Meiosis in Natural Decosaploid (22x) *Morus nigra* L.

Basavaiah, S. B. Dandia¹, Anil Dhar² and K. Sengupta

Mulberry Breeding and Genetics Laboratory,
Central Sericultural Research and Training Institute,
Mysore-570008, India

Accepted April 13, 1990

Morus nigra L., commonly called 'Black Mulberry' is a native of West Asia and cultivated in many countries for its edible fruits (Anonymous 1962). Its leaves are not preferred in sericulture as they are coarse and non-palatable to silkworms. But, in mulberry improvement programmes it is largely employed for breeding with other species of mulberry (Aliev 1979, Tojyo 1985). This species occupies a unique position amongst flowering plants in exhibiting decosaploidy (22 x) with chromosome number $2n=308$ (Janaki Ammal 1948, Seki and Oshikane 1960), the highest number recorded for any known species. The cytological informations available in this high polyploid are fragmentary and only preliminary observations have been made on its meiosis (Tojyo 1966, Agaev and Fedorova 1970). The information on breeding systems and evolution of different polyploids of the genus is highly useful in mulberry breeding programmes. In the present report, an attempt to understand the meiotic behaviour of natural decosaploid *M. nigra* has been made.

Material and methods

Male inflorescences were collected from wild trees of Srinagar valley, Kashmir. Flower buds of appropriate stages of development were fixed in 1:3 acetic alcohol for 24 hrs and preserved in 70% ethanol. Anthers were squashed in 2% propiono-carmin. Photomicrographs were made with temporary preparations. Pollen stainability was studied by staining the pollen grains with 2% aceto-carmin.

Observations

Meiosis proceeds normally even at this high ploidy level. The nucleus of pollen mother cells (PMCs) showed either single large nucleolus (Fig. 1) or 2–4 small nucleoli with size variation between them (Fig. 2). At diakinesis and metaphase I, chromosomes showed various types of associations. The small size and huge number of chromosomes in the complement offered a great handicap for critical analysis. But obviously, the bivalents were more frequent than the multivalents (Fig. 3) and frequency of univalents was very much less. Among the multivalents, no higher associations were observed thus being proportional to the ploidy of the polyploid and hexavalency was the highest. During metaphase I, majority of the PMCs showed regular alignment of entire chromosome complement on the equatorial plate except a few cells with lagging of either a multivalent (Fig. 4) or 2–4 univalents. Anaphase disjunction appears normal, while only about 5% of cells showed laggards during early anaphasic movement (Fig. 5). Regular daughter nuclei were formed at the end of telophase I (Fig. 6).

Meiosis II was also regular. All the chromosomes align perfectly at equatorial plate

Present Address: ¹ Karnataka State Sericulture Development Institute, Thalaghattapura, Bangalore-560 062, India.

² Regional Sericultural Research Station, Pampore-192121, India.

Figs. 1-6. Meiosis in decosaploid ($2n=22x=308$) *Morus nigra* L. 1, leptotene nucleus with single large nucleolus and coiled chromatin. 2, PI nucleus with 4 nucleoli of different sizes. 3, MI with various types of chromosome associations. 4, MI chromosomes at equatorial plate (one laggard). 5, AI showing laggards. 6, dyad nuclei of normal TI. ($\times 2000$).

Figs. 7-12. Meiosis in *Morus nigra* (contd.). 7 and 8, synchronous and normal M II in dyad. 9 and 10, A II showing 154 chromatids movement to each pole and different spindle orientation. 11, synchronous meiosis II divisions. 12, T II showing one laggard at each spindle. ($\times 2000$).

(Figs. 7 and 8). During anaphase II, 154 chromatids were clearly discernible moving to the respective poles (Figs. 9 and 10). The nuclear divisions were synchronous in all the meiocytes of anther lobe (Fig. 11). Only 2% of the cells were with 1 or 2 laggards (Fig. 12).

Pollen stainability was 85%. The pollen grains were uniform in size and with 2–5 germ-pores.

Discussion

Polyploidy is one of the best evolutionary processes in producing radically different and well adapted genotypes (Stebbins 1950). It has played a major role in the evolution of the genus *Morus* and attained a highest level of decosaploidy $2n=22x=308$ in *M. nigra* (Janaki Ammal 1948). Even among its other species high polyploidy such as hexaploidy ($2n=6x=84$) in *M. serrata* (Basavaiah *et al.* 1989) and *M. teliaefolia* (Seki 1952) and octoploidy in *M. cathayana* (Janaki Ammal 1948) have been reported. Agaev (1984) has reported that the previous chromosome counts $2n=22x=308$ for *M. nigra* are incorrect, as the actual number is $2n=22x=330$. He also stated that the large chromosomes of the low ploidy species appeared to originate from two small chromosomes fused in tandem and some of them separate in high polyploids, thus making the chromosome count variable one (Agaev 1985). But, in the present study, it was clear that the gametic chromosome number was $n=154$ with regular megachromosomes.

The large size of the single nucleolus and multiple nucleoli of various sizes, both the conditions observed are indicative of large number of secondary constrictions (NORs) in the chromosome complement. Usually the number of nucleoli in plants has been correlated with the number of secondary constrictions present in the complement and also the ploidy level (Darvey and Driscoll 1972, Thomas and Kaltsikes 1977). It is also attributed to the position of organisers within the nucleolus (Wilson and Morrison 1966). In this polyploid, it seems apparent that single large nucleolus results from comparative proximity of organisers during premeiotic convergence and multiple nucleoli result from spatial separation of organisers within the nucleus. Obviously, variation in the size of multiple nucleoli can be attributed to the variation in the number of organisers which takes part in each nucleolus.

While considerable chromosomal irregularities are expected in the metaphase I and anaphase I of a ploidy level such as this, surprisingly it exhibits normal meiosis with good pollen fertility. This clearly indicates the cytological stability attained by the polyploid to maintain its level. It is also assessed that bivalents were more frequent than multivalents and the frequency of univalents was negligible. But Agaev and Fedorova (1970) have recorded the occurrence of only bivalents and very few univalents in 22-ploid cultivar 'khar-toot' of *M. nigra*. The presence of both bivalents and multivalents in the complement indicates combined nature of auto- and allo-polyploidy. So, it is difficult to make out the extent of combination of autopolyploidy with allopolyploidy in this polyploid. Janaki Ammal (1948) based on stray polyploid races of *M. alba* and *M. cathayana* has predicted the interspecific hybrid origin of decosaploid *M. nigra*. However, it may be possible to understand the origin of this polyploid only when all its available chromosomal races and allied species are completely studied cytologically.

Summary

Male meiosis in natural decosaploid ($22x$) *Morus nigra* L. has been found regular with good pollen fertility. This is the clear indication of cytological stability attained by the polyploid to maintain its level. Young pollen mother cells showed 1–4 nucleoli. The frequency

of bivalents was more than that of multivalents and univalents occur rarely which clearly indicates the auto-allopolyploid nature of the taxon. The normal meiotic behaviour of this high polyploid is discussed in relation to its evolution.

References

- Agaev, Yu. M. 1984. Chromosome numbers in hybrids between the 22-ploid mulberry species *Morus nigra* and species and forms with low ploidy. *Tsitologia* **26**: 1131–1135.
- 1985. Cytological study of the 22x mulberry species *Morus nigra* L. and its hybrids. *Doklady Akademii Nauk SSR* **281**: 432–435 (Ru).
- and Fedorova, E. E. 1970. Study of meiosis in diploid (*Morus alba* L.), 22-ploid (*Morus nigra* L.) species of mulberry tree and their cross in relation to the problem of *Morus nigra* L. species origin. *Genetika* **6**: 88–100.
- Aliev, M. O. 1979. Use of crossing combinations of the genus *Morus* with different chromosome numbers. *Genet. Sel. Az.* **3**: 119–124 (Ru).
- Anonymus 1962. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products VI. 438–439. CSIR, New Delhi.
- Basavaiah, Dandin, S. B. and Mala, V. R. 1989. Microsporogenesis in hexaploid *Morus serrata* Roxb. *Cytologia* **54**: 747–751.
- Darvey, N. L. and Driscoll, C. J. 1972. Nucleolar behaviour in *Triticum*. *Chromosoma* **36**: 137–139.
- Janaki Ammal, E. K. 1948. The origin of Black Mulberry. *J. Roy. Hort. Soc.* **73**: 117–120.
- Seki, H. 1952. Cytological studies of *Moraceae* plants IV. On the chromosome number of *Morus teliaefolia* Makino. *Res. Rep. Fac. Text. Seric. Shinshu Univ. Jpn.* **2**: 13–17.
- and Oshikane, K. 1960. Studies in polyploid mulberry trees IV. Cytological and morphological studies in *Morus nigra* L. *Res. Rep. Fac. Text. Seric. Shinshu Univ. Jpn.* **10**: 7–13.
- Stebbins, G. L. 1950. Variation and Evolution in Plants. Columbia Univ. Press, New York.
- Thomas, J. B. and Kaltsikes, P. J. 1977. The effect of colchicine on chromosome pairing. *Can. J. Genet. Cytol.* **19**: 231–249.
- Tojyo, I. 1966. Studies on the polyploid mulberry trees IV. On the flower and pollen grains of one race of *Morus nigra* L. *J. Seric. Sci. Jpn.* **35**: 360–364.
- 1985. Research of polyploidy and its application in *Morus*. *JARQ* **18**: 222–228.
- Wilson, G. B. and Morrison, J. H. 1966. Cytology. 2nd ed. Reinhold Publishing Corp. New York. 319 pp.
-