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Studies on Cytomorphology of Two Mulberry Varieties (Moraceae)

KEYWORDS: Mulberry (Morus spp.), morphology, diploids, uneuploid, mitosis, karyotype analysis

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ABSTRACT *Regarding, micromorhological and karyomorphological studies of two mulberry verities namely, Channapatna local and M5 were selected. Morpho-criteria, stomatal frequency, somatic chromosome number, ploidy level, karyotype analysis, arm ratio and total haploid chromatin length were studied. Varieties studied exhibited considerable variations in height, internodal distance, leaf texture, leaf color and stomatal frequency. Stomatal frequency is lesser in uneuploid when compared to diploid variety. Channapatna local is diploid with 2n=28 and M5 is uneuploid with 2n=30 somatic chromosomes numbers respectively. Somatic chromosome length ranges from 1.46 µm to 2.86 µm where as arm ratio ranges from 0.52 to 1.00 µm. Karyotypes of these taxa are symmetric. Only metacentric and submetacentric chromosomes are found in the somatic complement.*

Introduction

In sericulture, the most important factor is the cultivation of elite mulberry varieties exhibiting desirable agronomical and commercial 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. In mulberry cultivation, attention must be given to both quality and quantity of leaves. They must be high yielding with low inputs. Most of the cultivated varieties of mulberry are diploids with 2n=28 chromosomes, but a few are polyploids (Gill & Gupta 1979, Venkatesh 2007). For a few Indian species, cytological investigations were carried out by Das et al. (1970), Kundu and Sharma (1976). Venkatesh & Munirajappa (2012 & 2013) studied the meiotic behaviors of triploid (2n=42) and tetraploid (2n=56) varieties of Morus. Triploids are developed through natural or controlled hybridization between diploid and tetraploid parents and are considered to superior than diploids and tetraploids in leaf yield and nutritive qualities of leaf. Venkatesh et al., (2013) studied the morphological, anatomical and reproductive parameters in different ploidy levels of mulberry varieties. These different chromosomes numbers has reflected on their micromophology, anatomy and reproductive characters of diploid, triploid and tetraploid varieties. In the present communication is the part of the investigation and details of morphology, anatomy and karyomorphological studies of two varieties of mulberry.

Materials and methods

Morphology

Three mulberry varieties namely, Channapatna local and M_5 which are maintained in the germplasm bank of Department of Sericulture, Bangalore University, Bangalore, India, were taken for the present study. Cuttings of these varieties were planted in pots for experimental use. Morphological characters are critically examined at different stages of growth and development. Following the procedure laid down in the mulberry descriptor (Dandin & Jolly 1986).

Mitosis

Somatic preparations were made from excised root tips of potted plants. Root tips were collected between 9.45 to 10.30 a.m. and pre-treated with 0.002 M 8 - hydroxyquinoline for 3 hours at 10^0c. After washing in water the root tips were hydrolyzed in 1N HCl for seven minutes at 50^0c and then stained with 2% aceto-orcein. Squash preparations were made in 45% acetic acid. Photomicrographs and drawings of good and clear plates were made immediately. To ascertain the chromosome number and their morphology, a number of preparations were examined in each variety. Ideograms were drawn using suitable scale. Karyotype classicification was made according to Leven et al. (1964) by taking arm ratio into an account.

Stomatal frequency

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).

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

Results and discussion

Variety Channapatna local: It is evolved at Kanva silk farm located at Channapatna, Karnataka. It is best suited for rain fed condition. It is low leaf yielding variety. Stem is woody, cylindrical, clothed with many lenticels and green to brown in color. Milky latex present in the stem. Leaves are simple, thin, light green, coriaceous, chordate, lobed serrate, acuminate, exhibited medium height, short, thick petiole and longer internode (Fig. 1). Stomatal frequency was found to be 194.26/mm^2 (Fig. 2). It is diploid genotype 2n=28 chromosomes (Fig. 3). Somatic chromosomes are measuring from 1.46.m to 2.86m in length and arm ratio ranges from 0.52 to 0.90 m. The karyotype formula of this genotype is $2n=28=4B^m+14B^{sm}+2C^m+8C^{sm}$ (Fig. 7). The total chromatin length of the haploid set is 29.80m.

Variety M_5: It is female an open pollinated hybrid selected from seedlings population of Mysore local variety at Kanva silk farm, Channapatna, Karnataka. This genotype is best suited for irrigated condition. Under ideal agro climatic condition it yields 35 tones of leaf per hectare in one year. Stem is woody, cylindrical, clothed with many lenticels and green to brown in color. Milky latex present in the stem. Leaves are simple, thin, light green, coriaceous, chordate, lobed serrate, acuminate, exhibited maximum height, short, thick petiole and shorter internode (Fig. 4). Stomatal frequency was found to be 180.30/mm^2 (Fig. 5). It is found to be uneuploid with 2n=28 chromosomes (Fig. 6). Somatic chromosomes are

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measuring from 2.00.m to 2.96m in length and arm ratio ranges from 0.60 to 1.00 m. The karyotype formula of this genotype is $2n=30=18B^{m}+12B^{sm}$ (Fig. 8). The total chromatin length of the haploid set is 38.84m. Details of the somatic chromosome number, ploidy level, range of chromosome length, karyotype formula, arm ratio and haploid chromatin length are presented in Table 1. Of the cultivars studied, one is diploid (2n = 28) and another one is uneuploid (2n = 30).

Table 1. Karyomorphological details of two mulberry verities

Mulberry varieties	Stomatal frequency/mm^2	chromosome number (2n)	Ploidy level	Karyotype formulae	Range of chromosome Length (m)	Arm ratio (m)	Haploid chromatin Length(m)
Channapatna local	194.26	28	Diploid	$2n=28=4B^{m}\ 14B^{sm}+2C^{m}+8C^{sm}$	1.46-2.86	0.52- 0.90	29.80
M_5	180.30	30	Uneuploid	$2n = 30 = 18B^{m}+12B^{sm}$	2.00-2.96	0.60- 1.00	38.84

Though, the taxa studied resembled generally in their gross morphological features, they exhibited a great deal of variation in phenotypic charecristics. In general, the architecture of the taxa is common. Plants are woody with tap root system. Basically they are trees but cultivated as shrubs or as low bushes by practicing pruning and training techniques. The frequency and size of stomata per unit area is significantly less in uneuploid compared to diploid. Stomatal frequency is an important parameter in selecting drought resistant genotype. Stomatal frequency correlated with drought and disease resistant (Hatalli et al. 1993; Nautiyal et al., 1994). Further lesser frequency and smaller size of stomata per unit area is more suitable for rain fed conditions.

Kastumata (1972) and Venkatesh et al., (2013) studied exomorphic features in some mulberry genotypes and recorded morphological variations. These variations are largely due to genetic flux operating on the evolution of different mulberry variants. Morphological characters are strongly heritable in nature and expected to manifest in the environment. These characters are used in genetic identity and to distinguish between varieties. (Dandin & Kumar; 1989).

Perusal of the existing literature on chromosome numbers for the genus Morus clearly indicates the occurrence of 2n=28 to 2n=308. However, Janaki Ammal (1948) has reported chromosome number of 2n=26 in M. alba. It is a stray report and this number (2n=26) has not been so far reported by other investigators. Das (1961) and Datta (1954) have reported basic number of x=7 for Morus based on the presence of secondary association in few varieties of M. indica. But in the present study as well as the observations made by others rule out the existence of secondary association of chromosomes in majority of Morus spp. (Venkatesh 2007).

Among two mulberry varieties studied, one is diploid with 2n=28 chromosomes and another one is uneuploid with 2n=30 chromosomes. In general chromosomes are smaller with a close range of length variation and form a graded series. The observation of uneuploid number like 2n=30 may be due to the vegetative propagation which invariably results in polysomaty as reported by Das (1963). The partial adaptation of a vegetative propagation has resulted in the maintenance of such altered nuclei in the somatic tissues as stated by Kundu and Sharma (1976). Only metacentric and sub metacentric chromosomes are found in the somatic complement of these taxa. The differences in the chromosome size within the respective complement are not very significant. The karyotype is symmetrical. The chromosome length ranges from m1.46 m to 3.16. Although gross similarities among the karyotypes suggest their homogenous assemblage, yet each cultivar shows certain chromosomal differences from the others retaining their individual pattern (Figs. 7, 8 & 9). Such karyotypic variation in different varieties/species of the genus Morus, L. clearly indicates that the chromosomal repatterning is involved in speciation. These karyomorphological investigations will be made use of while selecting the parents for evolving progeny of different ploidy level by hybridization.

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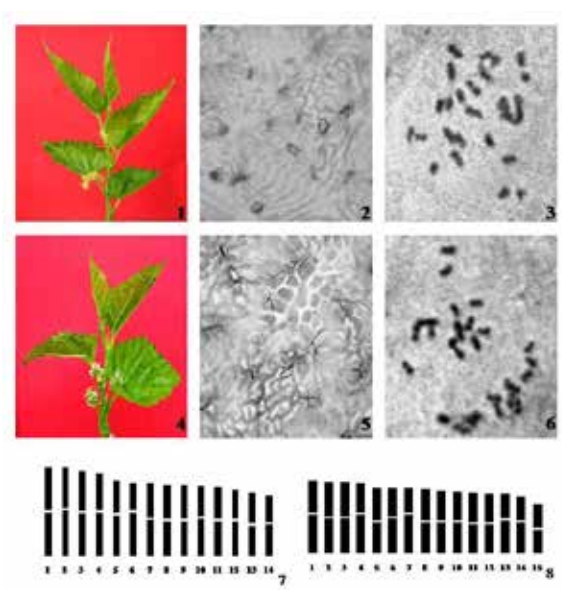

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