Comparative Micro-morphological and Karyomorphological Studies in Three Mulberry Varieties (*Morus* spp.)

K. H. Venkatesh* and Munirajappa

Department of Sericulture, Bangalore University, Bangalore-56006, Karnataka, India

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Summary This article reveals the micro-morphology and karyomorphological characters of three mulberry varieties, namely, Thysong, S41 and *Morus multicaulis*. Stomatal frequency, somatic chromosome number, ploidy level and karyotype analysis were studied for these varieties. Thysong is diploid with \(2n=28\), S41 is triploid with \(2n=42\) and *Morus multicaulis* is uneuploid with \(2n=30\) regarding somatic chromosomes numbers. The somatic chromosome length ranges from 1.26 to 2.83 \(\mu m\), whereas the arm ratio ranges from 0.48 to 1.00 \(\mu m\). Stomatal frequency is smaller in triploid varieties when compared to diploid and uneuploid mulberry varieties. In all the three varieties three to four types of chromosomes have been observed. Chromosomes are small sized with a narrow range of variation in length.

Key words Mulberry (*Morus* spp.), Stomatal frequency, Diploid, Triploid, Uneuploid, Karyotype analysis.

Mulberry (*Morus* spp.) of the family Moraceae is a multipurpose, predominantly dioecious, heterozygous and outbreeding tree. It is an economically important tree, and its leaf is the sole food for the silkworm (*Bombyx mori* L.). In addition to being fed to silkworms, mulberry is used in industry, medicine, aquaculture, agro-forestry, social forestry, water-shed management and drought prone area development programmes (Bari 1990, Dandin et al. 1992, Kannan and Misri 1990, Munirajappa et al. 1995, Tiku and Bindroo 1989, Philip 1989). For a few Indian species, cytogenetical investigations were carried out by Das et al. (1970), Kundu and Sharma (1976), Gill and Gupta (1979) and Venkatesh et al. (2013a). Most of the natural species of *Morus* is diploid with \(2n=28\) chromosomes, but few are polyploids (Venkatesh 2007). Cytogenetical information available today is confined only to chromosome counts and meiotic behaviour of a few species. Triploids \(2n=42\) are developed through natural or controlled hybridization between diploid and tetraploid parents and are considered to be superior than diploids in leaf yield and nutritive qualities of the leaf (Venkatesh and Munirajappa 2012). The information on breeding systems and evolution of different mulberry polyploids are highly useful in evolving elite genotypes required for commercial cultivation. In the present study, an attempt has been made to analyze the stomatal frequency and karyomorphological studies of three different mulberry varieties.

Materials and methods

Morphology Mulberry varieties used in the present study are Thysong, S41 and *Morus multicaulis* which are maintained in the germplasm bank, Department of Sericulture, Bangalore University, Bangalore, India. 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 and 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 h at 10°C. After washing in water the root tips were hydrolyzed in 1 N HCl for 7 min at 50°C and then stained with 2% aceto-orcein. Squash preparations were made in 45% acetic acid. Photomicrographs and drawings were made on the same day of preparation. For each variety, numbers of preparations were made to ascertain the chromosome number and their morphology. Ideograms were drawn using a suitable scale. Karyotype classification was made according to Levan et al. (1964).
Stomatal frequency

Stomatal frequency was determined by the nail polish impression method. Stomatal frequency was calculated by using the following formula and expressed as number of stomata/mm² (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 Thysong: It is an exotic mulberry introduced to India from China. Stem is green to greyish in colour. Leaves are unlobed ovate and green in colour. The stomatal frequency of this genotype was found to be 271.21/mm² (Fig. 5). This taxon revealed 2n=28 chromosomes (Fig. 6) which are small, measuring from 1.66 to 2.70μm in length. Even in this taxon only metacentric and sub-metacentric chromosomes are found in the somatic complement. However, metacentric chromosomes are more in number. The karyotype formula for this genotype is 2n=28=12Bm+8Bsm+6Cm+2Csm (Fig. 9). The total chromatin length of the haploid set is 30.84μm.

Variety S41: It is evolved through mutation breeding and selection. Stem is greenish-brown in colour. Leaves are unlobed, ovate and deep green in colour. The stomatal frequency was found to be 121.21/mm² (Fig. 3). This taxon revealed triploid chromosome number of 2n=42 (Fig. 4). The longest chromosome measured 2.83μm while the shortest measured 1.40μm. Only metacentric and sub metacentric chromosomes are found in the somatic complement. The karyotype formula of this

Figs. 7–9. Ideograms of Varieties Thysong, S41 and Morus multicaulis, respectively.
genotype is \(2n=42=10B_m+26B_{sm}+4C_m+2C_{sm}\) (Fig. 8). The total chromatin length of the haploid set is 46.80 \(\mu m\).

**Morus multicaulis**: It is an exotic mulberry variety introduced to India from Japan and propagated through grafting. Stem of this taxon is reddish brown in colour. Leaves are lobed, highly dissected, light green with long internodes. Stomatal frequency was found to be 196.96/mm\(^2\) (Fig. 1). It is a tetraploid cultivar with \(2n=30\) chromosomes (Fig. 2). Somatic chromosomes are small measuring 1.66 to 2.83 \(\mu m\) in length. Metacentric and sub-metacentric chromosomes are found in the somatic complement. The karyotype formula of this genotype is \(2n=30=8B_m+16B_{sm}+6C_m\) (Fig. 7). The total chromatin length of the haploid set is 34.72 \(\mu m\).

Details of the stomatal frequency, somatic chromosome number, ploidy level, range of chromosome length, karyotype formula, arm ratio and haploid chromatin length are presented in Table 1.

The basic chromosome number of the genus *Morus* L. as \(x=14\) for the majority of the species has been reported by Osawa (1920), Seki (1952), Janaki Ammal (1948), Datta (1954), Das (1961) and Kundu and Sharma (1976). 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 that the Thysong is diploid \((2n=28)\), \(S_41\) is triploid \((2n=42)\), and *Morus multicaulis* is uneuploid \((2n=30)\) regarding the chromosome number.

Although the taxa studied generally resembled each other in their gross morphological features, they exhibited a great deal of variation in phenotypic characteristics. 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. They have good tillering ability.

Stomatal size and frequency are important parameters in selecting drought resistant genotypes and these are also believed to regulate leaf yield. The frequency of stomata per unit area is significantly less in triploids compared to diploids (Venkatesh et al. 2013b). In the present findings higher stomatal frequency was recorded in diploid and uneuploid varieties, *viz*., Thysong (271.21/mm\(^2\)) and *Morus multicaulis* (196.96/mm\(^2\)) when compared to the triploid mulberry variety \(S_41\) (121.21/mm\(^2\)). The present findings are in agreement with the reports of Tikadar et al. (1999). Sastry et al. (1988) 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 a 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 a basic number of \(x=7\) for *Morus* based on the presence of secondary association in a 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 the majority of *Morus* spp. (Venkatesh 2007).

Mulberry varieties included in the present work exhibited variations in ploidy level and

### Table 1. Karyomorphological details of three mulberry varieties.

<table>
<thead>
<tr>
<th>Mulberry varieties</th>
<th>Stomatal frequency/ mm(^2)</th>
<th>(2n) chromosome number</th>
<th>Ploidy level</th>
<th>Karyotype formulae</th>
<th>Range of chromosome length ((\mu m))</th>
<th>Arm ratio ((\mu m))</th>
<th>Haploid chromatin length ((\mu m))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thysong</td>
<td>271.21</td>
<td>28</td>
<td>Diploid</td>
<td>(2n=28=12B_m+8B_{sm}+6C_m+2C_{sm})</td>
<td>1.66–2.70</td>
<td>0.58–1.00</td>
<td>30.84</td>
</tr>
<tr>
<td>(S_41)</td>
<td>121.21</td>
<td>42</td>
<td>Triploid</td>
<td>(2n=42=10B_m+26B_{sm}+4C_m+2C_{sm})</td>
<td>1.40–2.83</td>
<td>0.48–1.00</td>
<td>46.80</td>
</tr>
<tr>
<td><em>M. multicaulis</em></td>
<td>196.96</td>
<td>30</td>
<td>Uneuploid</td>
<td>(2n=30=8B_m+16B_{sm}+6C_m)</td>
<td>1.66–2.83</td>
<td>0.57–1.00</td>
<td>34.72</td>
</tr>
</tbody>
</table>
karyomorphology. Thysong and S$_{44}$ have revealed the diploid ($2n=28$) and triploid ($2n=42$) chromosome numbers, respectively. Mulberry variety Morus multicaulis has displayed the uneuploid chromosome number of $2n=30$. The observation of uneuploid number like $2n=30$ for Morus multicaulis confirm the observations made by earlier workers suggesting the inconsistency of chromosomes number, and the probable reason cited for the same is a high degree of 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). These karyomorphological investigations will be made use of while selecting the parents for evolving progeny of different ploidy level both by hybridization and colchicine treatment. In addition, the information will be of much use in establishing a phylogenetic relationship and evolution of mulberry and will also help in selecting mother plants for hybridization based on chromosome number.

References


