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# Somatic chromosomal studies in *Ocimum basilicum & Ocimum sanctum* L.

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## Abstract

The study of chromosomal number is a parameter commonly used to characterize cytological of a species, and may have implications for cultivar identification. The present study aimed to determining the chromosome number of Ocimum sanctum and Ocimum basilicum L. The survey conducted in the cytogenetic laboratory, department of genetics and plant breeding, CCS University, Meerut. We used seeds of four accessions of *Ocimum*, obtained from NBPGR, New Delhi were used in the present study. Accessions IC-436153 and EC-338575 are of Basilicum group, while Accessions IC-344638 and IC-344681 belong to Sanctum group. We used seeds of Ocimum basilicum and Ocimum sanctum. After 24 hrs of germination, radical tips were collected and immersed in saturated solution of para-dichlorobenzene (PDB) for about 3h. The material was fixed in freshly prepared Farmer's fluid ( 3 parts absolute alcohol: 1 part glacial acetic acid) solution for 24 h. After fixation, the material was transferred to 70% alcohol and stored in a refrigerator for long time preservation, until studied cytologically. Microphotographs were taken by digital camera at the magnification 1000X and karyotypes were generated with the computer-based programmers'- Adobe Photoshop, AutoCAD and Microsoft programmer. The measurements of the chromosomes were made with the help of Erma Tokyo objective micrometer. Chromosome measurements were made on – length of each arm, total chromosome length and the position of secondary constriction, F%, DI and TF%. The number of chromosome found for the species Ocimum basilicum was 2n =48 and Ocimum sanctum was 2n =32. The study of chromosome assumes the role as a restriction commonly used in cytological characterization of the species, and may also have implication for cultivars identification

**Keywords**: *Ocimum basilicum, Ocimum sanctum*,para dichloro benezen, farmer's fluid, aceto-orcein

## Introduction

Kary Wilhelm von Nageli first observed chromosomes in plant cells in 1842. Levitsky (1924) seems to have been the first to define the karyotype as the chromosome complement of an individual or of a related group of individuals and is characterized by the number, size and shape of its chromosomes. The chromosomes may differ in position of primary (centromere) and secondary (e.g. nucleolus organizer region) constrictions and in the distribution and size of hetero- and eu-chromatin segments. [1]. investigated the morphological and chemotaxonomic variability of 13 basil (*O. basilicum)* genotypes.

They also studied plant height, secondary branches, leaf, stem, flower ratio and essential oil content in different plant organs. [2]. observed inter-specific variation in the quantity and quality of the essential oils in the four botanical varieties (2n=48) of the aromatic herb *O. basilicum.*

Polyploids of *O. gratissimum* (rich in eugenol) x *O. viride* (rich in thymol) were induced [3]. too. The polyploids (2n=80) exhibited gigantism, showing heterosis for both vegetative and floral characters accumulating both eugenol (50-55%) and thymol (7-10%). High levels of both morphological and chemical variability exist within the genus due to interspecific hybridization, polyploidy, and the existence of chemotypes or chemical races that do not differ significantly in morphology [4].

To get better insight into intra- and interspecific taxonomy, a variety of different approaches based on geographic origin, morphology, karyotype, chemical composition, and crossability have been used [5-11]. RAPD markers have been used to characterize genetic diversity in a number of medicinal and aromatic plants including *Ocimum* [12-15]. AFLP markers are technically more demanding than RAPD but highly effective in detecting DNA polymorphism [16]. The capacity of the AFLP approach in the research of genetic variability within *Ocimum* genus was verified in an analysis of genetic distances among nine *O. basilicum* varieties done by [17].

Generally, diversification and speciation of flowering plants are accompanied by variation in the amount of nuclear DNA of the entire chromosome complement or haploid nuclear DNA content

together with changes in chromosome number and structure [18]. Flow cytometry enables fast screening of nuclear DNA content and establishment of DNA amounts in the large number of species. Taxonomy, population biology, and ecology require the analysis of large populations of plants, for which Flow Cytometry is ideally suited by [19].

According to chromosome number records summarized in the work of Khosla (1995), the *Ocimum* genus is characterized by more than two basic chromosome numbers and a variety of haploid chromosome numbers (12, 13, 16, 20, 24, 32, 36, and 38). These data could indicate an important role of aneuploidy and polyploidy, accompanied by the evolution of a series of new basic chromosome numbers, in the diversification of species in *Ocimum.* However, different chromosome numbers have been reported for the same species. For example, in the case of *O. sanctum* individuals with 2n = 32, 2n = 36, and 2n = 76 have been recorded, while individuals with 2n = 48 and 2n = 56 have been described for *O. minimum* [20-22].

Based on secondary chromosome association in meiosis, proposed that some *Ocimum* species might have undergone cytological diploidization in the course of evolution of functional diploids with x = 12, which probably evolved from x = 6. [23,24]. considered x = 8 as basic chromosome number for the genus *Ocimum* as a whole, while some other authors suggested that *Ocimum* species are characterized by the different basic chromosome numbers of x = 8, 10, 12, or 16 [25-27]. Most of the literature concerning the genus has dealt with the chemical and medicinal properties of *Ocimum* species [28-30], while phylogenetic studies of this genus have been relatively rare [31-34].

Analysis combining molecular markers, genome size, and chromosome number data produced groupings that give support to the existence of more groups within the genus *Ocimum*, as recognized, rather than only two i.e. Basilicum and Sanctum, as previously reported by [35,36]. Detailed studies on finer details of somatic chromosome structure and number in different species of *Ocimum* are not available.

## Material and methods

### Material

Seeds of four accessions of *Ocimum*, obtained from NBPGR, New Delhi were used in the present study. Accessions IC-436153 and EC-338575 are of Basilicum group, while Accessions IC-344638 and IC-344681 belong to Sanctum group.

### Methods

Karyotypes were prepared using somatic chromosomes of the root tip. Seeds, first soaked in water for 10-20 min, were germinated on moist filter paper in the Petri-dishes at $25^0$C. Young, healthy root tips of about 1-2 cm in length were cut and pre-treated with the saturated solution of para-dichlorobenzene (PDB) for about 3 h, initially they were chilled for 5 min at $4^0$C temperature and then placed at room temperature. After that, the material washed thoroughly under the running tap water for 5-10 min to remove extra PDB and the material was fixed in freshly prepared Farmer's fluid (3 parts absolute alcohol : 1 part glacial acetic acid) solution for 24 h. After fixation, the material was transferred to 70% alcohol and stored in a refrigerator for long time preservation, until studied cytologically.

For making a cytological preparation, the fixed root tips were followed by 3-7 minutes treatment in 45% acetic acid and stained with 2% aceto-orcein - 1N HC1 mixture (9:l) for 2 to 3 h. While staining, the vial is initially warmed over a flame for effective results. The stained root tips are then squashed in 45% acetic acid and the preparation was then temporarily sealed. At least 25-30 metaphase plates were screened of each material and well spread metaphase cells were randomly selected for collection of data on chromosome measurements.

Microphotographs were taken from temporary slide preparations with the help of digital camera at the magnification 1000X and karyotypes were generated with the computer based programmes- Adobe Photoshop, AutoCAD and Microsoft programmer. The measurements of the chromosomes were made with the help of Erma Tokyo objective micrometer. Chromosome measurements were made on – length of each arm, total chromosome length and the position of secondary constriction, if any.

Karyogram were prepared from the microphotographs of the respective metaphase plates. Idiograms were prepared based on the values obtained through image analysis. For chromosomal classification, the scheme proposed by [37]. was followed. According to them,

| Chromosometype | Arm ratio |
|---|---|
| Metacentric | 1.0-1.7 |
| Sub-metacentric | 1.71-3.0 |
| Sub-telocentric | 3.01-7.0 |
| Acrocentric | 7.01- |

Arm ratio was calculated using the formula:

$$\text{Arm ratio} = \frac{\text{Length of long arm}}{\text{Length of short arm}}$$

The disparity index (DI) of chromosomes in a karyotype is Calculated after Mohanty, *et al.,* (1991), by the formula:

$$\text{DI} = \frac{\text{Longest chromosome - Shortest chromosome}}{\text{Longest chromosome + Shortest chromosome}} \times 100$$

The variation coefficient among chromosome complements (VC) is determined after Verma (1980) as follows:

$$\text{VC} = \frac{\text{Standard deviation X 100}}{\text{Mean length of chromosomes}}$$

The total forma percentage or the mean Centromeric index value (TF%) is calculated in each taxon after [38], by the formula:

$$TF\% = \frac{\text{Total Sum of short arm length}}{\text{Total sum of chromosome length}} \times 100$$

In all the karyotypes, ratio of the short arm to the total length of the chromosome in percentage,
F% (Forma percentage or Centromeric index) is determined after [39]. Based on F% the nature of primary constriction in the chromosomes are classified as follows:

| F% | Nature of Primary Constriction |
|---|---|
| 50 | Median |
| 49.9 - 37.6 | Nearly median |
| 37.5 - 25.1 | Nearly submedian |
| 25 | Submedian |
| 24.9 - 18.76 | Nearly submedian |
| 18.75 - 12.6 | Nearly subterminal |
| 12.5 | Subterminal |
| 12.4 - 6.26 | Nearly subterminal |
| 6.25 - 1 | Extremely subterminal |
| 1 | Terminal |

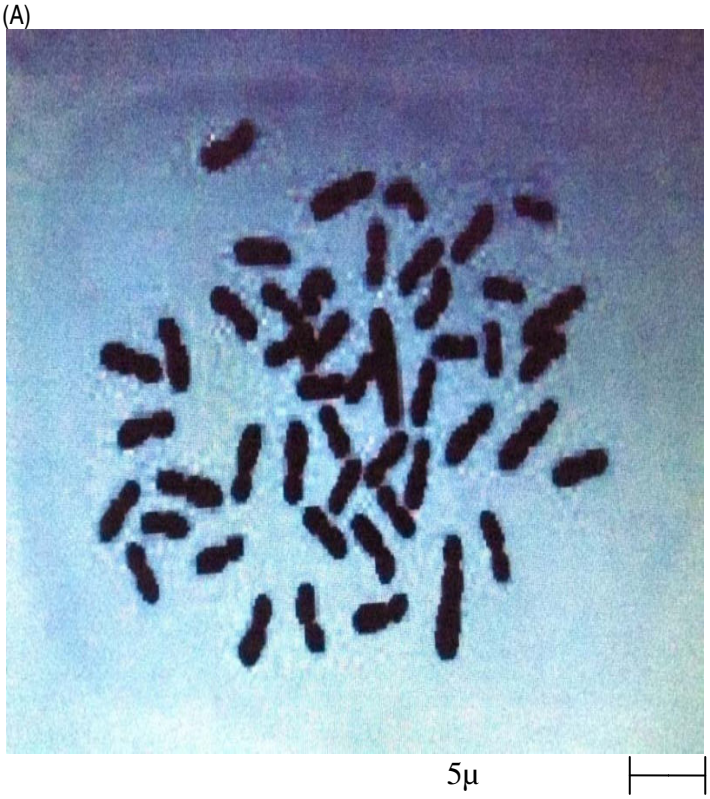
For the calculation of polyploidy, the base numbers given in [40,41], as well as Love and Love (1961b), are usually followed. In those cases where the basic numbers are not mentioned in these references, the latest literature is consulted.

The general description of the common chromosome types are given below, followed by a karyotype description for the members investigated.

**Type A:** Comparatively long chromosome with two constrictions, one median and the other nearly submedian in position (4.2 to 1.4).
**Type B:** Comparatively long chromosome with nearly median to nearly submedian primary constriction and a satellite at the distal end of shorter arm, joined by a SAT thread (2.8 to 2.2)
**Type C:** Relatively long chromosomes (4.2 - 2.0) with nearly median to nearly submedian primary constriction.

**Type D:** Medium to short chromosomes (< 2.0p) with nearly median to nearly submedian primary constriction.

(A)




(B)

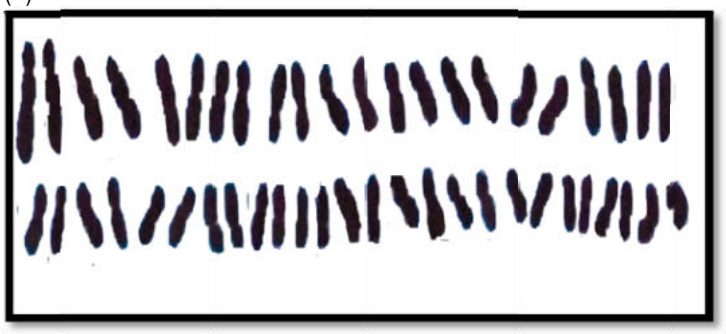
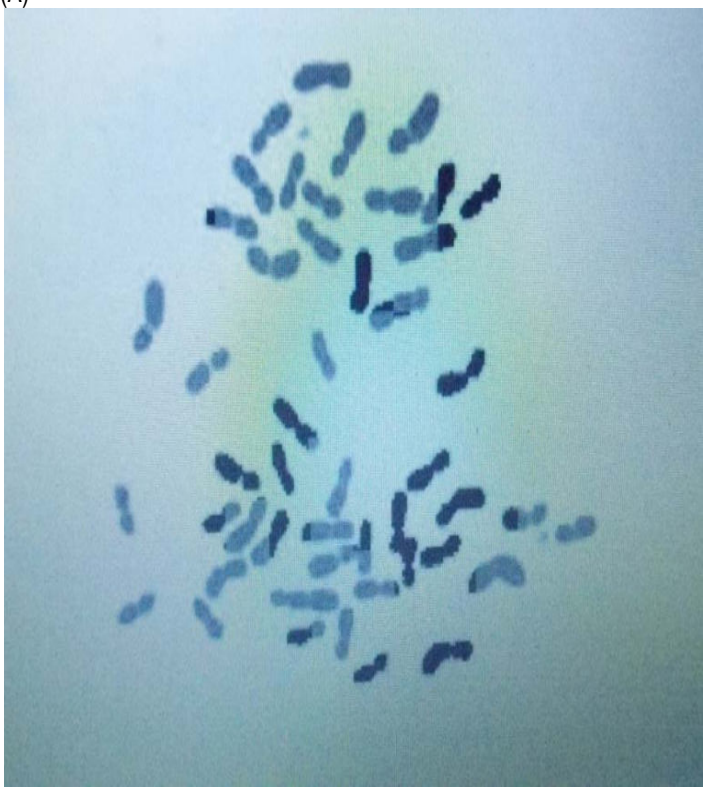


**Figure 1-Somatic metaphase chromosome in *O. basilicum* (accession IC-436153)**

(A) Somatic metaphase plate showing 48 chromosomes, (B) Karyogram

(A)




(B)

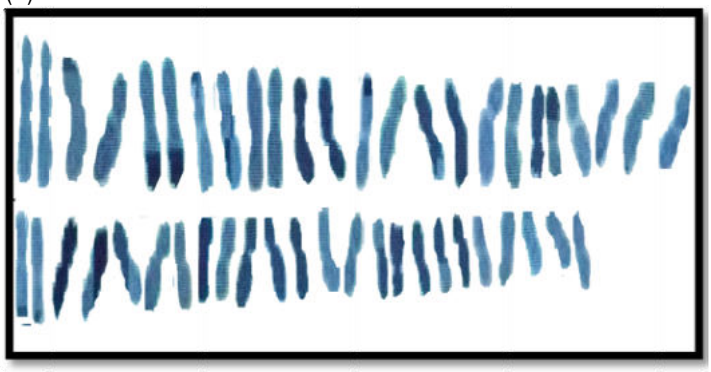
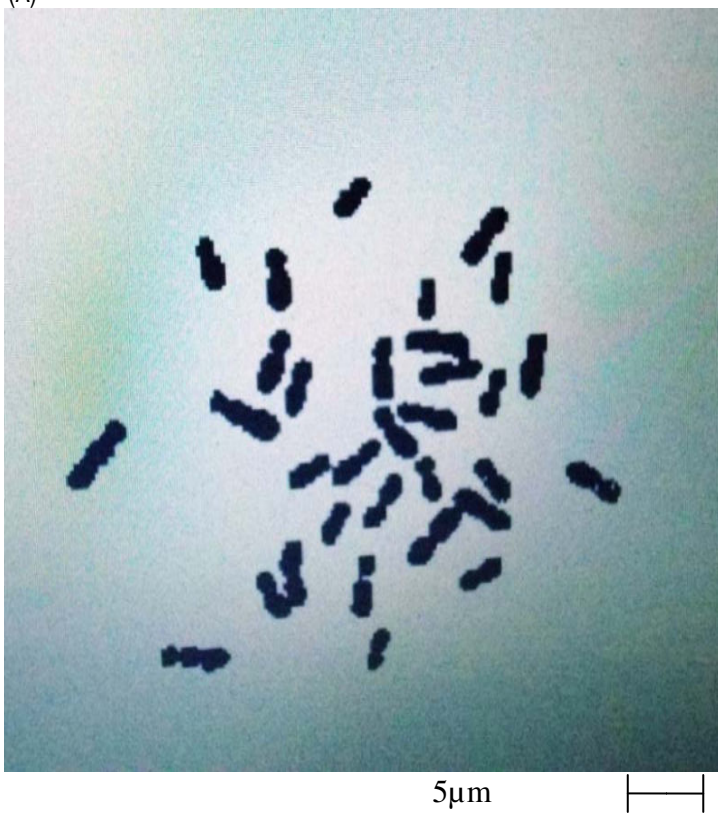


**Figure 2-Somatic metaphase chromosome in *O. basilicum* (accession EC-338575)**

(A)Somatic metaphase plate showing 48 chromosomes, (B) Karyogra

(A)

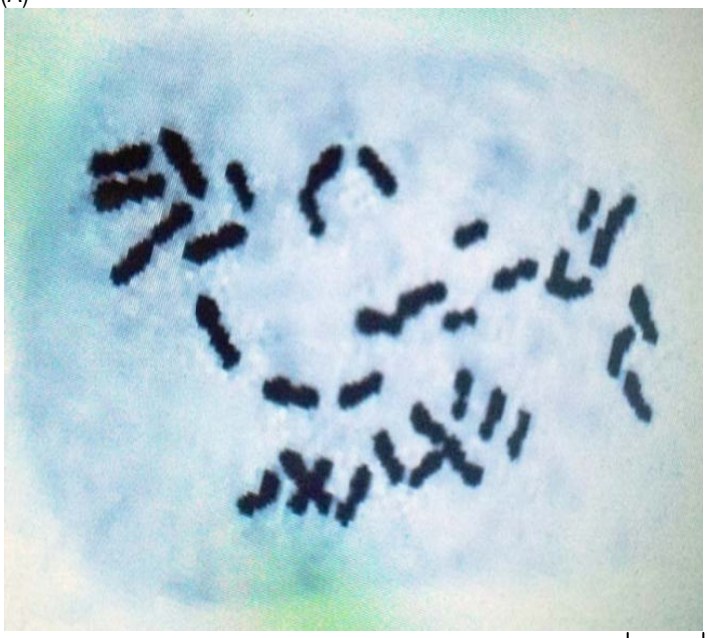



(B)

**Figure 3-somatic metaphase chromosome in *O. sanctum* (accession IC-344638)**

(A)Somatic metaphase plate showing 32 chromosomes, (B) Karyogram

(A)




(B)

**Figure 4-somatic metaphase chromosome in *O. sanctum* (accession IC-344681)**

(A)Somatic metaphase plate showing 32 chromosomes, (B) Karyogram

## Result and Discussion

Somatic karyotypes prepared on the root tip cells of four accessions of *Ocimum* were studied. The data on the finer details of the chromosome morphology are presented in the tables 1-4. The figures 1-4 represent the photographs of the representative somatic metaphase plates along with karyograms.

The somatic chromosome number in the two accessions IC 436153 and EC 338785 belonging to *O. basilicum* studied here was found to be constant with 2n=48 while in the other two accessions IC-344638 and IC-344681 belonging to *O. sanctum* was found to be 2n =32. In general, the chromosomes are very small and mostly metacentric in morphology. Secondary constriction was observed in 1-2 pairs of chromosomes in all the accessions. The chromosomes are comparatively longer (4.14 µ - 1.5 µ) in the accessions of Sanctum compared to the chromosomes in Basilicum group. A brief description on chromosome morphology in each of the four accessions is presented in the following text.

### Basilicum group

Accession IC-436153: Somatic chromosome number in this accession of *O. basilicum* is found to be 2n=48 and constant in all the metaphase cells screened. The chromosomes are small in size with individual chromosome length ranging from 3.95 µ (chromosome 1) to 1.44 µ (chromosome 24). The value of DI is 44.23 and TF% is 44.05. The longest chromosome is more than double the length to the shortest one and carried secondary constriction. Because of its length and secondary constriction, it can be easily identified. In this accession, two pairs of longer chromosomes carried secondary constriction. Most of the chromosomes are of metacentric type (Figure.1; Tables 1).

Accession EC-338575: Somatic chromosome number in this accession of *O. basilicum* is also found to be 2n=48 and constant in all metaphase cells screened. The chromosomes are short in size with individual chromosome length ranging from 3.33 µ (chromosome 1) to 1.45 µ (chromosome 24). The longest chromosome is double the length to the shortest one and carried secondary constriction. The value of DI is 39.33 and TF% is 40.94. In this accession also, two pairs of longer chromosomes had secondary constriction. The chromosomes are mostly metacentric type (Figure.2; Tables 2).

### Sanctum group

Accession IC-344638: The somatic chromosome number in this accession is 2n=32. Individual chromosome length varied from 4.16 µ to 1.87 µ. The value of DI is 37.97 and TF% is 44.05. This accession has two pairs of chromosomes with secondary constriction. The chromosomes, in general are small and mostly metacentric type (Figure.3; Tables 3).

Accession IC-344681: The chromosome number in this accession is also 2n=32. Individual chromosome length varied from 4.10 µ to 1.66 µ with an average value of 2.5 µ. The value of DI is 37.97 similar to above accessions and TF% is 41.90. This accession also has two pairs longer chromosomes with secondary constriction. The chromosomes are small and mostly metacentric (Figure.4; Table 4).

The present study on karyotypes of two groups of *Ocimum* confirms that the somatic chromosome number in the *O. basilicum* is 48 (n=24) and in *O. sanctum* is 32 (n=16), same as reported by the earlier workers. This wide range of chromosome numbers may be due to the difference in number of chromosomal biotypes belonging to the different groups [42].Previous chromosome counts suggest that the genus tolerates a high chromosomal diversity in its constitution. In *O. basilicum* L. the somatic chromosome number of 2n = 48, which seem to be constant in the two accessions i.e, EC &

IC studied along with the absence of any somatic variation numbers suggest the stability of the chromosomal biotype. The absence of somatic variation numbers might have been probably due to the cytologically diploid behavior and normal disjunction of chromosomes in these polyploids, during meiosis [43]. On the contrary, *O.sanctum* is characterized by interspecific variations. The chromosome number is found to be 2n=32 in both the cultivars. The role of polyploidy in the mechanism of speciation is obvious in the tribe Ocimeae [44]. *Ocimum* L. The cytological data's obtained from the genus *Ocimum* L. indicated that eleven out of twelve members (91.67%) are polyploids. All the varieties of *O. basilicum* are characterized by the absence of variants. The stability of the chromosome number (2=48) indicates that distinctly well differentiated genomes are involved in the origin of these polyploids [45].The cultivars of *O. sanctum* exhibit interspecific polyploidy. This is due to the existence of two different sets of chromosome numbers 2n = 32 and 36 derived through two different series, from the primary base figures X1 = 8 and X1 = 9, by autopolyploidy [46]. As in our study we found chromosome number 2n=32 in this group similar as above data. The size of chromosomes in both the groups of *Ocimum* is relatively small and generally lies between 4.00 µ - 1.50 µ. The longer chromosomes generally carried secondary constriction. Because of small size, locating centromere in some chromosomes was difficult. Similarly, measurement of chromosome volumes was not undertaken due to difficulty in measuring the chromosome width. The DI values were found to range from 37.97 to 44.23; Total Centromeric index TF% ranges from 40.94 to 44.05. Thus the various micromorphological details of the karyotype like , difference in absolute chromosome size , difference in the position of centromere, difference in TF%, difference in karyotypes formula & difference in the number as well as position of satellite vary from cytotypes to cytotypes.

**Table 1: Data on somatic metaphase chromosomes in root tip cells of *O. basilicum* (Accession IC-436153)**

| ChromosomeNo. | Chromosome length (µ) | | | Arm ratio | F% | Chromosome Classification |
|---|---|---|---|---|---|---|
| | Long arm | Short arm | Total | | | |
| 1. | 2.29 | 1.67 | 3.96* | 1.37 | 42.17 | Metacentric |
| 2. | 1.45 | 1.25 | 3.90* | 1.16 | 32.05 | " |
| 3. | 1.45 | 1.25 | 3.90* | 1.16 | 32.05 | " |
| 4. | 1.35 | 1.14 | 2.49 | 1.17 | 45.78 | " |
| 5. | 1.25 | 1.04 | 2.29 | 1.20 | 45.41 | " |
| 6. | 1.25 | 1.04 | 2.29 | 1.20 | 45.41 | " |
| 7. | 1.25 | 1.04 | 2.29 | 1.20 | 45.41 | " |
| 8. | 1.45 | 0.83 | 2.28 | 1.74 | 36.40 | Submetacentric |
| 9. | 1.25 | 0.93 | 2.18 | 1.33 | 42.66 | Metacentric |
| 10. | 1.25 | 0.83 | 2.08 | 1.50 | 39.90 | " |
| 11. | 1.14 | 0.93 | 2.08 | 1.22 | 44.71 | " |
| 12. | 0.93 | 0.93 | 1.87 | 1.00 | 49.73 | " |
| 13. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |
| 14. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |
| 15. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |
| 16. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |
| 17. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |
| 18. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |
| 19. | 0.83 | 0.83 | 1.66 | 1.00 | 50.00 | " |
| 20. | 1.50 | 0.62 | 1.56 | 1.50 | 39.74 | " |
| 21. | 0.83 | 0.62 | 1.45 | 1.33 | 42.75 | " |
| 22. | 0.72 | 0.72 | 1.42 | 1.00 | 50.70 | " |
| 23. | 0.72 | 0.62 | 1.35 | 1.66 | 45.92 | " |
| 24. | 0.52 | 0.52 | 1.04 | 1.00 | 50.00 | " |

Chromosome with *secondary constriction.
DI = 44.2
Total F% = 44.05
Chromosomal formula = A4B36C8

Table 2. Data on somatic metaphase chromosomes in root tip cells of *O. basilicum* (Accession EC-338575)

| | Chromosome length (µ) | | | Arm ratio | F% | Chromosome Classification |
|---|---|---|---|---|---|---|
| | Long arm | Short arm | Total | | | |
| 1. | 2.18 | 1.14 | 3.33* | 1.90 | 34.23 | Submetacentric |
| 2. | 2.18 | 1.14 | 3.33* | 1.90 | 34.23 | " |
| 3. | 1.97 | 1.14 | 3.12 | 1.20 | 36.53 | Metacentric |
| 4. | 1.97 | 1.14 | 3.11 | 1.20 | 36.65 | " |
| 5. | 1.97 | 1.14 | 3.11 | 1.20 | 36.65 | " |
| 6. | 1.67 | 1.25 | 2.92 | 1.33 | 42.80 | " |
| 7. | 1.45 | 1.25 | 2.90 | 1.16 | 43.10 | " |
| 8. | 1.56 | 1.04 | 2.60 | 1.50 | 40.00 | " |
| 9. | 1.56 | 1.04 | 2.60 | 1.50 | 40.00 | " |
| 10. | 1.35 | 1.14 | 2.49 | 1.39 | 45.78 | " |
| 11. | 1.45 | 1.04 | 2.49 | 1.39 | 41.76 | " |
| 12. | 1.35 | 1.04 | 2.39 | 1.39 | 43.51 | " |
| 13. | 1.35 | 1.04 | 2.39 | 1.39 | 43.51 | " |
| 14. | 1.35 | 1.04 | 2.39 | 1.39 | 43.51 | " |
| 15. | 1.25 | 1.04 | 2.29 | 1.20 | 45.41 | " |
| 16. | 1.25 | 1.04 | 2.29 | 1.20 | 45.41 | " |
| 17. | 1.25 | 0.93 | 2.18 | 1.33 | 42.66 | " |
| 18. | 1.25 | 0.83 | 2.08 | 1.50 | 39.90 | " |
| 19. | 1.25 | 0.83 | 2.08 | 1.50 | 39.90 | " |
| 20. | 1.25 | 0.83 | 2.08 | 1.50 | 39.90 | " |
| 21. | 1.04 | 0.83 | 1.97 | 1.24 | 42.13 | " |
| 22. | 1.04 | 0.83 | 1.87 | 1.23 | 44.38 | " |
| 23. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |
| 24. | 0.72 | 0.72 | 1.45 | 1.00 | 49.65 | " |

Chromosome with *secondary constriction.DI = 39.33Total F% = 40.94 Chromosomal formula =B38C10

Table3. Data on somatic metaphase chromosomes in root tip cells of *O. sanctum* (Accession IC-344638)

| ChromosomeNo. | Chromosome length (µ) | | | Arm ratio | F% | Chromosome Classification |
|---|---|---|---|---|---|---|
| | Long arm | Short arm | Total | | | |
| 1. | 2.29 | 1.87 | 4.16* | 1.22 | 44.95 | Metacentric |
| 2. | 2.08 | 1.67 | 3.75* | 1.24 | 44.53 | " |
| 3. | 1.90 | 1.35 | 3.35 | 1.46 | 40.29 | " |
| 4. | 1.67 | 1.25 | 2.92 | 1.33 | 42.80 | " |
| 5. | 1.67 | 1.25 | 2.92 | 1.33 | 42.80 | " |
| 6. | 1.67 | 1.04 | 2.71 | 1.60 | 38.37 | " |
| 7. | 1.35 | 1.25 | 2.60 | 1.08 | 48.07 | " |
| 8. | 1.50 | 0.83 | 2.39 | 1.70 | 35.62 | " |
| 9. | 1.34 | 1.93 | 2.29 | 1.44 | 58.95 | " |
| 10. | 1.14 | 1.14 | 2.29 | 1.00 | 49.78 | " |
| 11. | 1.35 | 0.72 | 2.08 | 1.85 | 34.61 | Submetacentric |
| 12. | 1.25 | 0.83 | 2.08 | 1.50 | 39.90 | Metacentric |
| 13. | 1.14 | 0.93 | 2.08 | 1.22 | 44.71 | " |
| 14. | 0.93 | 0.93 | 1.87 | 1.00 | 49.73 | " |
| 15. | 0.93 | 0.93 | 1.87 | 1.00 | 49.73 | " |
| 16. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |

Chromosome with *secondary constriction.
DI = 37.97
Total F% = 44.06
Chromosomal formula = A1B14C1

Table 4: Data on somatic metaphase chromosomes in root tip cells of *O. sanctum* (Accession IC-344681)

| ChromosomeNo. | Chromosome length (µ) | | | Arm ratio | F% | Chromosome Classification |
|---|---|---|---|---|---|---|
| | Long arm | Short arm | Total | | | |
| 1. | 2.08 | 2.08 | 4.16* | 1.00 | 50.00 | Metacentric |
| 2. | 2.08 | 1.67 | 3.75* | 1.24 | 44.53 | " |
| 3. | 2.60 | 1.04 | 3.64* | 2.50 | 28.57 | Submetacentric |
| 4. | 1.87 | 1.67 | 3.54 | 1.11 | 47.17 | Metacentric |
| 5. | 1.87 | 1.25 | 3.12 | 1.49 | 40.06 | " |
| 6. | 1.67 | 1.25 | 2.92 | 1.33 | 42.80 | " |
| 7. | 1.56 | 1.35 | 2.91 | 1.53 | 46.39 | " |
| 8. | 1.87 | 1.04 | 2.91 | 1.79 | 35.73 | Submetacentric |
| 9. | 1.67 | 1.04 | 2.71 | 1.60 | 38.37 | Metacentric |
| 10. | 1.45 | 1.25 | 2.70 | 1.16 | 46.29 | " |
| 11. | 1.56 | 0.83 | 2.33 | 1.87 | 35.62 | Sub metacentric |
| 12. | 1.04 | 1.04 | 2.08 | 1.00 | 50.00 | Metacentric |
| 13. | 1.25 | 0.83 | 2.08 | 1.30 | 39.90 | " |
| 14. | 1.35 | 0.72 | 2.08 | 1.85 | 34.61 | Sub metacentric |
| 15. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | Metacentric |
| 16. | 1.04 | 0.83 | 1.87 | 1.24 | 44.38 | " |

Chromosome with *secondary constriction.DI = 37.92 Total F% Chromosomal Formula = A2B10C4

The higher DI value found in *O. basilicum* correspond to the heterogeneous assemblage of chromosome in these taxas whereas the lower value of DI found in other members point towards the general homogeneity found in various species of

*Ocimum* L. Normally a low DI value corresponds to the homogeneity of chromosome is most of the higher as well as lower plants [47]. In addition to the above the high mean Centromeric index (TF %) value undoubtedly confirms the primitive status of *Ocimum* L.

A high TF% value represent a high symmetric karyotype, which is a primitive condition [48]. However, the comparatively higher variation coefficient & TF% values exhibited by *O. sanctum* shows that it represent the climax of evolution among the different taxa investigated in *Ocimum* L. In *O. basilicum* L. the karyotypes of a high number of medium sized chromosome (type B) found in both groups accessions & low number of type A&C. The comparative inert heterochromatin segments might have been deleted resulting in shorting of the chromosome size [49]. Moreover, the value of DI & TF% found in *O. basilicum* & further

confirms that both these are two extremes in the evolutionary pathway.

The phylogenetic changes occurring in the length of the chromosome could cause shortening in size of one of the arms leading to the shifting of the Centromeric position as well as reduction in the absolute length of chromosome [50]. It has also been found that irrespective of the degree of polyploidy, chromosomes always exhibit bivalent formation. Polyploidy meiosis characterized by bivalent formation point towards allopolyploidy. However, autopolyploid on long standing behave like allopolyploids. The presence of a wide range of chromosome numbers, numerical variations and structural changes of chromosomes found in many genera mark the significant role that both aneuploidy and polyploidy have played in the evolution of various taxa of the family at the generic and species level. Both mitotic and meiotic aberrations have played a major role in the evolutionary diversification of the family. Individuals with same chromosome number but with differences in karyomorphological details reflect the ongoing evolutionary processes at micro level.

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