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Cytomorphological studies of two mulberry varieties (Moraceae)

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Two mulberry varieties, namely, S_{34} and Tr-$_{10}$ were selected for cytomorphological studies. Stomatal frequency, somatic chromosome number, ploidy level and meiotic behaviour were studied for these varieties. S_{34} is diploid with 2n=28 and Tr-$_{10}$ is triploid with 2n=42 chromosomes. Meiosis was irregular. Various anomalies like univalents, bivalents, trivalents laggards, loose association and unequal separation of chromosomes were observed in some pollen mother cells (PMCs) studied. Stomatal frequency was found to be lesser in triploid when compared to diploid variety.

Key words: Mulberry (*Morus* spp.), stomatal frequency, diploid, triploid, mitosis, meiosis.

INTRODUCTION

Mulberry is an outstanding bio energy plant of the family Moraceae. In addition to being fed to silkworms (*Bombyx mori* L.) mulberry is used in industry, medicine, aqua-culture, agro-forestry, social forestry and drought prone area development programmes (Dandin et al., 1992; Munirajappa et al., 1995). In sericulture, the most important factor is the cultivation of elite mulberry varieties exhibiting desirable agronomical and commer-cial traits. It is an established fact that about 60% of the total cost of silk production is attributed to mulberry production alone. Therefore, it is very important to select high yielding varieties with better quality leaves. Most of the cultivated varieties of mulberry are diploids with 2n=28 chromosomes, but few are polyploids (Venkatesh, 2007). For a few Indian species, cytogenetical invest-tigation was carried out by Kundu and Sharma (1976) and Venkatesh et al. (2013). Cytotaxonomy of the genus *Morus* L. clearly indicates the lacunae. Triploids (2n=42) are developed through natural or controlled hybridization between diploid and tetraploid parents and are considered to be superior than diploids and tetraploids in leaf yield and nutritive qualities of leaf. In the present study, an attempt to understand the stomatal frequency, somatic chromosome number and meiotic behaviour of two different varieties of mulberry have been discussed.

MATERIALS AND METHODS

Mulberry varieties S_{34} and Tr-$_{10}$ were used in the present study and maintained in the germplasm bank attached to Department of Sericulture, Bangalore University, Bangalore. Somatic preparations were made from excised root tips of potted plants. Root tips were collected at 10.15a.m. and pre-treated with 0.002 M 8- hydroxy quinoline for 3 h at 10°C.

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After thorough washing, root tips were hydrolysed in 1 N HCl for seven minutes at 40°C and then stained with 2% aceto-orcein. Squash preparations were made in 45% acetic acid. Photographs were made on the same day of preparation to ascertain the chromosome number and ploidy level. For meiosis, flower buds of appropriate stages of development were collected between 9.20 to 10.30 a. m. during sunny days and fixed in 1:3 acetic-alcohols for 24 h and preserved in 70% ethanol. Anthers were squashed in 2% aceto-carmine stain. Photo-micrographs were taken using labomed microscope fitted with Nikon cool fix digital Camera. Stomatal frequency was determined by nail polish impression method. Stomatal frequency was calculated by using the formula and expressed as number of stomata/mm^2 (Aneja, 2001; Sikdar et al., 1986).

$$\text{Stomatal frequency} = \frac{\text{Number of Stomata}}{\text{Area of microscopic field}} \times \text{mm}^2$$

RESULTS AND DISCUSSION

Variety S_{34}

Variety S_{34} evolved through artificial hybridization between S_{30} and C_{776} at Central Sericultural Research and Training Institute, Mysore. This variety is best suited for rain fed condition. It has better rooting and sprouting abilities and it is capable of thriving well both in temperate and tropical climatic conditions. Stem is green to brown in colour. Leaves are unlobed ovate and light green in colour. The stomatal frequency of this genotype was found to be 190.97/mm^2 (Figure 1a). This genotype revealed 2n=28 somatic chromosomes number (Figure 1b) and it is a diploid genotype. Mitosis was regular with equal separation. Meiotic behaviour was also regular. In metaphase I majority of the pollen mother cells (PMCs) exhibited 14 bivalents are scattered in the cytoplasm without any additional association, of which one bivalent was larger than the others (Figure 1c). Anaphase I showed equal separation of chromosomes were clearly discernible to the respective poles (Figure 1d). Telophase and prophase II were also observed in Figure 1e and f, respectively. Anaphase II was quite abnormal due to the presence of laggards, unequal separation and precocious movement of chromosomes (Figure 1g). Pollen stain ability was found to be 97.23% (Figure 1h).

Variety Tr_{-10}

It evolved through ploypoid breeding technique. It is being cultivated as a perennial bush especially in hilly tract. Stem is green to brown in colour. Leaves are unlobed ovate and dark green in colour. The Stomatal frequency of this genotype was found to be 161.21/mm^2 (Figure 2a). This genotype revealed 2n=42 somatic chromosomes number (Figure 2b) and it is a triploid genotype. Meiosis was irregular. Metaphase I showed loose association and various types of chromosomal associations (Figure 2c). Depending on the pairing of chromosomes, uni, bi, and trivalents showed different configurations. Anaphase I, showed unequal separation of chromosomes (Figure 2d). Majority of the PMCs showed unequal separation of chromosomes at anaphase I. Chances of equal distribution was meagre and 22:20 and 23:19 distribution were common among the PMCs observed. In this variety, the trivalents frequency was high and various types of chromosomal configurations (‘ >-’ ‘---’ and ‘v’) were observed. Different types of chromosomal configurations found in the present study corroborated the findings of Das et al. (1984), Basavaiah et al. (1990) and Venkatesh and Munirajappa (2012) in triploid mulberry varieties. Telophase I was also observed (Figure 2e). Pollen stain ability was found to be 92.23% (Figure 2f).

Cultivars S_{34} and Tr_{-10} have similarity in their adaptation that is, unlobed and light green leaves, good rooting, etc. Triploid forms are better rooting, grow more quickly and posses larger leaves when compared to diploids and tetraploids (Eswar Rao et al., 2000). Stomatal frequency is an important parameter in selecting drought resistant genotype and also belived to regulate leaf yield. The frequency of stomata per unit area is significantly less in triploid compared to diploid. Basavaiah and Murthy (2001) reported that, moisture retention capacity will be higher in those mulberry varieties possessing smaller and lower stomatal frequency. The present findings are also in agreement with the reports of Vijayan et al. (1998). Stomatal frequency also correlated with drought and disease resistant (Hatalli et al., 1993; Nautiyal et al., 1994). Further lesser frequency per unit area is more suitable for rain fed conditions. However, reduction in the internodal and number of stomata per unit area indicates that the increased dosage of genes does not always increase in size but may also reduce it (Dwivedi et al., 1986).

In the present investigation in the genus *Morus* analyzes the chromosome number, ploidy level, meiotic behaviour and stomatal frequency in two different mulberry genotypes. Among these, the present study recorded the S_{34} is diploid (2n=28) and Tr_{-10} is triploid (2n=42) chromosome number of the mulberry varieties studied.

In general meiosis in diploid was regular and it was clear that the gametic chromosomes number was n=14 with regular mega chromosomes. There were no secondary associations and multivalents in metaphase I. Presence of 14 bivalents in most of the PMCs at metaphase I confirms the diploid nature of the variant. The high frequency of bivalents is suggestive of a fair degree of homology between the constituent genomes and allo diploid nature of the variety. One bivalent was found bigger in comparison to others as also reported by Gill and Gupta (1979) in *Morus.* Anaphase I was quite abnormal in triploid variety due to unequal separation of chromosomes, is attributed to irregularities in basic process

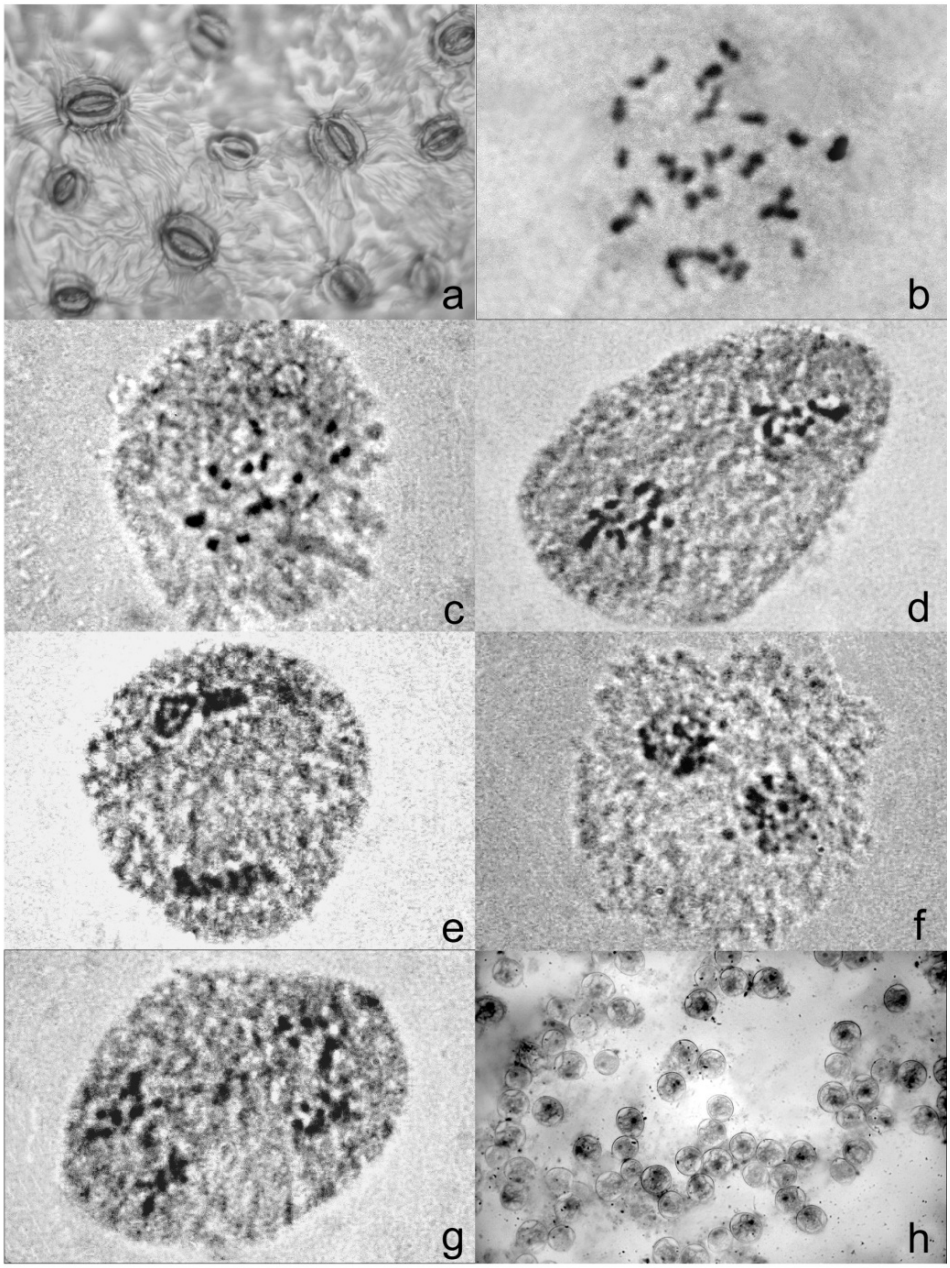

Figure 1. a and b. Stomatal frequency and somatic chromosomes (2n=28) of variety S_{34}, **c.** metaphase I (13 bivalents and one large bivalent scattered in the cytoplasm). **d.** anaphase I, **e.** telophase I, **f.** prophase II, **g.** anaphase II (laggards and unequal separation of chromosomes), h. pollen grains.

like chromosome pairing and alignment. The presence of higher percentages of trivalents in metaphase I is a suggestive of fair degree of homology between the constituent genomes and auto triploid nature of the variety. Meiotic abnormalities such as unequal separation, presence of uni, bi and trivalents, loose association and irregular distribution of chromosomes to different poles have resulted in the size variation of pollen and their low fertility compared to diploids. Reduced pollen fertility observed in triploid here is in confirmation with the findings of Tikader and Dandin (2007). Reduction in pollen fertility in triploid variety can be attributed as multivalents association during synopsis and invariably results in loss of chromatin materials (Gottschalk, 1978). These findings will be much use in establishing a phylogenetic relationship, evolution of mulberry and selecting mother plants for hybridization on the basis of micro morphology, somatic chromosome number and

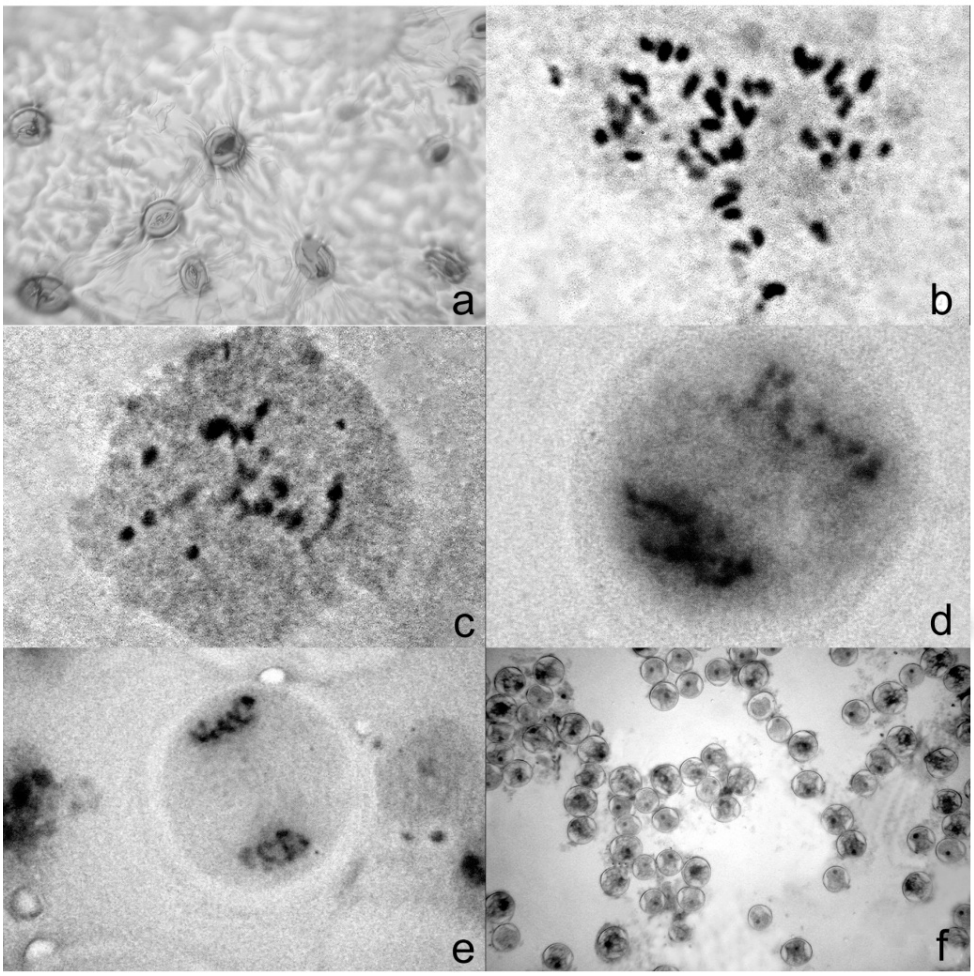

Figure 2a and b. Stomatal frequency and somatic chromosomes (2n=42) of variety Tr-$_{10,}$ **c.** metaphase I (loose association of trivalents), **d.** anaphase I (unequal separation of chromosomes), **e.** telophase I, f. pollen grains.

ploidy level of the mulberry varieties.

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