Microsporogenesis in Hexaploid *Morus serrata* Roxb.

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Morus serrata Roxb., is found widely distributed in temperate Himalaya between 4 to 9 thousand ft. altitude (Hooker 1885). It is commonly known as Himalayan Mulberry and recognised as a valuable timber species. The wood is used for furniture, sporting requisites, agricultural implements etc., (Anonymous 1962). The tree is lopped for fodder and the leaves are also rarely used for feeding silkworms in Khasi and Jaintia hills (Kanjilal 1940). Except for these floristic reports, no other information is available for the commercial exploitation of this native species as a food source of silkworms. Further, the only available cytological information on this species is the diploid chromosome number ($2n=28$) report by Janaki Ammal (1948). The present communication is the first report of hexaploidy in *M. serrata*. In addition, the meiotic behaviour of this hexaploid mulberry is also discussed.

Material and methods

Cuttings from a giant mulberry (*Morus serrata*) tree of Joshimath, Chemoli District (U. P., India) were collected and planted in mulberry germplasm of the institute. The species identification was got confirmed by National Herbarium, BSI, Calcutta. Male inflorescences of appropriate developmental stages from these plants were fixed in Cornoy's II solution for 24 hours and preserved in 70% ethanol. Anthers were squashed in 2% propionocarmine. Photomicrographs were made with temporary preparations. Number of observations were made to get confirmative results.

Observations

Pollen mother cells (PMCs) revealed the gametic chromosome number of $n=42$ and exhibited normal meiosis. At diakinesis, 42 bivalents are clearly seen and in majority of the cells two or three bivalents are found associated with the nucleolus (Fig. 1). In some cells, one hexavalent is found associated with nucleolus (Fig. 2). Various types of chromosomal associations ranging from uni- to hexavalents are observed during diakinesis and metaphase I (Figs. 3 and 4). The range and mean frequency of such chromosomal associations scored in 100 PMCs is given in Table 1. Majority of cells (48%) showed 42 bivalents and frequency of multivalents per cell is much less compared to that of bivalents. Among the multivalents, the frequencies of trivalents (1.28) and tetravalents (1.20) are more than that of penta- (0.04) and hexavalents (0.28). Univalents are observed in only 5% of cells with a frequency of 0.4. At metaphase I, all the bivalents regularly align on the equatorial plate (Fig. 5). Anaphase I disjunction (Fig. 6) and telophase I (Fig. 7) are regular. During prophase II and metaphase II also, 42 chromosomes in each daughter nuclei are clearly seen (Fig. 8). Subsequent stages of meiosis II are found to be normal leading to the formation of isobilateral or tetrahedral tetrad (Figs. 9 and 10). Pollen grains are of uniform size and showed 78% stainability.

Figs. 1-6. Meiosis in hexaploid *Morus serrata*. 1, diakinesis showing 42 bivalents (two bivalents associating with nucleolus). 2, diakinesis showing one hexavalent (associating with nucleolus + 4 IV + 31 II). 3, metaphase I showing 2 IV + 38 II. 4, metaphase I showing 1 VI + 4 IV + 2 III + 28 II. 5, metaphase I showing entire chromosome complement on the equatorial plate. 6, anaphase I. ($\times 2000$).

Table 1. Frequency of chromosome associations at diakinesis and metaphase I in *Morus serrata*

	VI	V	VI	III	II	I	No. of cells observed
	2	1	2	4	23	1	4
	1	—	2	4	29	—	6
	1	—	4	2	28	—	10
	1	—	3	2	29	2	4
	—	—	2	2	34	2	14
	—	—	2	—	38	—	6
	—	—	1	4	34	—	8
	—	—	—	—	42	—	48
Mean	0.28	0.04	1.20	1.28	36.54	0.40	Total 100 cells
Range	0-2	0-1	0-4	0-4	23-42	0-2

Figs. 7-10. 7, telophase I. 8, prophase II showing 42 chromosomes in each daughter nucleus. 9, telophase II. 10, isobilateral and tetrahedral tetrads. ($\times 1200$).

Discussion

Among the various species of *Morus* L., a high degree of polyploidy ranging from diploidy ($2n=2x=28$) to 22 ploidy ($2n=22x=308$) has been reported (Cf. Federov 1969). Further,

hexaploidy has been reported only in *M. cathyana* (Janaki Ammal 1948) and *M. tiliaefolia* (Seki 1959). In *Morus serrata*, only diploid chromosome number $2n=28$ has been reported by Janaki Ammal (1948) and hence the present report is the first record of hexaploidy in this species. In general, this hexaploid exhibits normal meiosis and is characterised by high frequency of bivalents. However, the occurrence of multivalents such as hexa-, penta-, tetra- and trivalents indicates its polyploid nature. But the frequency of these is quite small compared to that of bivalents. This suggests that, the taxon may be a segmental allohexaploid as per the classification of Stebbins (1947).

The concept of assessing the nature of polyploidy based on sole criterion of multivalents frequency appears to have a limited value. Several workers have shown that, pairing of chromosomes is mainly governed by genes. Gilles and Randolph (1951) and Swaminathan and Sulbha (1954) found that with 'evolution' a significant reduction in multivalent frequency occurred in the colchicine induced autotetraploids of *Zea mays* and *Brassica campestris* var. *toria* respectively. Further, gene controlled diploidizing mechanism was found in bread wheat (Riley and Chapman 1958) and polyploid cotton (Kimber 1961). Another significant evidence was the findings of Riley and Law (1965) that specificity of synopsis can be widened or narrowed by gene action to permit the pairing of chromosomes related genetically or evolutionarily. Stebbins (1950) has also pointed out that polyploids undergo several secondary modifications in the course of evolution. One such modification commonly noticed in many tetraploid species is the regular meiosis and total absence of multivalents similar to normal diploids. So, the diploid like meiotic behaviour of hexaploid *M. serrata* may be regarded as secondary modification and genetically controlled as observed in *Phleum* by Müntzing and Prakken (1940). The low incidence of multivalents might be due to small size of the chromosomes as observed by Kostoff (1940) who established a direct relation between multivalent formation and chromosome length. But Morrison and Rajhathy (1960) contradicted this in their studies on autotetraploids and observed that multivalent association was higher in plants with smaller chromosomes than those with larger ones. In the present hexaploid mulberry, high pollen fertility (78%) may also be attributed to low incidence of multivalents and their normal segregation.

At present, only diploid chromosome number report is available with *Morus serrata* and it would be interesting if this diploid progenitor is discovered. Any interpretation on phylogeny of this hexaploid race is possible only when diploid and other polyploid races of this species are fully understood. Such cytogeographical and evolutionary taxonomic studies of different species of *Morus* and populations of *M. serrata* collected from different geographical regions are also under progress at this institute.

Summary

Detailed microsporogenesis of hexaploid *Morus serrata* has been studied. This is the first record of natural hexaploidy ($2n=6x=84$) in the species. Meiosis has been found regular with high frequency of bivalents. The frequency of hexa-, penta-, tetra- and trivalents is considerably less suggesting its allopolyploid nature. Diploid like behaviour of this species with high frequency of bivalents, regular meiosis and high pollen stainability are discussed in relation to its evolution.

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