Secondary Chromosome Associations in *Ocimum* spp.

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**Summary** Secondary association of chromosomes at metaphase I was noted in 61.94%, 60.00%, 50.38% and 21.74% meiocytes of *Ocimum tenuiflorum*–green type (2n=36), *O. gratissimum* (2n=40), *O. canum* (2n=26) and *O. kilimandscharicum* (2n=76) respectively. Secondary polyploidy has been attributed as the possible cause of secondary association of chromosomes and statistical analysis of cytological data revealed that the basic chromosome number in the species is 12 (x=6 for *O. canum*) with probable origin from x=6 through polyploidy.

**Key words** *Ocimum tenuiflorum*, *O. gratissimum*, *O. canum*, *O. kilimandscharicum*, Basic chromosome number, Secondary association of chromosomes.

Meiotic chromosome analysis made in cultivars of *Ocimum basilicum* and *O. tenuiflorum*–purple type has suggested that the species were secondary polyploids having x=12 as base chromosome number with probable origin from x=6 (Mukherjee and Datta 2005, Mukherjee et al. 2005). Such studies have been extended to other species/cultivars of *Ocimum* (tropical genus of the family Labiatae, including plants with rich source of essential oils) represented from India and this communication describes persistent occurrence of secondary chromosome association in meiocytes of *O. tenuiflorum* L.–green type (holy basil), *O. gratissimum* L. (shrubby basil), *O. canum* Sims (hoary basil) and *O. kilimandscharicum* Guerke (camphor basil) and reports on the basic chromosome number of the species, as a part of research initiated in *Ocimum* species and cultivars for their cytological characterization aiding to taxonomy and identification of chemotypes of potential commercial interest.

**Materials and methods**

For meiosis, flower buds of suitable sizes from 5 randomly selected plants of each species (plants raised in the Experimental plots of Kalyani University from seed samples: *O. tenuiflorum*–wild collection, *O. gratissimum*–National Bureau of Plant Genetic Resources, accession no. EC–213933, *O. canum*–wild collection, *O. kilimandscharicum*–NBPGR collection, accession no. P–2086; voucher specimens deposited in the Herbarium, Botany Department, Kalyani University) were fixed (6 to 7 a.m.) in Carnoy’s fluid and three changes were given in the fixative at an interval of 48 h. Anthers were squashed in 2% propionocarmine solution. Cytological data presented in the text has been pooled over the plants. Photomicrographs were taken from temporary squash preparations.

**Results and discussion**

**Meiotic analysis**

*Ocimum tenuiflorum*–green type: The species had 2n=36 chromosomes always with an aver-
Figs. 1–12. Meiotic metaphase I chromosomes (fig. 7-diplotene) of Ocimum spp. 1–4. O. tenuiflorum–green type. 1) 18 II (2n=36). 2–4) forming secondary groups of 3 (fig. 2), 6 (fig. 3) and 12 (fig. 4). 5–6. O. gratissimum. 5) 2n=40 chromosomes. 6) 12 secondary groups. 7–8. O. kilimandscharicum. 7) 38 II (2n=76). 8) formation of 3 groups. 9–12. O. canum. 9) 2n=26 chromosomes. 10–12) formation of 6 (figs. 10–11) and 8 (fig. 12) secondary groups. In few cases the groups have been marked (→→) for convenience.
age of 0.27 univalents (0.00 to 0.93) and 17.87 bivalents (17.53 to 18.00) per cell at MI (134 cells scored). Mostly MI cells showed 18 II (92.54%) formation; while, the rest had 17 II+2 I (2.24%), 16 II+4 I (4.48%) and 15 II+6 I (0.75%). Secondary chromosome associations have been studied in 61.94% MI cells and the chromosomes formed groups of 3 (2.41%), 4 (2.41%), 6 (24.10%) 8 (10.84%), 9 (19.28%), 10 (12.05%) and 12 (28.92%). Formation of 3 II(2)+2 II (4)+1 II (4) has been the most frequent (10.84%) association among the group classes. About 95.12% of AI cells were cytologically (18 : 18) balanced (123 cells studied); while the rest formed laggard(s) (1–2) and sticky bridges.

Mukherjee and Datta (2005) reported the same chromosome number (2n=36) for purple type cultivar of O. tenuiflorum. Bir and Sagoo (1980) and Singh (1980) also reported 2n=36 chromosomes for the species but it was also stated to be as n=16 (Mehra and Gill 1972, Khosla and Sobti 1985), n=16+0–3 B (Vij and Kashyap 1976), n=17 (Singh 1980) and n=32 (Tischler 1938).

O. gratissimum: The species revealed 20 II formation (2n=40) at MI (315 cells scored) in 81.27% cells; while, the rest showed chromosomal associations ranging from 19 II+2 I (7.62%) to 13 II+14 I (0.95%). Bivalent frequency per cell was noted to be 19.50 (18.42 to 19.91) and those bivalents tended to form variable groups (60.00% MI cell) of 3 (2.12%), 6 (39.68%), 8 (14.29%), 9 (8.99%), 10 (6.88%), 12 (24.87%) and >12 (3.17%); although univalents (1.00/cell, range–0.18 to 3.16/cell) were often associated in groups with bivalents. Formation of 2 II (8)+1 II (4) under 12 group class was the most predominant (10.05%) chromosomal association studied. Cytologically balanced AI cells (20 : 20) was observed in 96.63% PMCs (123 cells noted); while, the rest had laggards and bridges.

The chromosome number 2n=40 is in accordance to earlier reports (Morton 1962, Khosla and Sobti 1985, Singh 1980); however, diploid chromosome number 24 (Mitra and Datta 1967), 34 (Pal 1971), 48 (Morton 1962) and 64 (Tischler 1938) have also been documented for the species.

O. canum: PMC squashes showed 2n=26 chromosomes in all cases. The plants formed 13 II in 93.98% MI cells (266 cells scored); while, the rest had chromosomal association of 12 II+2 I (5.64%) and 10 II+6 I (0.38%). Bivalents (12.93/cell, range: 12.89 to 13.00/cell) and univalents (0.14/cell, range: 0.00 to 0.22/cell) tended to form variable groups of 3 (19.40%), 4 (7.46%), 6 (53.73%), 8 (4.48%), 9 (1.49%) and 12 (13.43%) in 50.38% MI cells. The most frequent (14.18%) association noted among the group classes has been 4 II (1) and 8 II (2) (3.16/cell) were often associated in groups with bivalents. Formation of 2 II (2) under 12 group class was the most predominant (10.05%) chromosomal association studied. Cytologically balanced AI cells (20 : 20) was observed in 96.63% PMCs (123 cells noted); while, the rest had laggards and bridges.

Pushpangadan et al. (1975) reported 2 cytotypes of O. canum with 2n=24 and 26 chromosomes, the former was introduced from Kenya while the latter was indigenous to India. Chromosome number 2n=34 (Pal 1971), 2n=64 (Sanjappa 1979) and 2n=64+0–4 B (Vij and Kashyap 1976) have also been reported for the species.

O. kilimandscharicum: Meioocytes formed 38 II (2n=76) regularly at MI (138 PMCs analyzed). About 21.74% MI cells had groupings of 3 (10.00%), 6 (10.00%), 9 (10.00%), 10 (10.00%), 12 (50.00%) and >12 (10.00%). AI cells were cytologically (38 : 38) balanced (97.33%, 75 cells studied) with occasional formation of 1 to 3 laggard(s) in 2.67% cells.

Statistical analysis of cytological data relating to secondary groupings of chromosomes: χ² test performed between group classes of O. tenuiflorum (χ²=36.83 at 6 DF, p<0.001), O. gratissimum (χ²=147.02 at 6 DF, p<0.001), O. canum (χ²=149.19 at 5 DF, p<0.001) and O. kilimandscharicum (χ²=29.0 at 5 DF, p<0.001) revealed heterogeneity, thereby suggesting that chromosomes have assorted themselves more preferentially into certain number of groups (predominantly 6 and 12 group classes) than they do into others much against random distribution. In the species, observed cell frequencies for 12 and 6 group classes have been found to be significantly higher than expected and it was verified by comparing the cell frequency of 12 (O. tenuiflorum: observed 12
group class 24, rest 59, expected 12 group class 11.86, rest 71.14, total 83, $\chi^2=14.50$ at 1 DF, $p<0.001$; *O. gratissimum*: observed 12 group class 47, rest 142, expected 12 group class 27.0, rest 162.0, total 189, $\chi^2=17.28$ at 1 DF, $p<0.001$; *O. kilimandscharicum*: observed 12 group class 15, rest 15, expected 6 group class 22.33, rest 111.67, total 134, $\chi^2=99.56$ at 1 DF, $p<0.001$; *O. tenuiflorum*: observed 6 group class 20, rest 63, expected 6 group class 11.86 rest 71.14, total 83, $\chi^2=6.52$ at 1 DF, $p<0.05$; *O. gratissimum*: observed 6 group class 75, rest 114, expected 6 group class 27.0, rest 162.0, total 189, $\chi^2=99.56$ at 1 DF, $p<0.001$; *O. canum*: observed 6 group class 72, rest 62, expected 6 group class 22.33, rest 111.67, total 134, $\chi^2=132.58$ at 1 DF, $p<0.001$) group classes against the pooled frequency of rest of the classes.

Secondary association of chromosomes suggested secondary polyploid nature of the species. The species might have underwent cytological diploidization in course of evolution showing diploid like meiotic behaviour. Statistical analysis of cytological data revealed that the basic chromosome number in the species is 12 ($x=6$ in *O. canum*) which probably evolved from $x=6$ through polyploidy. However Darlington and Wylie (1955) and Mehra and Gill (1972) considered $x=8$ as the base number for the genus *Ocimum* as a whole; while, it was reported to be 12 and 8, 10, 12 and 16 in Basilicum and Sanctum groups of Labiateae respectively (Morton 1962, Sobti and Pushpangadan 1977, Khosla and Sobti 1985).

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