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 Hemisphe- res. 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 cathayana 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. cathayana 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 mulberry by Kedarnath and Lakshmikanth (1965), Tojyo (1966), Sastry et al. (1968), Das et al. (1970), Dzhafurov, 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.
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
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. cathayana* (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

### Table 1 Chromosome distribution at anaphase I in tetraploid

<table>
<thead>
<tr>
<th>Distribution of chromosomes</th>
<th>No. of cells</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>B = lagging bivalents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U = lagging univalents</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. 28 : 28</td>
<td>21</td>
<td>70.00</td>
</tr>
<tr>
<td>2. 29 : 27</td>
<td>2</td>
<td>6.66</td>
</tr>
<tr>
<td>3. 27 : 29</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>4. 28 : 27 :1U</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>5. 27 : 27 : 1B</td>
<td>2</td>
<td>6.66</td>
</tr>
<tr>
<td>6. 28 : 26 : 1B</td>
<td>2</td>
<td>6.66</td>
</tr>
<tr>
<td>7. 26 : 27 : 1B + 1U</td>
<td>1</td>
<td>3.33</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>99.97</td>
</tr>
</tbody>
</table>
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, Thomaskaltisches 1977). Thus high frequency of tetravalents is suggestive of a fair degree of homology between the constituent 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 & Munirajapppa 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.

**LITERATURE CITED**


