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Cytogenetical studies in two tetraploid mulberry varieties (Moraceae)

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ABSTRACT. *Morus* L. an economically important genus has more than 60 species distributed in both the Hemispheres. Chromosome number varies from 2n=28 to 2n=308 with ploidy level from x to 22x. In the present investigation two tetraploid mulberry varieties namely, *Morus cathyana* and *M. macroura* have been analyzed for detailed meiotic studies. Based on the chromosome configuration and other meiotic behaviour x = 14 has been considered as basic number of the genus. Meiosis was irregular. Various anomalies like univalents, bivalents, trivalents and quadrivalents are associated with nucleolus at diakinesis. Tetra, tri and bivalents in addition to univalents are noticed in metaphase I. Unequal separation of chromosomes are observed in anaphase I and II. Laggards and precocious movement of chromosomes at metaphase II and unoriented chromosomes at anaphase II have been observed.

KEYWORDS: Meiotic behavior, Mulberry, Tetraploids

For the rearing of silkworms (*Bombyx mori*) mulberry cultivation is the pre requisite. The foliage of the plant is used mainly as a unique source of silkworm (*Bombyx mori* L.) feed and cultivated in over 40 countries (Machii and Katagari, 1991). Cytogenetical studies, which is a prerequisite for genetic improvement in plant species. Most of the species of *Morus* are diploid having 2n=2x=28 chromosomes, but a few species, namely, *M. tiliaefolia* Makino, *M. cathyana* and *M. nigra* L., are higher polylpoids (Darlington and Wylie 1955). *Morus laevigata Wall* is a natural tetraploid occurring in the wild and in the cultivated forms in the eastern Himalayas (Dutta 1954; Das 1961). Its leaves are unsuitable for silkworm feeding. The occurrence of natural tetraploids of *Morus* has not been reported from any other part of the world than India. Besides utilizing the tetraploids for higher leaf yield and silkworm feeding, they may serve as a source of breeding material for the production of triploid varieties. Autotetraploid have been indicated in induced in mulberry by Kedarnath and Lakshmikanth (1965), Tojyo (1966), Sastry *et al*. (1968), Das *et al*. (1970), Dhafurov, and Alekperova (1978) and Alekperov (1979). There have been number of reports on the induction of tetraploidy in mulberry through colchicine treatment of germinating seeds, seedlings and vegetative buds (Das *et al*. 1970; Dwivedi *et al*. 1986; Verma *et al*.1986; Sikdar and Jolly 1994).

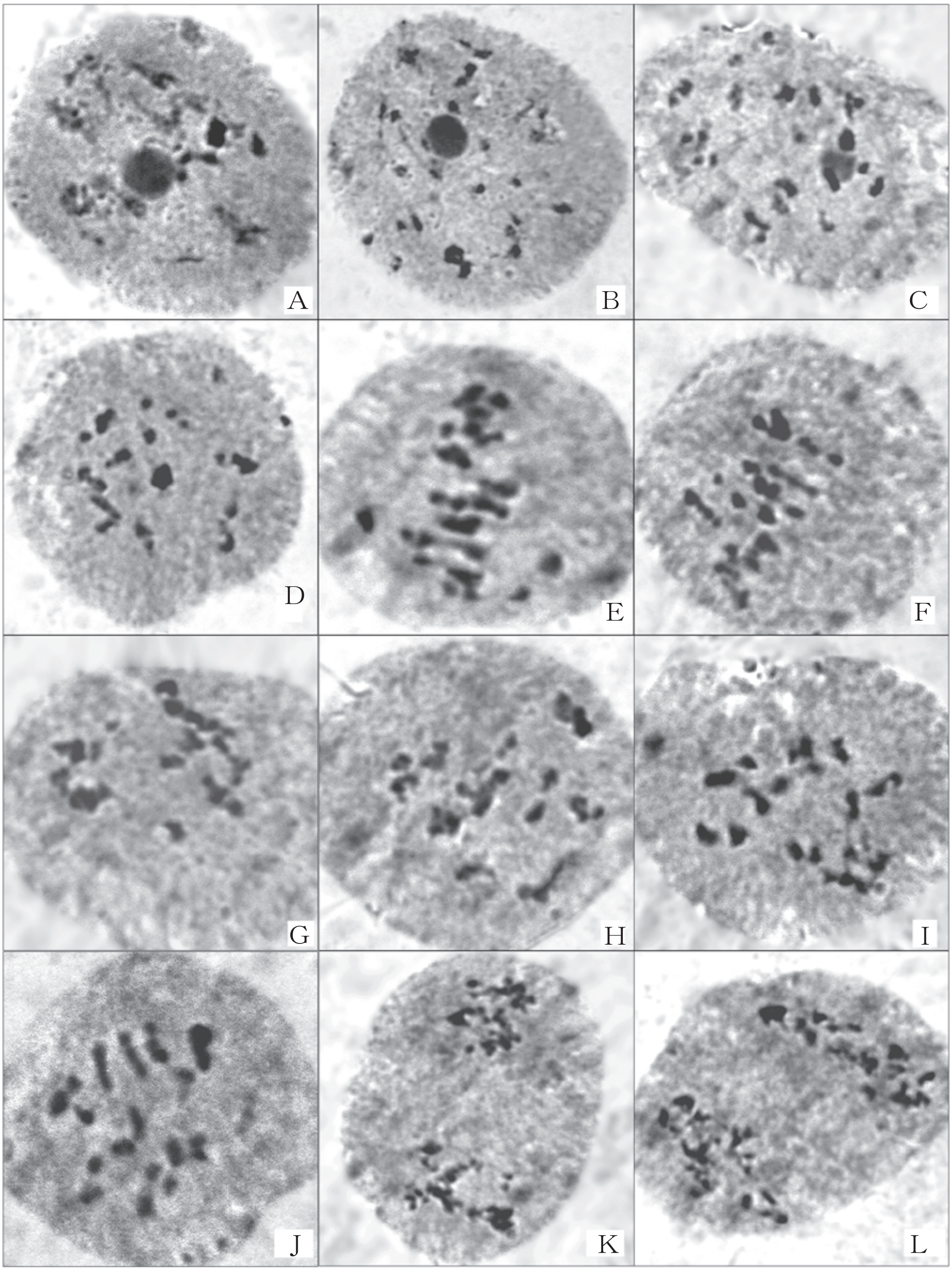
Hazama (1968) reported that due to slow shoot growth of tetraploids the yield of leaf is less than that of diploid. But the superior nutritive quality of leaves of tetraploid and triploid varieties were reported were by Seki and Oshikane (1959) and Dzhafurov and Alekperov (1978). In the present study, an attempt to understand the chromosome association and meiotic behaviour of two natural tetraploid varieties of mulberry have been discussed.

MATERIALS AND METHODS

Mulberry varieties used in the present study are *Morus cathyana* and *Morus macroura*, which are maintained in the germplasm bank attached to Bangalore University, Bangalore, India. For meiosis flower buds of appropriate stages of development were harvested at 9.30 AM. during sunny days and fixed in 1:3 acetic-alcohols for 24 hours and preserved in 70% ethanol. Anthers were squashed in 2% aceto-carmine stain. Photomicrographs were taken using labomed microscope fitted with Nikon cool fix digital camera

RESULTS AND DISCUSSION

The taxa investigated in the present study, *Morus cathyana* and *M. macroura* displayed tetraploid chromosome number with 2n=4x=56. Meiotic behaviour was slightly irregular. At diakinesis chromosomes showed various types of association. The small size and huge number of chromosomes in the component offered a great handicap for critical analysis. But obviously, the bivalents were more frequent than the multivalents and frequency of univalents was very much less. In some PMCs one or two quadrivalents are found associated with the nucleolus and quadrivalents were frequent than, bivalents and univalents. Apart from the regular occurrence of single large nucleolus, few pollen mother cells (PMCs) showed two nucleoli, one large and another small. During metaphase I majority of the PMCs exhibit, chromosomes are scattered in the cytoplasm without aligning on the equatorial plate except, a few PMCs shows regular alignment of chromosomes complement on the equatorial plate and showed irregular meiosis. Four homologous "mega chromosomes" characteristically exhibit early condensation and deep staining. Chromosome pairing appears to be normal for tetraploid with three homologous being synapsed at any one point while the fourth homologue remains unpaired with them. And one quadrivalent found associated with nucleolus (Fig. 1A). At diakinesis large nucleolus and formation of quadrivalents, trivalents, bivalents and univalents are clearly seen (Fig. 1B) and the majority of the cells, bivalents and quadrivalents were found associated with the nucleolus (Fig. 1C). Very few PMC's showed single small nucleolus (Fig. 1D). Metaphase I showed various types of chromosomal configurations (Figs. 1E-J) and showed various types of chromosomal configurational shapes, ('>--', '>-', '---' and '--'). The range of quadrivalents, trivalents, bivalents and univalents was 4-14, 1-6, 0-26 and 0-2 respectively. And their mean being 8.420, 3.674, 6.92 and 0.024, respectively. The frequencies of quadrivalents and bivalents was higher than that of the trivalents and univalents. Quadrivalents were mostly of either chain type or ring type. Out of 30 cells analyzed for anaphase I, 21 cells were normal with equal distribution of chromosomes (28:28, Figs. 1K and L) and other nine cells abnormalities like unequal separation (Figs.1M and N). Regular daughter nuclei were formed at the end of



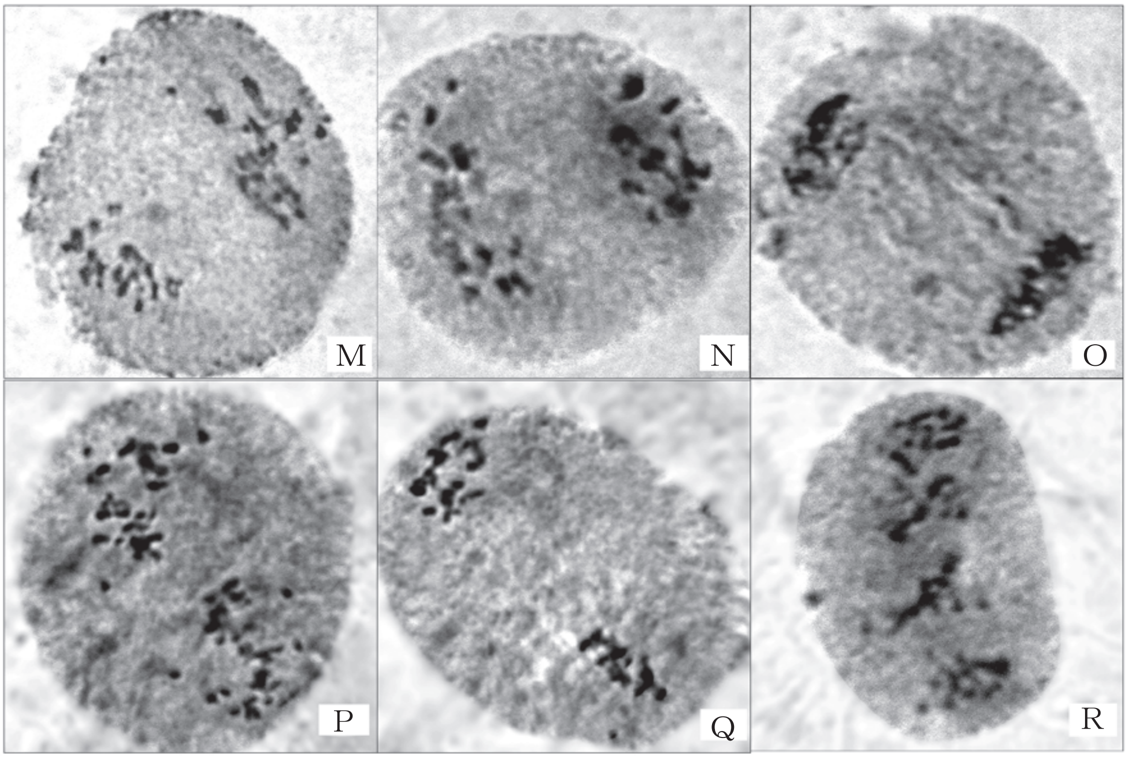


Fig. 1. Chromosome association and meiotic behavior during meiosis I and II in tetraploid varieties of mulberry. A. Diplotene (deeply stained quadrivalents of mega chromosome). B. Diakinesis. C. Diakinesis showing one bivalent associated with nucleolus. D. Diakinesis with micro nucleolus. E-J. Metaphase I showing different types of chromosomal configurations. K-L. Anaphase I (equal separation). M and N. Anaphase I (unequal separation). O. Telophase I. P and Q. Metaphase II (laggards). R. Anaphase II, showing unoriented and unequal separation of chromosomes.

Table. 1 Chromosome distribution at anaphase I in tetraploid

Distribution of chromosomes B = lagging bivalents U = lagging univalents	No. of cells	Percentage
1. 28 : 28	21	70.00
2. 29 : 27	2	6.66
3. 27 : 29	1	3.33
4. 28 : 27 :1U	1	3.33
5. 27 : 27 : 1B	2	6.66
6. 28 : 26 : 1B	2	6.66
7. 26 : 27 : 1B + 1U	1	3.33
Total	30	99.97

telophase I (Fig. 1H). Meiosis II was also slightly irregular. Metaphase II showed many chromosomes scattered in the cytoplasm without aligning on the equatorial plate and unequal number of chromosomes in daughter cells respectively (Figs.1 P and Q). This has lead to unoriented and unequal separation of chromosomes during anaphase II (Fig. 1R). Subsequently leading to the formation of isobilateral tetrad.

Among the various species of *Morus* L., a high degree of poly ploidy ranging from diploid (2n=2x=28) to 22 ploidy (2n=22x=308) has been reported (Fedorov 1969). Further hexoploid has been reported only in *M. cathyana* (Janaki Ammal 1948) and *M. tiliafolia* (Seki 1959). In *M. serrata*, only diploid chromosome number 2n=28 has been reported by Janaki Ammal (1948) and hence the present report is tetraploidy in this species. In general this tetraploid exhibits slightly irregular meiosis and is characterized by high frequency of quadrivalents and bivalents. However, the occurrence of multivalents such as tetra and trivalents indicates its polyploidy nature. This suggests that, the taxon may be segmental allo tetraploid as per the classification of Stebbins (1947). Stebbins (1950) pointed out that polyploids undergo several secondary modifications in the course of evolution. One such modification commonly noticed in many tetraploid species in the regular meiosis and total absence of multivalents similar to normal diploids.

In respect to meiotic behaviour tetraploids can be considered as multiple tetrasomics and their greatly reduced fertility makes genetic research quite difficult (Sybenga 1972). In general meiosis in tetraploids was highly irregular. PMCs at diakinesis showed two unequal sized nucleoli. This indicates that two homologues nucleolar organizers of diploid complement organize a large nucleolus, while the other organizer of the 4th genome forms the extra nucleolus. The number of nucleoli in plants has usually been correlated with number of secondary constrictions present in the complement and also the ploidy level (Darvey and Driscoll 1972, Thomaskaltsikes 1977). Thus high frequency of tetravalents is suggestive of a fair degree of homology between the constituient of genomes and the tetraploid nature of these varieties.

Theoretically tetraploids should form more quadrivalents in meiosis due to the presence of four homologous chromosomes but it is not so in the present tetraploids where in the quadrivalents range 4-14 with the mean value of 8. 420 and bivalents range 0-26 with the mean value of 6.92. The number of quadrivalents observed during diakinesis suggest the possible hybrid origin of the putative diploid. The other four configurations of quadrivalents such as trivalent plus univalent, one bivalent plus two univalents, two bivalents and four univalents suggest the segmental homology of the chromosomes which in turn also indicates the allo tetraploid nature of induced tetraploids. Formation of 26 bivalents and two univalents in some PMCs suggest that the presence of more than two homologous chromosome is not the only pre requisite for multivalent association. This observation also supports the genetic control of chromosome pairing.

Meiotic abnormalities such as irregular distribution and unequal separation of chromosomes in anaphase I and II, precocious movement and disturbed metaphase I and II resulted in the size variations of pollen and their low fertility which was reduced to half in the triploids and tetraploids as compared to diploids (Venkatesh & Munirajappa 2012, Venkatesh *et al*., 2012). Decreased fertility in tetraploids of *Morus* has also been reported by Seki (1959). The reduced fertility in polyploidy is generally attributed to the multivalent association of chromosomes during synopsis (Darlington 1937). Stebbins (1947; 1950) and various other meiosis abnormalities which invariably result in loss of chromatin material (Das *et al*. 1970, Gottschalk 1978).

Conclusion

Tetraploids are characterized by stunted growth, large, thick, coarse and dark green leaves. Tetraploids of mulberry were produced by the treatment of young seedlings and bud treatment with colchicine solution. Chromosomal association and behaviour during microsporogenesis were studied in two tetraploid varieties namely, *Morus macroura* and *Morus cathyana* (2n=4x=56). The meiosis in these varieties was slightly irregular. The high frequencies of quadrivalents suggested allotetraploid nature of this variant. Various types of chromosomal associations, presence of univalents, unequal separation and unoriented chromosomes at anaphase I and II, precocious movement of chromosomes at metaphase I and II have been frequently observed in the PMCs. The reduced pollen fertility has been attributed to the loss of chromatin material due to meiotic abnormalities and multivalent association.

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