Secondary Chromosome Associations in *Ocimum basilicum* L. and *Ocimum tenuiflorum* L.

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Summary Meiotic analysis performed in *O. basilicum* L. ($2n=72$) and *O. tenuiflorum* L. ($2n=36$) demonstrated persistent presence of secondary association of chromosomes in 94.74 and 85.16 per cent metaphase I cells respectively. Secondary polyploidy has been attributed as the possible cause of secondary association of chromosomes and the basic chromosome number for both species has been suggested to be $x=12$ with probable origin from primitive base number $x=6$ through polyploidy.

Key words *Ocimum basilicum*, *O. tenuiflorum*, Secondary association of chromosomes, Basic chromosome number, Secondary polyploidy.

Bivalents and univalents lying in close proximity due to genetic homoeology between associating members (Riley 1960, Kempanna and Riley 1964) but always without any distinct material connections (Darlington 1965) has been referred to as secondary association or secondary pairing of chromosomes and the phenomenon was first reported by Kuwada (1910) in *Oryza sativa* followed by Ishikawa (1911) in *Dahlia variabilis*. Secondary association of chromosomes often encountered in meiotic processes of plant has been used in tracing basic chromosome number and polyploid nature of different plant species.

Based on karyomorphology and chromosome counts, the basic chromosome number in Basilicum and Sanctum groups of Labiatae has been reported to be 12 and 8, 10, 12 and 16 respectively (Morton 1962, Sobti and Pushpangadan 1977, Khosla and Sobti 1985). Darlington and Wylie (1955) and Mehra and Gill (1972) considered $x=8$ as the base number for the genus *Ocimum* as a whole. Present investigation describes persistent occurrence of secondary chromosome associations in meiocytes of *Ocimum basilicum* L. and *O. tenuiflorum* L. (syn. *O. sanctum* L.), aromatic herbs yielding essential oil of commerce, and reports on the basic chromosome number of the species.

Materials and methods

For meiosis, flower buds of suitable sizes from ten randomly selected plants of *O. basilicum* and *O. tenuiflorum* (plants raised in the Experimental plots of Kalyani University from seeds obtained from Medicinal Plant Garden, Narendrapur Ramkrishna Mission, West Bengal, India; voucher specimens deposited in the Herbarium, Botany Department, Kalyani University) were fixed in Carnoy's fluid (*O. basilicum*: 4 to 5 PM; *O. tenuiflorum*: 1 to 2 PM) and 3 to 4 changes were given in the fixative at an interval of 48 h. Anthers were squashed in 2% acetocarmine solution. Cytological data presented in the text has been pooled over the plants. Photomicrographs were taken from temporary squash preparations.

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Results and discussion

Meiotic analysis

O. basilicum: PMC squashes showed $2n=72$ chromosomes (Fig. 1) in all cases. The plants formed 36 II in 49.34% MI cells (304 cells scored); while the rest showed chromosomal associations of 35 II+2 I (24.34%), 34 II+4 I (17.11%), 33 II+6 I (7.89%), 32 II+8 I (0.66%) and 31 II+10 I (0.66%). Bivalent frequency among the plants was 35.12 (range: 34.76 to 35.33) per cell and those bivalents (random distribution evidenced from χ^2 test of heterogeneity, $\chi^2=0.265$ at 9 DF, $p>0.95$) tended to form variable groups at MI; although univalents (average 1.76/cell, range 1.33 to 2.48/cell; non-randomly distributed, $\chi^2=21.27$ at 9 DF, $p>0.01$) were often associated in groups with bivalents. About 94.74% MI cells demonstrated the formation of secondary groups of chromosomes (Figs. 2–6) in the form of 3 (3.13%), 6 (20.83%), 9 (12.50%), 12 (43.75%), 15 (8.33%), 18 (9.38%) and >18 (2.08%). Out of 94.74% MI cells showing chromosomal groupings, 75.00% cells could be analyzed to show 18 different types of associations of which 6 II(1)+5 II(1)+4 II(3)+3 II(2)+2 II(3)+1 I(2) under 12 group class (30.56%) being most predominant (Fig. 5). Metaphase I groupings seems not to have affected AI segregation of chromosomes, which were mostly balanced (36:36) cytologically (86.14%, 404 cells studied). Remaining (13.86%) cells formed only laggards (1 to 4).

Present investigation reports on $2n=72$ chromosomes in *O. basilicum* which is rather contrary to $2n=16$ (Sz.-Boros 1970) and $2n=48$ (Morton 1962, Mehra and Gill 1972, Pushpangadan *et al.* 1975, Sanjappa 1979, Pushpangadan and Sobti 1982); however, the number $2n=72$ has earlier been reported in *Ocimum* for the species *O. americanum* (Pushpangadan and Sobti 1982). Critical analysis of taxonomic data on the genus *Ocimum* provided correct nomenclature indicating that *O. basilicum* and *O. americanum* are synonyms (Banerjee and Maiti 2000).

O. tenuiflorum: The species had $2n=36$ chromosomes in the meiocytes (Figs. 7–8) always with an average of 17.83 bivalents (range 17.40 to 18.00; random distribution, $\chi^2=0.504$ at 9 DF, $p>0.95$) and 0.34 univalents (range 0.00 to 1.20; non-random distribution, $\chi^2=104.96$ at 9 DF, $p>0.001$) per cell at MI (310 cells scored). Mostly MI cells showed 18 II (87.74%) formation; while, the rest had 17 II+2 I (7.74%) and 16 II+4 I (4.52%). Secondary chromosome associations have been studied in 85.16% MI cells and the chromosomes tended to form variable groups (Figs. 9–12) of 3 (4.55%), 6 (25.00%), 8 (9.09%), 9 (11.36%), 10 (19.32%) and 12 (30.68%). Seventeen different types of chromosomal associations were studied in different group classes of which 13 II(1)+4 II(1)+1 II(1) under 3 group class (4.55%), 5 II(2)+4 II(1)+2 II(1)+1 II+1 I(1)+1 I(1) under 6 (Fig. 9) group class (10.98%), 3 II(3)+2 II(4)+1 II(1) under 8 group class (4.17%), 3 II(3)+2 II(4)+1 I(2) under 9 group class (11.36%), 3 II(2)+2 II(4)+1 II(4) under 10 (Fig. 11) group class (6.82%) and 2 II(6)+1 II(6) under 12 (Fig. 12) group class (11.36%) were most frequent. AI cells (Fig. 8) were cytologically (18:18) balanced (98.14%, 322 cells scored) with occasional formation of a laggard in 1.86% cells.

Present findings corroborated with earlier reports on chromosome number ($2n=36$) of the species (Singh 1980, Bir and Saggoo 1980); however, it was also reported as $n=16$ (Mehra and Gill 1972, Khosla and Sobti 1985), $n=16+0-3B$ (Vij and Kashyap 1976), $n=17$ (Singh 1980) and $n=32$ (Tischler 1938).

Statistical analysis of data relating to secondary groupings of chromosomes

χ^2 test performed between group classes of *O. basilicum* ($\chi^2=251.45$ at 6 DF, $p>0.001$) and *O. tenuiflorum* ($\chi^2=80.05$ at 5 DF, $p>0.001$) revealed heterogeneity, thereby indicating that chromosomes have assorted themselves more preferentially into certain number of groups (predominantly 12 group class in both species) than they do into others much against random distribution. In both species, observed cell frequencies for 12 and 6 group classes have only been found to be sig-

Figs. 1–12. Meiotic chromosomes of *O. basilicum* (1–6) and *O. tenuiflorum* (7–12). 1) Diplotene with 34 II+4 I. 2–6) MI chromosomes ($2n=72$) forming secondary groups of 3 (2), 6 (3), 9 (4) and 12 (5–6) in different associations. 7) Diplotene with 16 II+4 I. 8) 18:18 segregation at AI. 9–12) MI chromosomes ($2n=36$) forming groups in different associations. 9) 5 II(2)+4 II(1)+2 II(1)+1 II+1 I(1)+1 I(1), 6. 10) 3 II+2 I(1)+3 II(2)+2 II(3)+1 II(2), 8. 11) 3 II(2)+2 II(4)+1 II(4), 10. 12) 2 II(6)+1 II(6), 12. Univalents ($\rightarrow$) and in few cases the groups ($\longrightarrow$) have been marked for convenience.

nificantly higher than expected and it was verified by comparing the cell frequency of 12 (*O. basilicum*: observed 12 group class 126, rest 162, expected 12 group class 41.14, rest 246.86, total 288, $\chi^2=204.21$ at 1 DF, $p>0.001$; *O. tenuiflorum*: observed 12 group class 81, rest 183, expected 12 group class 44.00, rest 220.00, total 264, $\chi^2=37.34$ at 1 DF, $p>0.001$) and 6 (*O. basilicum*: observed 6 group class 60, rest 228, expected 6 group class 41.14, rest 246.86, total 288, $\chi^2=10.09$ at 1 DF, $p>0.001$; *O. tenuiflorum*: observed 6 group class 66, rest 198, expected 6 group class 44.00,

rest 220.00, total 264, $\chi^2=13.20$ at 1 DF, $p>0.001$) group classes against the pooled frequency of rest of the classes.

Persistent occurrence of secondary association of chromosomes in *O. basilicum* and *O. tenuiflorum* has suggested secondary polyploid nature of the species and statistical analysis of cytological data has indicated that the basic chromosome number in both species is 12 which possibly evolved from ancestral basic number $x=6$ through polyploidy. Total lack of multivalent formation supports the view that both the species might underwent cytological diploidization through a potential genetic mechanism in course of evolution (Riley and Chapman 1958, Riley and Kempanna 1963), thereby showing diploid like meiotic behaviour.

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