring ingredient etc. have been extensively catalogued for *Ocimum basilicum*. Reviews of the phytochemical and pharmacological studies on the plant indicate that it possesses analgesic, anti-inflammatory, antimicrobial, antioxidant, antiulcerogenic, cardiac stimulant, chemomodulatory, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulatory and larvicidal activies [8-17]. Cardioprotective activity of ethanolic extract of sweet basil against isoproterenol-induced myocardial infarction has also been recently demon-

strated [18]. Reports of cytogenetic studies have shown that polyploidy and chromosome number variations are common among *Ocimum* species. However, two basic chromosome numbers (x = 8 and x = 12) have been reported, on the basis of which the various species of the genus have been classified into two groups: *basilcum* group (x = 12) and *sanctum* group (x = 8) [19,20]. This cytogenetic variability presents a veritable resource to geneticists and plant breeders to develop plant varieties well adapted to specific agro-ecological zones in response to the ever increasing demand for this all-important plant species.

However, in Nigeria, where there exists a great genetic diversity of *Ocimum* species, recognized and utilized for various purposes by the locals, information regarding their cytogenetic features had, prior to this work, been limited or completely unavailable. This paper, therefore, reports on the cytogenetic characteristics (karyotype) of *Ocimum basilicum* accessions in the humid forest vegetation zone of Nigeria.

2. Materials and Methods

2.1. Plant Materials

Seeds of *O. basilicum* were obtained from different locations in Cross River State, South-eastern region of Nigeria (Akpabuyo-KTA, Calabar South-ANA, Calabar Municipality-MUN and Akamkpa-AKP) and raised in polybags in the experimental farm of the University of Calabar, Nigeria between the months of February and March, 2010. **Table 1** provides geographic information of specific locations where plant materials were collected.

2.2. Karyotype Study

Axillary buds were obtained from the growing plants and pretreated in 8-hydroxyquinoline (0.002M), fixed in carnoy's solution and hydrolyzed in 1N hydrochloric acid at 60˚C. The hydrolyzed materials were rinsed in 70% alcohol and stained with FLP orcein before squashing. Prepared slides were viewed under a light microscope digitized with a Chinese made power shot A630 Canon camera (80 mega pixel, Canon PC 1201, NO 4126202101) and photographs of good cells were taken. Chromosomes were measured using R1370-19 ocular micrometer calibrated with OB2001 objective micrometer at 0.77 per unit. Ideograms of the species were accordingly constructed. Description of chromosomes was based on size, arm length, haploid set length (HSL), relative length, symmetry and form [21-23].

3. Results

The mitotic chromosome numbers, haploid set length, total long arm, total short arm, and karyotype formulae are presented in **Table 2**, while **Table 3** shows the karyotype form (%) of the species.

Table 1. Geographic information of locations of plant collection.

State	Local government area	Accession group tag	Community	Lat (˚N)	Long (˚E)
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	05.61	10.32
			Awi	05.63	10.32
			Ako	05.60	10.31

Table 2. Chromosome numbers, haploid set length, total long arm, total short arm and karyotype formulae of *O. basilicum*.

Species	2n	HSL (µm)	L (µm)	S (µm)	Karyotype formula
O. basilicum (CR_1)	48	89.34	59.86	29.48	4m + 6sm + 8a + 1st + 5t
O. basilicum (CR_2)	60	108.21	71.53	36.68	7m + 5sm + 10a + 4st + 4t

Table 3. Karyotype form (%).

	O. basilicum	
	2n = 48	2n = 60
Metacentric	16.67	23.33
Submetacentric	25.00	16.67
Acrocentric	33.33	33.33
Sub-telocentric	04.17	13.33
Telocentric	20.83	13.33

3.1. Karyotype Details of *O. basilicum*

Two chromosome numbers, 2n = 48 and 60 (**Plates 1** and **2**), were obtained in the four accession groups. The accessions with 24 pairs (2n = 48) of chromosomes (KTA, ANA and AKP) revealed a karyotype formula of 4m + 6sm + 8a + 1st + 5t. The mean total length of the chromosomes was 3.72 µm, while the means of the short and long arms were 1.23 µm and 2.49 µm, respectively (**Table 4**). The chromosomes ranged in size from 1.54 µm (chromosome 24) to 5.4 µm (chromosome 1), 16.67% of which were metacentric. The longest chromosome occupied 6.03% of the genome, while the shortest occupied 1.72% of the genome (**Figure 1**).

Table 4. Karyotype details of *O. basilicum*.

	CR_1 Population (2n = 48)					CR_2 Population (2n = 60)				
Chromosome Pair	L (m)	S (m)	TL (m)	R	RL (%)	L (m)	S (m)	TL (m)	R	RL (%)
1	2.7	2.7	5.4	1	6.04	2.7	2.7	5.4	1	4.99
2	3.85	1.54	5.39	2.5	6.03	3.85	1.54	5.39	2.5	4.98
3	3.08	2.31	5.39	1.33	6.03	2.7	2.31	5.01	1.17	4.63
4	2.70	2.31	5.01	1.17	5.61	3.08	1.54	4.62	2	4.27
5	2.31	2.31	4.62	1	5.17	2.31	2.31	4.62	1	4.27
6	2.31	2.31	4.62	1	5.17	2.31	2.31	4.62	1	4.27
7	3.08	1.54	4.62	2	5.17	2.31	2.31	4.62	1	4.27
8	2.31	2.31	4.62	1	5.17	3.08	1.54	4.62	2	4.27
9	3.08	1.16	4.24	2.7	4.75	3.85	0.77	4.62	5	4.27
10	3.08	0.77	3.85	4	4.31	1.93	1.93	3.86	1.5	3.57
11	2.31	1.54	3.85	1.5	4.31	2.31	1.54	3.85	1.5	3.56
12	2.31	1.54	3.85	1.5	4.31	3.08	0.77	3.85	4	3.56
13	1.93	1.54	3.47	1.25	3.88	2.31	1.54	3.85	1.5	3.56
14	1.93	1.54	3.47	1.25	3.88	3.08	0.77	3.85	4	3.56
15	3.08	-	3.08	-	3.45	2.31	1.54	3.85	1.5	3.56
16	2.31	0.77	3.08	3	3.45	2.7	0.77	3.47	3.5	3.21
17	2.31	0.77	3.08	3	3.45	1.93	1.54	3.47	1.25	3.21
18	3.08	-	3.08	-	3.45	2.31	0.77	3.08	3	2.85
19	3.08	-	3.08	-	3.45	2.31	0.77	3.08	3	2.85
20	2.31	0.77	3.08	3	3.45	1.54	1.54	3.08	1	2.85
21	2.31	-	2.31	-	2.59	2.31	0.77	3.08	3	2.85
22	1.54	0.77	2.31	2	2.59	3.08	-	3.08	-	2.85
23	1.54	0.77	2.31	2	2.59	2.31	0.77	3.08	3	2.85
24	1.54	-	1.54	-	1.72	2.31	0.77	3.08	3	2.85
25						1.93	0.77	2.7	2.5	2.50
26						2.7	-	2.7	-	2.50
27						1.16	1.16	2.32	1	2.14
28						2.31	-	2.31	-	2.13
29						0.77	0.77	1.54	1	1.42
30						1.54	-	1.54	-	1.42
Mean	2.49	1.23	3.72			2.38	1.23	3.61		

L—Long arm; S-short arm; TL—Total length; R—Arm ratio; RL—Relative length.

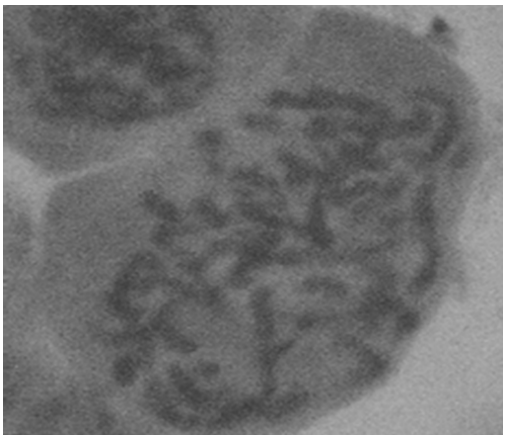

Plate 1. Somatic metaphase chromosomes of *Ocimumbasilicum* (CR_1 population, 2n = 48, Magnification 7600×).

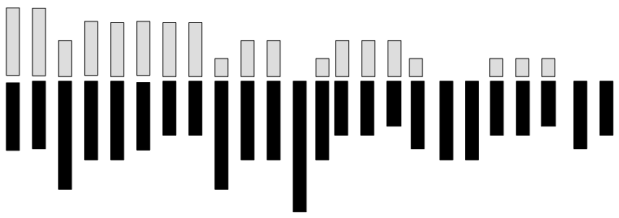

Figure 1. Idiogram of *Ocimumbasilicum* (CR_1 population, 2n = 48).

On the other hand, 30 pairs (2n = 60) of chromosomes were recorded in the MUN accession group, with 23.33% of metacentric chromosomes. Chromosomes of this group ranged from 1.54 µm (chromosome 30) to 5.4 µm (chromosome 1) in size, with a total mean length of 3.61 µm, mean short arm of 1.23 µm and mean long arm of

2.38 µm (Table 4). The population revealed a karyotype formula of 7m + 5sm + 10a + 4st + 4t, with 1.42% of the genome being occupied by the shortest chromosome, whereas the longest chromosome occupied 4.98% of the genome (Table 4, Figure 2).

4. Discussion

4.1. Variable Chromosome Numbers and Polyploidization in *Ocimum basilicum*

The chromosome counts of 2n = 48 and 60 recorded in this research groups the evaluated accessions into two cytotypes: group I—KTA, ANA and AKP (2n = 48) and group II—MUN (2n = 60). Chromosome number, 2n = 48 obtained in this work for this plant species agrees with earlier reports which posited that *O. basilicum* occurs in nature as a tetraploid species with chromosome count of 2n = 4x = 48 [7,24]. However, 2n = 60 in this plant species is a new number obtained in this study for the species. Other research results had shown variable chromosome numbers for this species: 2n = 48, 52 and 72 [7,25]. Based on the basic number of 12 for *O. basilicum* [20,24], it can be suggested that polyploidization, occurring at different levels, is a feature of this plant species. Hence, 2n = 48 and 60 indicate tetraploid and pentaploid, respectively.

The importance of this study, therefore, is that in the *O. basilicum* population in South-eastern Nigeria, at least one new cytotype (2n = 60) has evolved. This is an indication of the occurrence of polyploidy and new cytotypes in this species.

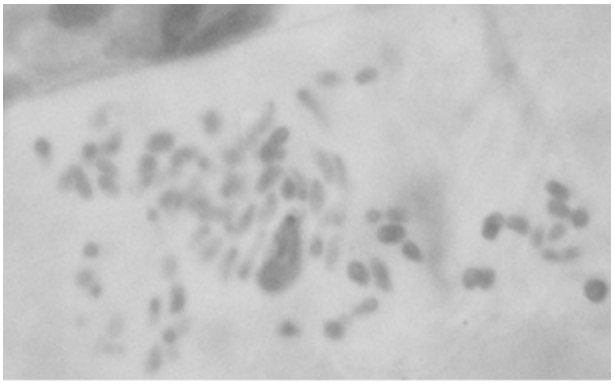

Plate 2. Somatic metaphase chromosomes of *Ocimumbasilicum* (CR_2 population, 2n = 60, Magnification 7300×).

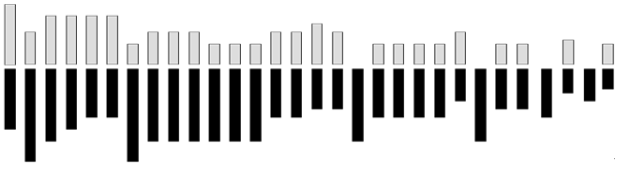

Figure 2. Idiogram of *Ocimumbasilicum* (CR_2 Population, 2n = 60).

The absence of diploid species in this species may be attributed to the observable fluctuations in the climatic conditions of this humid ecological zone, since polyploids are at a selective advantage in habitats which have been subjected to frequent changes in climatic and edaphic factors [26].

4.2. Karyotype Symmetry and Evolution

From the karyotype details obtained from this study, the plant species has asymmetrical karyotype. Asymmetrical karyotypes as those possessing many chromosomes with subterminal centromeres or greater differences in size between the largest and smallest chromosomes [26]. The karyotypes showed higher proportions of acrocentric chromosomes as compared to metacentric chromosomes and by implication cannot be said to be primitive. Chromosomes become more asymmetrical as evolution progresses, moving from submetacentric state towards acrocentric state in extreme cases [27]. However, a reversal of this trend occurs periodically, giving rise to a reduction in chromosome number [26]. This is possible because at the peak of asymmetry, all the chromosomes can be acrocentric and/or telocentric. Hence, two telocentric chromosomes can fuse (centric fusion) to form large metacentric or submetacentric chromosomes, resulting in a reduced number of chromosomes in a species. Although variable chromosome numbers have been earlier on reported for this species (2n = 48, 52 and 72), it is not certain that the difference in number of chromosomes observed in this study is attributable to centric fusion since there is no pronounced difference in the size of the chromosomes of the two karyotypes. However, polyploidy may have been operative at different levels.

5. Conclusion

From this work, it has been established that the population of *Ocimum basilicum* growing in the South-eastern region of Nigeria are characterized by polyploidy and chromosome number variation, with at least two cytotypes. The chromosome count of 2n = 60 is a new number discovered in this study for this species. The plant species has asymmetrical karyotype with a higher proportion of acrocentric chromosomes, as compared to other forms of chromosomes, indicative of advancement in its evolutionary trend. However, further investigation utilizing flow cytometric analysis and molecular markers is strongly recommended.

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