Karyotype and Stomatal Studies on Three Genotypes of *Morus* spp.

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Summary  Three mulberry genotypes viz., MR2, C763 and *Morus macroura* were analyzed for the stomatal frequency and karyotypic attributes. MR2 and C763 were found to be diploids with somatic chromosome number of $2n=28$, while variety *Morus cathayana* revealed tetraploid chromosome number of $2n=56$. Somatic chromosomes measured 1.26 to 2.86 $\mu$m in length. Karyotypes of these taxa are symmetric. Only metacentric and sub-metacentric chromosomes are found in the complement. Stomatal frequency was found to be high in diploid mulberry varieties compared to tetraploid variety.

Key words  Mulberry, Mitosis, Diploids, Tetraploid, Karyotype analysis.

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. The existing literature on cytotaxonomy of the genus *Morus*, L. clearly indicates the lacunae (Basavaiah et al. 1989, 1990a, 1990b). Cytogenetic information is important in identifying and evolving promising cultivars. Most of the cultivated varieties of mulberry are diploid with $2n=28$ chromosomes; a few are polyploids (Gill and Gupta 1979, Venkatesh 2007). Venkatesh and Munirajappa (2012, 2013) studied the meiotic behaviours of triploid ($2n=42$) and tetraploid ($2n=56$) varieties of *Morus*. Venkatesh et al. (2013a) studied the micro morphology and reproductive characteristics of different ploidy levels of the mulberry varieties and considered diploid parents to be superior to triploid and tetraploid. Stomatal frequency and karyotypic studies have been reported by Venkatesh et al. (2013b). Karyotypes of these taxa are symmetric; only metacentric and submetacentric chromosomes are found in the somatic complement. This data is essential in selecting suitable species/cultivars for breeding programmes, resolving taxonomic confusions, tracing the evolutionary tendencies and evolving polyploidy varieties with better agronomic qualities. Keeping these views, the present investigation was undertaken to unravel the stomatal frequency and karyomorphological data in three different mulberry genotypes.

Materials and methods

Three mulberry varieties viz., MR2, C763 and *Morus macroura* which are maintained in the mulberry germplasm bank of Department of Sericulture, Bangalore University, Bangalore, India, were chosen for present study. Cuttings of these varieties were planted in earthen pots for experimental use. Somatic preparations were made from excised root tips. Root tips were pre-treated with aqueous solution of 0.002 M 8-hydroxyquinoline for 3 h at 10°C and fixed in 1 : 3 acetic acid : alcohol for 24 h. After washing in water the root tips were hydrolysed in 1 N HCl for seven minutes at 60°C and stained in 2% aceto-orcein. Squash preparations were made in 45% acetic acid. Photomicrographs and drawing of good and clear plates were made immediately to ascertain the chromosome number and their morphology. Ideograms were drawn using suitable scale. Karyotype classification was made according to Levan et al. (1964). Stomatal frequency was determined by nail polish impression method. Stomatal frequency was calculated by using the formula below 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 MR2**

It is a mildew resistant, open pollinated hybrid. It is evolved at Coonoor Sericulture Form, Tamil Nadu. Stem of this taxon is greenish-brown in colour. Leaves are...
lobed, light green with long internodes. Stomatal frequency was found to be 190.87 mm$^{-2}$ (Fig. 2). It is a diploid cultivar with 2$n$=28 chromosomes (Fig. 1). Somatic chromosomes are small measuring 1.96 to 2.86 $\mu$m in length. Metacentric and sub-metacentric chromosomes are found in the somatic complement. The karyotype formula of this genotype is $2n=28=22B^m+4B^{sm}+2C^m$ (Fig. 7). The total chromatin length of the haploid set is 34.11 $\mu$m.

Variety C763

It is a male clone grown in tropical regions. Stem is greenish-brown in colour. Leaves are unlobed, ovate and deep green in colour. The stomatal frequency was found to be 206.66 mm$^{-2}$ (Fig. 4). This taxon also revealed diploid chromosome number of 2$n$=28 (Fig. 3). The longest chromosome measured 2.86 $\mu$m while the shortest measured 1.59 $\mu$m. Only metacentric and sub-metacentric chromosomes are found in the somatic complement. The karyotype formula of this genotype is $2n=28=18B^m+8B^{sm}+2C^m$ (Fig. 8). The total chromatin length of the haploid set is 32.43 $\mu$m.

Morus macroura

It is a tetraploid male variety. Stem is purple green to grey brown in colour. The leaves are smaller, thin, upper surface is dark green and lustrous with a pale green under surface, unlobed, margin is crenate-dentate, acuminate and having thin long internodes. Many minute pubescences were found on young stem and leaves. This variety exhibited reduction in height and number of branches when compared to diploids and triploids. Stomatal frequency was found to be 150.65 mm$^{-2}$ (Fig. 6). This taxon revealed 2$n$=56 chromosomes (Fig. 5) which are small measuring from 1.26 to 2.48 $\mu$m in length. Even in this taxon only metacentric and sub-metacentric chromosomes are found in the somatic complement. However, sub-metacentric chromosomes are more in number. The karyotype formula for this genotype is $2n=56=2B^m+8B^{sm}+14C^m+32C^{sm}$ (Fig. 9). The total chromatin length of the haploid set is 48.60 $\mu$m.

Mulberry is a heterogeneous, predominantly unisexual, cross pollinated and as well as vegetatively propagated perennial plant. It exhibits a wide range of variations in each and every phenotypic characteristic. It
is a plastic species; for instance, often the same genotype reveals the presence of both lobed and unlobed leaves. Even with regard to inflorescence, flower and floral parts there exists a wide range of variability within the same genotype. Sex reversal is also a common phenomenon. These stretchable exomorphic features create a lot of confusion among systematic botanists to precisely identify a particular genotype. With this prevalent plasticity of phenotype, genotype tagging is very essentially required for selecting promising ones for cultivation, phytopathological, agronomical, breeding, genetic engineering and transgenic research. The perusal of the available literature clearly revealed a wide gap in taxonomy, nomenclature, cytogenetics and such other related aspects of the genus *Morus*.

To evolve a dependable system of classification, a study of all the three types of relationship viz., phylogenetic, phenotypic and geotropic is imperative as stated by Chennaveeraiah (1983). Baker (1970) stated that for the determination of biological species in cultivated plants, the observational bases are full description of chromosome number and karyotype analysis and evidence of natural hybridization.

Mulberry varieties included in the present work exhibited variations in ploidy level and karyomorphology. In this backdrop, the present investigation on cytological studies in the genus *Morus* analyzes the chromosome number, ploidy level, karyotype, and stomatal frequency in three different mulberry genotypes. Among these, the present study recorded the diploid number (2n=28) in two mulberry varieties (MR$_2$ and C$_{763}$) and tetraploid chromosome numbers (2n=56) in one mulberry variety (*Morus macroura*) studied.

Stomatal size and frequency are important parameters in selecting drought resistant genotypes and these are also believed to regulate leaf yield. In the present findings, higher stomatal frequency was recorded in diploid varieties viz., MR$_2$ (109.87 mm$^{-2}$) and C$_{763}$ (206.66 mm$^{-2}$) when compared to triploid mulberry variety *Morus macroura* (150.65 mm$^{-2}$). The present findings are in agreement with the reports of Tikadar et al. (1999). Sastry et al. (1998) also recorded the variation in number of stomata/unit area in different mulberry varieties. 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 the present study as well as observations made by others rule out the existence of secondary association of chromosomes in majority of *Morus* spp. (Venkatesh 2007).

In general, the chromosomes are short, fairly uniform in their size and form a graded series. 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 1.26 to 2.86 µm. Although gross similarities among the karyotypes suggest their homogenous assemblage, each cultivar shows certain chromosomal differences from the others retaining their individual pattern. Such karyotypic variation in different varieties/species of the genus *Morus* L. clearly indicates that chromosomal repatterning is involved in speciation.

References


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