

COMPARISON OF DIFFERENT CYTOLOGICAL PARAMETERS OF THREE SPECIES OF *OCIMUM*

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Abstract

Karyomorphological analysis of three species of the genus *Ocimum* (*O. basilicum* L., *O. americanum* L. and *O. tenuiflorum* L.) was carried out. Gross chromosome morphology of these species are nearly sub metacentric (Sm), nearly metacentric (m) and metacentric (M) in nature. The high values of mean chromosome length, total chromosome length of *O. americanum* followed by *O. basilicum* show their primitiveness. Whereas, lower values of these parameters in *O. tenuiflorum* denotes evolved nature of the species. All the three species of *Ocimum* showed high values of total forma percentage (TF%) in conjunction with low coefficient of variation (CV), which corresponds to the primitive status in the evolution of flowering plants and was placed in 3A class of Stebbins' system, indicating the presence of symmetrical karyotype in them. Karyotyping along with UPGMA based dendrogram analysis clearly separated *O. basilicum* L. and *O. americanum* L. in one cluster and *O. tenuiflorum* L. in a separate cluster with greater Euclidian distance.

In general, the genus *Ocimum* showed a wide range of chromosomes with graded karyotype (Kundu and Sharma 1988). In the present investigation comparative karyotype analysis of three related species has been used to describe patterns and directions of chromosomal evolution within the genus *Ocimum* and to infer the evolutionary role of karyotype changes. Detailed information regarding the chromosome architecture in higher plants can thus serve as a useful tool to understand their systematic relationships and for tracing the trend and direction of their evolution.

Cytological studies were made following propionic orcein squash technique (Sharma and Sharma 1980). For somatic chromosome analysis, healthy root tips of *O. basilicum* were pretreated in a solution of saturated aqueous paradichlorobezene (PDB) and with a trace of aesculin at 18 - 20°C for 3.5 hrs. Whereas, in case of *O. americanum* and *O. tenuiflorum* pretreatment was done in a solution of saturated aqueous paradichlorobezene (PDB) and 2 mM hydroxyquinolene (1 : 1). The root tips were initially chilled at 4°C for 4 - 5 min and then kept at 18 - 20°C for 3.5 hrs. The root tips were fixed in 1 : 2 propionic ethanol for 3 hrs and hydrolyzed in 1(N) HCl for 12 min at 60°C. After thorough washing in distilled water and removing all traces of acid, root tips were treated in 45% propionic acid and then stained in 2% propionic orcein solution for 3 hrs. Karyotype analysis was carried out by critically examining about 20 clear, well-scattered metaphase plates from different root tips of 10 plants of each species. Based on the karyotype data, a cluster analysis was carried out to examine karyotype similarity among species. A data matrix of 3 operational taxonomic units (OTUs) × 8 variables were used. The variables were mean chromosome length (MCL), mean chromosome volume (MCV), total forma percentage (TF%), coefficient of variation (CV), number of chromosomes with median primary constriction (M), chromosomes with nearly median (m) primary constriction, number of chromosomes with submedian primary constriction (Sm), and number of satellite chromosomes. The similarity matrices (Stat Soft, 2002. *STATISTICA for Windows*, computer program manual Stat Soft, Tulsa, Oklahoma) were used to construct dendrogram using the unweighed paired group method with arithmetic average (UPGMA) method.

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The present cytological investigation on chromosome number determination and karyotype analysis of three species of *Ocimum* revealed different chromosome numbers as *O. tenuiflorum*, *O. basilicum* and *O. americanum* with $2n = 32$, 48 and 72, respectively (Fig. 1) The chromosome number of these species clearly indicated different ploidy status though the basic numbers were

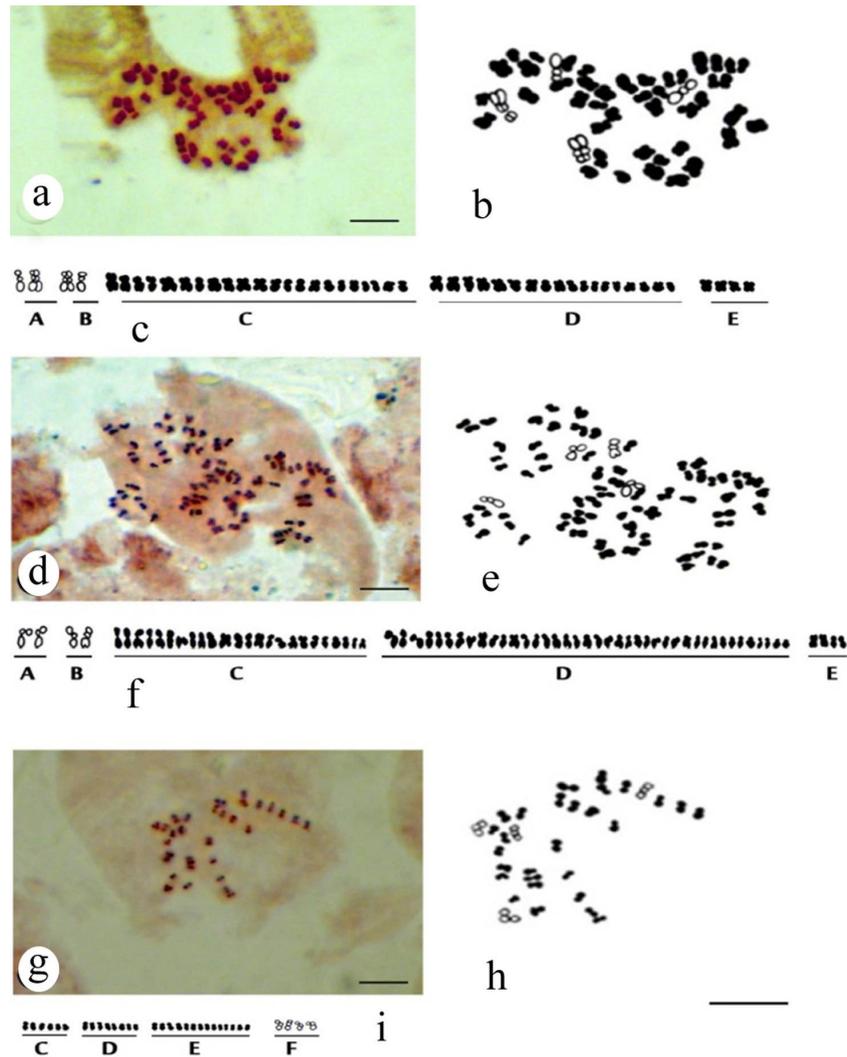


Fig. 1. Photographs of somatic cells at metaphase, drawing of the same stage and karyograms of different species of *Ocimum* (bar = 5.0 μm). a - c *O. basilicum*, d - f *O. americanum*, g - i. *O. tenuiflorum*.

different (Singh and Sharma 1982, Bir and Sagoo 1985). *O. tenuiflorum* with $2n = 32$ chromosomes, were tetraploids as the basic number was $x = 8$. Similarly, *O. basilicum* and *O. americanum* were tetraploids and hexaploids, respectively with respect to their basic chromosome number $x = 12$ (Table 1). The general chromosome morphology studied in these species differed, though the karyotype was graded or symmetrical. The chromosomes were distinctly smaller in size (0.7 - 1.04 μm) in *O. tenuiflorum* as compared to the other two species where chromosomes

were little larger (1.08 - 2.08 μm). In several species of angiosperms, a reduction in chromosome size has often been recorded along with polyploidy. Such fact was correlated with differential condensation, differential polynemy and other factors (Sharma 1970).

Table 1. Comparison of different cytological parameters of three species of *Ocimum*.

Cytological parameters	Plant material			F value*
	<i>O. tenuiflorum</i>	<i>O. basilicum</i>	<i>O. americanum</i>	
Somatic chromosome number	2n = 32	2n = 48	2n = 72	
Chromosome pair with secondary constriction	2	2	2	
Range of chromosome length (μm)	0.70 - 1.04	1.08 - 2.08	0.58 - 2.00	
Range of centromeric index (<i>i</i> - value)	31.58 - 50.0	25.0 - 50.0	25.25 - 50.0	
Total chromosome length (TCL) (μm)	25.520	76.240	116.208	106.32
Mean chromosome length (MCL) (μm)	0.798 \pm 0.017	1.589 \pm 0.049	1.614 \pm 0.030	26.10
Total chromosome volume (TCV) (μm^3)	14.302	107.232	179.251	
Mean chromosome volume (MCV) (μm^3)	0.447 \pm 0.013	2.234 \pm 0.220	2.489 \pm 0.180	
Total Forma percentage (TF %)	41.193	42.058	40.060	
Coefficient of variation (CV)	13.904	21.219	17.640	
Karyotype formula	C ₈ D ₆ E ₁₄ F ₄	A ₂ B ₂ C ₂₂ D ₁₈ E ₄	A ₂ B ₂ C ₂₄ D ₄₀ E ₄	
Degree of asymmetry (Stebbins 1971)	3A	3A	3A	

* $p \leq 0.05$. Means were compared by one-way ANOVA of fixed effect and on the basis of analysis, it can be said that above F values are higher than the critical F value at the 5% level of significance.

However, the similar chromosome size among diploids and polyploids were also reported (Sharma 1974). The similarity in chromosome size and gross morphology between *O. basilicum* and *O. americanum* might have been due to the similar condensation index of chromosomes. All these species revealed graded karyotype with medium to short chromosome which may suggest their advance nature (Fig. 1) (Mukhopadhyay and Sharma 1987, Lahiri *et al.* 2009, 2010, Ray *et al.* 2009). So far as the chromosome's types were concerned *O. tenuiflorum* exhibited a complete different set of combination of chromosomes types in comparison to the other two species. *O. basilicum* and *O. americanum* revealing similar types and almost mere numbers of different chromosomes. In *O. tenuiflorum* the metacentric chromosomes were predominant (E₁₄) than median and sub median chromosomes. Moreover, a separate type of nucleolar chromosomes was observed in this species. On the other hand, both median and sub median chromosomes

constituted the majority of the total chromosome complement than metacentric chromosomes in both *O. basilicum* and *O. americanum*. In *O. basilicum* and *O. americanum* the nucleolar chromosomes were of two different types (A_2B_2) (Fig. 1). All these facts clearly suggested the involvement of minute structural alterations of chromosomes in the karyotype evolution of this species (Mukhopadhyay and Sharma 1984, Lahiri *et al.* 2010, Mukhopadhyay and Ray 2013). The range of centromeric index shows similarity between *O. basilicum* and *O. americanum* with a clear difference from *O. tenuiflorum*. The total chromosome length and total chromosome volume differed remarkably among three species. Similar results were also observed in mean chromosome length and volume, however, values of total form percentage (TF %) revealed similarity among these three species of *Ocimum*. The high values of mean chromosome length, total chromosome length of *O. americanum* followed by *O. basilicum* show their primitiveness. Whereas lower values of these parameters in *O. tenuiflorum* denotes their higher evolutionary status than other two. All three species of *Ocimum* studied showed high values of total form percentage (TF%, 40.06 - 42.058) in conjunction with low coefficient of variation (CV, 13.904 - 21.219), which corresponds to the primitive status in the evolution of flowering plants and were placed in 3A class of Stebbins' system, indicating the presence of symmetrical karyotype in them. Variation in karyotype details revealed phylogenetic relationship and affinities of three different species. UPGMA based dendrogram analysis clearly separated *O. basilicum* and *O. americanum* in one cluster and *O. tenuiflorum* in a separate cluster with greater Euclidian distance. Several other investigations using molecular marker, nuclear DNA values and chromosome morphology revealed the existence of more infra generic groups within this genus. The section *Ocimum* was divided into two separate clades; the genotypes belonging to *O. basilicum* clade were tetraploids, while varieties belonging to *O. americanum* clades were hexaploids. *O. tenuiflorum* belonging to a separate clade represented the most divergent species as far as the genetic distance was concerned. This group adds the smallest genome size organized in a few small chromosomes. The differences in genome size and chromosome numbers among *Ocimum* species suggested evolution of genomes might have been responsible due to sequence deletion or amplification, chromosome rearrangement and polyploidization as well (Carović-Stanko *et al.* 2006, 2010).

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