

Chromosome Studies and Estimation of Nuclear DNA in Different Varieties of *Cuminum cyminum* L. and *Carum copticum* Benth. and Hook.

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The species *Cuminum cyminum* L. and *Carum copticum* Benth. and Hook. belong to the subtribe Carinae of the tribe Ammineae of Umbelliferae (Drude 1898). *Carum copticum* commonly called as 'ajwain', is reported to be the native of Egypt. Its seeds are a commercial source of essential oil which mainly contains 40–45 per cent of thymol, chiefly used in the pharmaceutical industry. The seeds are also used as a spice or condiment. Seeds of *Cuminum cyminum* (Cumin) have an aromatic odour and are extensively used as condiment. Both the species are widely cultivated throughout the world for their economic values.

Cytological studies on these two species were carried out earlier by several authors (Shah 1953, Sharma and Ghosh 1954, Baijal and Kaul 1973, Hore 1975, Khanolkar 1977, Subramanian 1986). However, karyotypic details including cryptic difference, if any, at the varietal level and the nuclear DNA value are not available. These data provide with an understanding of inter- and intraspecific affinities and the mode of evolution.

Materials and methods

Seeds of three different varieties of *Cuminum cyminum* namely 'Karnataka local', 'RS-1' and 'UC-19' and two different varieties of *Carum copticum* Benth. and Hook., namely, 'Karnataka local' and 'Globe's variety' were collected from different localities of Karnataka, India; Department of Genetics and Plant Breeding, Jobnar, India; and Globe Nursery, Calcutta, India.

The seeds were germinated on moist filter paper in petri-dishes at room temperature (20–25°C), and fresh healthy root tips were taken for the study of karyological details and estimation of 4C nuclear DNA.

The root tips which showed peak meristematic activity between 10 A.M. to 12 Noon were cut into 2–3 mm segments and pretreated with a saturated solution of para-dichlorobenzene and 0.002M oxyquinoline (1: 1) for 3 hours in case of different varieties of *Cuminum cyminum* and 3.15 hours in case of different varieties of *Carum copticum* at 14–16°C. The materials were fixed in 1: 3 acetic ethanol for overnight at room temperature. Prior to staining in 2% acetic orcein for 3 hours, the root tips were hydrolysed in 1 N HCl at 60°C for 5 minutes, thoroughly washed with distilled water and kept in 45% acetic acid for 2–3 minutes. The materials were finally squashed in a drop of 45% acetic acid.

Chromosome volume was measured using the formula:

$$V = \pi r^2 h$$

where, V is the chromosome volume, r is the radius and h is the length of the chromosome.

For Feulgen cytophotometric estimation of *in situ* DNA, fresh healthy root tips of the

same five varieties of *C. cyminum* and *C. copticum* and of *Allium cepa* were fixed in 1:3 acetic ethanol mixture for overnight after pretreatment in saturated paradichlorobenzene for 1 hour, hydrolysed in 1 N HCl for 15 minutes at 60°C, washed in distilled water, kept in 45% acetic acid for 2–3 minutes and stained in Schiff's reagent for 2 hours at 16–18°C. The root tips were finally squashed in a drop of 45% acetic acid. Cytophotometric estimation was done with the help of a Leitz Wetzlar Aristophot with microspectrophotometer following single wavelength (550 nm) method (vide Sharma and Sharma 1980). The 4C DNA values were calculated from 100 metaphase plates, scanned in each varieties, on the basis of optical density, in terms of relative arbitrary units of absorbance. Taking the 4C DNA value of 67 picogram for *Allium cepa* (Van't Hof 1965) as a standard, the arbitrary values were converted to absolute units in picograms.

Observations

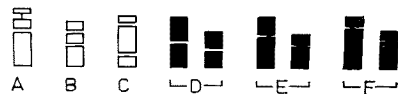
Cytological data

All the three varieties of *Cuminum cyminum* showed the somatic chromosome number to be $2n=14$ (Figs. 2–6). Chromosomes were classified into four types, namely A, C, E and F (Fig. 1). The two varieties of *Carum copticum* showed the somatic chromosome number that is $2n=18$. The chromosomes of *C. copticum* were typified into 5 types, namely A, B, D, E and F (Fig. 1). The descriptions of all the chromosome types of *Cuminum cyminum* and *Carum copticum* is given below:

- Type A: Chromosomes within 3.48 μm to 3.70 μm in length with two constrictions, one nearly submedian and the other nearly terminal or subterminal at the distal end of the shorter arm. The end segment is a satellite.
- Type B: Chromosome is 2.39 μm in length with two constrictions, one nearly median, other nearly submedian, the middle and proximal segments are almost equal in size.
- Type C: Chromosome is 3.26 μm in length with two constrictions, both submedian at opposite ends. Of the two distal segments, one is smaller than the other.
- Type D: Chromosomes within 2.17 μm to 3.26 μm in length with nearly median constrictions.
- Type E: Chromosomes between 1.74 μm to 3.48 μm in length with nearly submedian constrictions.
- Type F: Chromosomes within 2.39 μm to 3.04 μm in length with nearly subterminal constrictions.

Total chromosome length and volume, average chromosome length and volume, mean of total F% of three varieties of *Cuminum cyminum* vary from 34.34 μm to 39.14 μm , 22.12 cu. μm to 25.86 cu. μm , 2.45 μm to 2.80 μm , 1.58 cu. μm to 1.85 cu. μm and 22.58% to 26.08% respectively (Table 1). One pair of chromosomes with secondary constrictions is present in all the strains.

In the two varieties of *Carum copticum*, total chromosome lengths vary between 47.82 μm (Karnataka local) to 55.66 μm (Globe's variety). There are one to two pairs of chromosomes with secondary constrictions. Total chromosome volume, mean of total F%, average chromosome length and average chromosome volume in the variety 'Karnataka local' are 30.34 cu.



CHROMOSOME TYPES

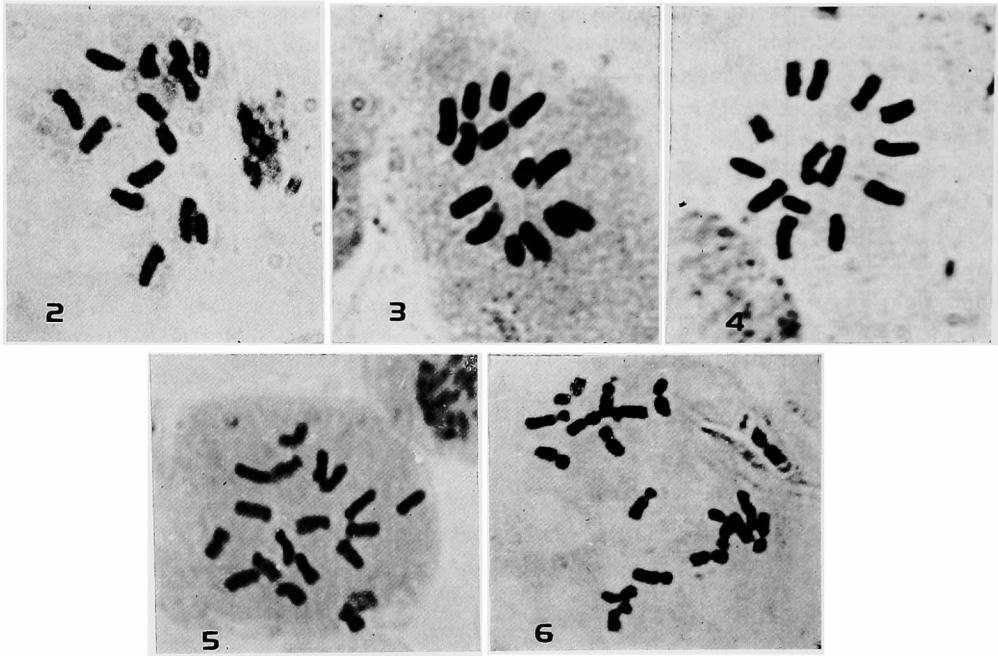
Fig. 1. Diagrammatic representation of chromosome types.

Table 1. Details of karyotype of different varieties of *Cuminum cyminum* L. and *Carum copticum* Benth. and Hook.

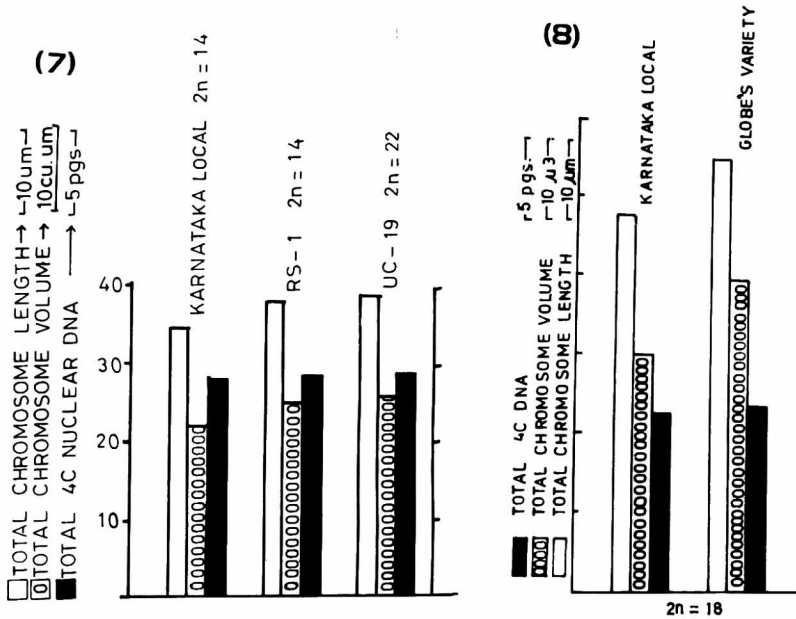
Name of the variety	Somatic chromosome number (2n)	Karyotype formula	No. of chromosomes with secondary constrictions	Mean of total F%	Total chromosome length (μm)	Range of chromosome length (μm)	Average chromosome length (μm)	Total chromosome length (μm)	Range of chromosome volume (cu. μm)	Average chromosome volume (cu. μm)
<i>Cuminum cyminum</i>										
Karnataka local	14	$2A_1+12E$	2	24.26	34.34	1.74-3.48	2.45	22.12	0.84-2.50	1.58
RS-1	14	$2A_1+2C+10E$	4	26.08	37.82	1.74-3.70	2.70	24.98	1.04-2.66	1.78
UC-19	14	$2C+10E+2F$	2	22.58	39.14	1.74-3.48	2.80	25.86	1.25-2.50	1.85
<i>Carum copticum</i>										
Karnataka local	18	$2A_1+2B_2+4D+10E$	4	30.02	47.82	2.17-3.48	2.66	30.34	1.29-2.07	1.69
Globe's variety	18	$2A_1+6D+8E+2F$	2	28.24	55.66	2.61-3.70	3.09	39.98	1.88-2.66	2.22

Table 2. Value of 4C nuclear DNA in root meristem in different varieties of *Cuminum cyminum* L. and *Carum copticum* Benth. and Hook.

Name of the variety	Somatic chromosome No. (2n)	Total chromosome length (μm)	Average chromosome length (μm)	Total chromosome volume (cu. μm)	Average chromosome volume (cu. μm)	Amount of 4C nuclear DNA in root (pgs) \pm S.E.	Amount of 4C nuclear DNA per genome (pgs)	Amount of 4C nuclear DNA per chromosome (pgs)
<i>Cuminum cyminum</i>								
Karnataka local	14	34.34	2.45	22.12	1.58	13.98 ± 0.15	6.99	1.00
RS-1	14	37.82	2.70	24.98	1.78	14.10 ± 0.15	7.05	1.01
UC-19	14	39.14	2.80	25.86	1.85	14.42 ± 0.14	7.21	1.03
<i>Carum copticum</i>								
Karnataka local	18	47.82	2.66	30.34	1.69	11.37 ± 0.15	5.69	0.63
Globe's variety	18	55.66	3.09	39.98	2.22	11.74 ± 0.12	5.87	0.65



Figs. 2-4. Somatic metaphase plates showing $2n=14$ chromosomes respectively of the three different varieties of *Cuminum cyminum* L. 2, Karnataka local ($\times 1900$). 3, RS-1 ($\times 2300$). 4, UC-19 ($\times 1720$). Figs. 5-6. $2n=18$ chromosomes respectively of the two different varieties of *Carum copticum* Benth. and Hook. 5, Karnataka local ($\times 1720$). 6, Globe's variety ($\times 1600$).



Figs. 7-8. Comparative bar diagrams showing total chromosome length, total chromosome volume and 4C nuclear DNA value respectively in different varieties of *Cuminum cyminum* L. and *Carum copticum* Benth. and Hook.

μm , 30.02%, 2.66 μm and 1.69 cu. μm respectively and those in 'Globe's variety' are 39.98 cu. μm , 28.24%, 3.09 μm and 2.22 cu. μm respectively (Table 1).

Nuclear DNA

4C nuclear DNA in three varieties of *Cuminum cyminum* varies from 13.98 pgs to 14.42 pgs. Between the two varieties of *Carum copticum*, 'Karnataka local' contains 11.37 pgs and 'Globe's variety' contains 11.74 pgs of 4C nuclear DNA.

Discussion

The somatic chromosome number in three different varieties of *Cuminum cyminum* has been found to be $2n=14$ and in two different varieties of *Carum copticum* it is $2n=18$ which is in conformation to the previous reports (Shah 1953, Sharma and Ghosh 1954, Bajjal and Kaul 1973, Hore 1975, 1979, Khanolkar 1977). However, Subramanian (1986) reported $2n=18$ chromosomes in *Cuminum cyminum* from South India. If confirmed, this would indicate the existence of cytotype in this species.

Most of the chromosomes in *C. cyminum* possess submedian or subterminal centromere. One pair of chromosomes with secondary constrictions are present in two varieties, namely 'Karnataka local' and UC-19 but in the variety 'RS-1', two pairs of chromosomes with secondary constrictions are present. Karyotype analysis shows gross morphological similarity in this species despite the evolution of different varieties.

As far as the length and volume of the chromosome is concerned, no marked variations among the varieties are found. The total chromosome length and volume range between 34.34 μm and 39.14 μm and 22.12 cu. μm and 25.86 cu. μm . The maximum of total chromosome length and volume is found in the variety 'UC-19'.

The study of DNA content also reveals varietal constancy with slight variation in the different varieties. All the varieties contain approximately 14 picograms of 4C DNA; the highest amount is recorded in the variety 'UC-19', being 14.42 pgs. The validity of the present results has been ensured through repeated experiments under identical conditions. Such intraspecific constancy in nuclear DNA amount has also been reported by various authors in several species (Bennett and Smith 1976, Price 1976, Sharma and Mukhopadhyay 1984, Mukherjee and Sharma 1986 a, b; Ohri and Khoshoo 1986, Chattopadhyay 1989). However, there are also reports of intraspecific variation in the amount of DNA (Raina and Rees 1983, Greenlee *et al.* 1984, Bennett 1985, Ohri and Khoshoo 1986, Mukherjee and Sharma 1990). A proportionate increase in DNA content is recorded with the increase of length and volume. Though there are minute differences in karyotypic details, no marked variations in chromosome length, volume and 4C DNA amount are found among the three varieties of *Cuminum cyminum*.

A gross homogeneity in chromosome morphology is also found in the two varieties of *Carum copticum*. Majority of the chromosomes possess either nearly median or nearly submedian centromere with little size difference of the chromosome complement. Thus the karyotype may be described as symmetrical.

One pair of satellited chromosomes are found in the both varieties. In the variety 'Karnataka local' one additional pair of chromosomes with secondary constrictions are also present. However, Sharma and Ghosh (1954) reported five pairs of chromosomes with secondary constrictions in *C. copticum*. Hore (1975) reported four pairs of chromosomes with secondary constrictions in the same species. Khanolkar (1977) and Shubhada *et al.* (1984) reported only one satellited pair and Subramanian (1986) noticed two pairs of chromosomes with secondary constrictions. The discrepancy between all these reports may be ascribed to the use of different varieties that might represent different cytotypes. The presence of such cytotypes at the

interspecific level is of special significance.

Along with minor karyotype differences in two varieties of *C. copticum* a marked variation is also found in total chromosome length and volume. Differences of approximately $8 \mu\text{m}$ and $9 \text{cu. } \mu\text{m}$ in total chromosome length and volume are calculated between the two varieties. The Globe's variety shows highest chromosome length and volume.

However, 4C DNA value between the two varieties of *C. copticum* reveals a rather consistent picture. More or less similar DNA value i.e., 11.37 picograms in the 'Karnataka local' and 11.74 picograms in the 'Globe's variety' is found. As both the varieties possess same chromosome number and almost similar DNA value, the differences in chromosome length and volume may be attributed to differential spiralization and condensation of chromosomes along with the content of protein in addition to DNA, respectively. All these factors are under genetic control. The constancy in the amount of DNA in different varieties of same species is a clear index of not only the selective value of this amount but its utility as an identifying parameter.

Summary

The somatic chromosome number in three different varieties of *Cuminum cyminum* L. and two varieties of *Carum copticum* Benth. and Hook. are $2n=14$ and $2n=18$ respectively. Most of the chromosomes in *C. cyminum* possess submedian or subterminal constrictions. Karyotype analysis shows gross morphological similarity in *C. cyminum* despite the evolution of different varieties. In length and volume of chromosomes, no marked variations among the varieties are found. The study of DNA content also reveals varietal constancy with slight variation in the different varieties of *C. cyminum*. A proportionate increase in DNA content is recorded with the increase in length and volume in the varieties.

A gross homogeneity in chromosome morphology is also found in the two varieties of *Carum copticum*. Majority of the chromosomes possess either nearly median or nearly submedian centromere with little size difference of the chromosome complement. Along with minor karyotype differences in two varieties, a marked variation is found in total chromosome length and volume. However, 4C DNA value between the two varieties of *C. copticum* reveals a rather consistent picture. Therefore, the differences in chromosome length and volume may be attributed to differential spiralization and condensation of chromosomes along with the content of protein and DNA. The constancy in the amount of DNA in different varieties of same species is a clear index of the selective value of this amount.

Acknowledgement

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