Meiotic behavior and chromosomal association of two diploid mulberry varieties, (Moraceae)

K. H. Venkatesh*, G. S. Pragathi and M. Shivashankar

Plant Breeding and Genetics Laboratory, Department of Life Science, Bangalore University, Bengaluru -560056, Karnataka, India

*Author for correspondence (khvenki1972@gmail.com)
Received August 18, 2018, accepted September 25

ABSTRACT: Two diploid mulberry varieties namely, V₁ and M₁ have been analyzed for detailed meiotic studies. Based on the chromosome configuration and other meiotic behavior x = 14 have been considered as basic number of the genus Morus. Meiosis shows marginal differences. Various meiotic abnormalities like occurrence of one mega chromosome pair at diakinesis, chromosomal bridge and chromosomes grouped more than four poles at anaphase II have been observed.

KEYWORDS: Mulberry (Morus spp.), Diploids, Meiotic behaviour.

Mulberry of the family Moraceae is an economically important tree, its leaves are the sole food for the silkworms (Bombyx mori L). In addition to being fed to Silkworms mulberry is used in industry, medicine, aqua-culture, agro-forestry, social forestry and drought prone area development programmers (Dandin et al. 1992; Munirajappa et al. 1995). Most of the cultivated varieties of mulberry are diploids with 2n = 28 chromosomes, but a few are ployploids (Das 1961; Gill & Gupta 1979; Venkatesh 2007). The first cytological research about plants of the genus Morus L. was done by Tahara (1909) who observed that they generally had a diploid number of chromosomes equal to 14 (2n = 28). For a few Indian species cytogenetical studies were carried out by Das et al. (1970), Kundu & Sharma (1976), Gill & Gupta (1979) and Venkatesh et al. (2013). Diploid mulberry varieties are considered to be superior than triploid and tetraploid mulberry varieties in both leaf yield and nutritive qualities. In the present study, an attempt to understand the chromosome association and meiotic behaviour of two diploid varieties of mulberry have been discussed.

MATERIALS AND METHODS

Mulberry varieties used in the present study are V₁ and M₁ which are maintained in the germplasm bank attached to Department of Life Science Bangalore University, Bengaluru, India. For meiosis sprouting buds with young catkins of appropriate stage of development were fixed in Carnoy’s solution for 12-24 hrs between 8.30 A M to 9.30 A M on sunny days. Fixed materials were preserved in 70% alcohol. Young flower buds were squashed in 2% aceto-carmine and a cover slip was placed on the smear. Gentle pressure was applied over the cover slip to get uniform spreading of chromosomes. Photomicrographs were taken using Sony digital Camera.

RESULTS

Mulberry varieties V₁ and M₁ revealed diploid chromosomes number with 2n= 28 in their pollen mother cells (PMCs) and showed marginal differences in meiosis. Regular occurrence of single large nucleolus and 14 bivalents and mega-chromosome pair was found associated with nucleolus (Figs.1 & 2) at diakinesis. In metaphase I PMC exhibited 12 bivalents, one tri and one univalent scattered in the cytoplasm (Figs. 3). Depending upon the pairing of chromosomes tri and bivalent showed different configurational shape. Like wise anaphase I showed unequal separation of chromosomes (Fig. 4) and chromosomal bridge (Fig. 5) respectively. Metaphase II was also observed (Fig. 6). Anaphase II was quite abnormal due to five groups of chromosomes suggesting the improper action of spindle fibres (Fig. 7). Telophase II was also observed (Fig. 8). Tapetal cells showing multinucleate condition (Fig. 9). At the end of meiosis II we observed pollen tetrads (Fig. 10; Tetrahedral & isobilateral) and pollen grains (Fig. 11).

The taxa investigated in the present study, V₁ and M₁ displayed diploid chromosome number of 2n = 2 x= 28 and marginal differences in meiosis. Genus morus has wide range of chromosome number from 2n = 28 to 2n = 308 and ploidy from x to 22x. Tahara (1909) has reported 2n = 28 for M. alba and M. indica. The present findings are in agreement with the previous report (Gill & Gupta 1979). Hence the highest chromosome number of 2n = 308 (22x) was established by Janaki Ammal (1948) with a polyploidy series of x = 14 to 22x. Regular occurrence of single large nucleolus and association of megachromosome pair to the nucleolus at diakinesis indicates that two homologous nucleolar organizers of diploid complement organize a large nucleolus. The number of nucleoli in plants has usually been correlated with the number of secondary constrictions present in the complement and also the ploidy level (Darvey and
Figures 1-11. 1: Pollen mother cell showing chromosome association and nucleolus, 2: Diakinesis-one megachromosome pair associated with nucleolus, 3: Metaphase-I showing 12 bivalents, one trivalent and one univalent, 4: Anaphase-I showing unequal separation of chromosomes, 5: Anaphase-I showing chromosome bridge, 6: PMC at metaphase-II, 7: Anaphase-II showing chromosomes grouped into five poles, 8: Telophase II, 9: Tapetal cells showing multinucleate condition, 10: Pollen tetrad, 11: Pollen grains.
Driscoll 1972; Thomas and Kaltsikes 1977). Presence of 14 bivalents in most of the PMCs at metaphase I confirms the diploid nature of the Plants. One pair of megachromosomes was found bigger in comparison to other as also reported by Gill and Gupta (1979) and Dwivedi et al. (1986) in the genus Morus. The present investigation also showed that there were no secondary associations and multivalents in diakinesis and metaphase I in both the varieties studied. Sinoto (1929) while studying the behaviour of allosomes, except a large pair of chromosomes in meiosis, has also not reported by any such secondary associations. Observation made by Dutta (1954) may be due to the large size of one pair chromosomes which may be mistakes for trivalent/tetravalent. The high Frequency of bivalents is suggestive of a fair degree of homology between the constituent genomes and the diploid nature of these varieties.

As expected, the disturbance in the meiosis I has adverse effect on the subsequent stages of meiosis II, in both the varieties. Meiotic abnormalities like presence of tri and univalent at metaphase I, unequal separation and chromosomes grouped at more than four poles at anaphase I and II suggesting the action of extra spindle fibers is attributed to irregularities in basic process like chromosome pairing and alignment leads to the formation of aberrant pollen grains with unequal number of chromosomes. High percentage of pollen fertility in male and seed set in female plants with 2n=28 chromosomes indicate the dibasic nature of these varieties.

**Conclusion**

Chromosomal association and meiotic behavior during microsporogenesis were studied in two diploid varieties of mulberry. Based on the present findings, V₁ and M₅ are diploids have 2n = 28 chromosomes. It can be concluded that 14 as the basic chromosome number. Marginal irregular meiosis, association of mega chromosome pair with nucleolus, anaphasic separation was unequal leading to the formation of aberrant pollen grains.

**Literature Cited**

Dandin, S. B., Ranganatha Sastry, K. N. and Bongale, U. D. 1992. Mulberry in waste land development programme scale. Indian silk, 26(3): 12-14.

Darvey, N. L. and Driscoll, C. J. 1972. Nuclear behaviour in *Triticum*. Chromosoma 36: 137-139.

Das, B. C. 1961. Cytological studies in *Morus indica*. L. and *Morus laevigata wall*. Cytologia 19: 159 – 165.

Das, B. C., Prasad, D. N. and Sikdar, A. K. 1970. Colchicines induced tetraploids of mulberry. Caryologia 23 (3): 283 - 293.

Dutta, M. 1954. Cytological studies in the species of *Morus*. Cytologia 19: 86 - 95.

Dwivedi, M. K. Sikdar, A. K., Dandin, S. B., Sastry, C. R. and Jolly, M. S. 1986. Induced tetraploidy in mulberry 1, Morphological, anatomical and cytological investigations in cultivar RFS – 135, Cytologia 51 (2): 393 - 401.

Gill, G. S. and Gupta, R. C. 1979. Cytological studies the sex types of *Morus alba*, L. (*Moraceae*). Current Science 48 (1): 35 - 36.

Janaki Ammal, E. K. 1948. The origin of black mulberry. J. Roy. Hort. Soc., Vol. 75: 117 - 120.

Kundu, D. and Sharma, A. 1976. Chromosome studies in some Indian Moraceae. In P. Kachroo (ed.), Recent advances in Botany Bishen Singh Mahendra Pal Singh Dehradun. pp. 348-369. Munirajappa, Ramesh, H. L. and Nijagunaiah, R. 1995. Revitalizing dwindling ecosystem through mulberry cultivation. National seminar on Man, Industry and Environment Organised by Dept. of Environmental Science, Bangalore University Bangalore-56, 5th-8th April 1995.

Sinoto, Y. 1929. Chromosomes studies in some dioecious plants with special reference to the allosomes. Cytologia 1. 109-191.

Tahara, M. 1909. On chromosomes of *Morus alba*. Bot. Mag. Tokyo, 23: 343 - 353.

Thomas, J. B. and Kaltsikes, P. J. 1977. The effect of colchicines on chromosome paring. Con. J. Genet. Cytol. 19: 231-249.

Venkatesh, K. H. 2007. Cytogenetical investigations in the Genus *Morus* L. Ph.D., Thesis Bangalore University, Bangalore.

Venkatesh, K. H., Venu, N., Dinesh, B. and Munirajappa. 2013. Stomatal frequency and karyotypic studies in three varieties of mulberry J. Cytol. Gen., 14 (NS): 101-107.