Morpho-criteria and karyotype studies in three mulberry varieties

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ABSTRACT: Morpho-criteria, karyotype analysis, chromosome number and ploidy levels were studied for three mulberry varieties. Mysore local and RFS-₁₇₅ is diploid with $2n=28$ and BC₂₋₅₉ is triploid with $2n=42$ somatic chromosomes numbers respectively. Somatic chromosome length ranges from 1.29 μm to 3.29 μm where as arm ratio ranges from 0.59 to 1.00 μm . Karyotypes of these taxa are symmetric. Only metacentric and submetacentric chromosomes are found in the somatic complement. Stomatal frequency is lesser in triploid variety when compared to diploid varieties.

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

The foliage of the mulberry plant is used mainly as a unique source of silkworm (*Bombyx mori* L.) feed. In addition to feed to silkworms, mulberry is also used in industry, medicine, agro forest and social forest (Bari 1990, Dandin *et al.* 1992, Kannan and Misri 1990). Most of the cultivated varieties of mulberry are diploids with $2n = 28$ chromosomes, but a few are polyploids (Gill and Gupta 1979, Venkatesh 2007). Venkatesh and 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 micro morphology and reproductive characteristics of different ploidy level of the mulberry varieties and are considered diploid parents are superior to triploid and tetraploid. Venkatesh *et al.* (2014) studied the morphological, anatomical and reproductive parameters in different ploidy levels of mulberry varieties. These different chromosomes numbers has reflected on their micromorphology, anatomy and reproductive characters of diploid, triploid and tetraploid varieties. Venkatesh *et al.* (2012) studied the meiotic studies of diploid ($2n=28$) varieties of *Morus* and confirmed the extreme difference between the 13 small pairs and one large pair of chromosomes. In the present study, is focused on the morphology and karyomorphological details in three varieties of mulberry.

MATERIALS AND METHODS

Morphology Mulberry varieties used in the present study are Mysore local, RFS-₁₇₅ and BC₂₋₅₉ 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 hours at 10⁰c. After washing in water the root tips were hydrolyzed in 1N HCl for seven minutes at 50⁰c 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 suitable scale. Karyotype classification was made according to Leven *et al.* (1964).

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/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 Mysore local This variety is also called “Natikaddi”. It was introduced to Karnataka during the period of Tippu Sultan. It is best suited for rain fed condition. 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. Stomatal frequency was found to be 174.24/mm² (Fig. 1) and pollen stain ability was 96.32%. It is diploid genotype $2n=28$ chromosomes (Fig. 2). Somatic chromosomes are measuring 2.20 μm to 3.29 μm in length. The karyotype formula of this genotype is $2n =$

$28 = 22B^m + 6B^{sm}$ (Fig. 3). The total chromatin length of the haploid set is $38.26\mu m$.

Variety RFS₁₇₅ This variety is recommended for cultivation under rain fed condition. Stem is woody, cylindrical, clothed with many lenticels. It is purple green to greenish brown in color, filled with milky latex. Leaves are larger, thin, dark green, coriaceous, chordate, lobed, serrate, and acuminate, it exhibited medium in height, shorter internode, short and slender petiole. Stomatal frequency was found to be $181.81/mm^2$ (Figs. 4) and pollen stain ability 96.18%. It is also diploid genotype with $2n=28$ chromosomes (Fig. 5). Somatic chromosomes are measuring $1.32\mu m$ to $2.26\mu m$ in length. The karyotype formula of this genotype is $2n = 28 = 2B^m + 8B^{sm} + 14C^m + 4C^{sm}$ (Fig. 6). The total chromatin length of the haploid set is $26.20\mu m$.

Variety BC₂₋₅₉ It is a triploid variety. Stem of this taxon is woody, cylindrical, clothed with many lenticels and green in color. Milky latex present in the stem. Leaves are simple, thick, dark green, cortaceous, chordate, unlobed, dentate, acuminate, exhibited maximum height, long, thick petiole and shorter internode. Stomatal frequency was found to be $133.33/mm^2$ (Fig. 7) and pollen stain ability 91.42%. It is triploid genotype with $2n=42$ chromosomes (Fig. 8). Somatic chromosomes are small measuring $1.29\mu m$ to $3.19\mu m$ in length. Metacentric and sub-metacentric chromosomes are found in the somatic complement. The karyotype formula of this genotype is $2n = 42 = 6B^m + 26B^{sm} + 6C^m + 4C^{sm}$ (Fig. 9). The total chromatin length of the haploid set is $47.55\mu m$. Morphological comparison and karyomorphological details between the three varieties is given in Tables 1 and 2.

Tikadar and Dandin (2007) 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 and Kumar; 1989).

Varieties Mysore local, RFS₁₇₅ and BC₂₋₅₉ are morphologically distinct and exhibited some similarities in stem and leaves color with identical leaf margin. Dissimilarities in their height, internodal distance, leaf texture and nature, stomatal frequency, size and pollen stain ability. Variety BC₂₋₅₉ is triploid have similarity in their adaptation i.e. better rooting, grow more quickly and posses larger leaves. Leaves are unlobed, thick, dark green and course in texture when compared to diploid varieties. However, leaves of diploids are lobed, thin, light green and course in texture. The frequency and size of stomata per unit area is significantly less in triploid compared to diploids. 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.

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).

In 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 ($2n=48$) in two mulberry genotypes (Mysore local and RFS₁₇₅) and triploid chromosome number ($2n=42$) in one of the mulberry variety (BC₂₋₅₉) studied.

Mulberry varieties included in the present work exhibited variations in ploidy level and karyomorphology. Mysore local and RFS₁₇₅ have revealed the diploid chromosome of $2n=28$. Mulberry variety BC₂₋₅₉ has displayed the triploid chromosome number of $2n=42$. 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 $1.29\mu m$ to $3.29\mu m$. 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 and 9). Such karyotypic variation in different varieties/species of the genus *Morus* L. clearly indicates that the chromosomal repatterning is involved in speciation.

CONCLUSION

Morphology and karyomorphological studies of three cultivars namely, Mysore local, RFS₁₇₅ and BC₂₋₅₉ belonging to *Morus alba*. Morphological variations are attributed to genetic drift, it can be resolved that morphological variations and evolution of *Morus spp.* is mainly due to structural changes in the ploidization and mutation. Of the three cultivars, two are diploid ($2n=28$) and one is triploid ($2n=42$). 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. Morphological variation and evolution of taxa were attributed to structural changes in the karyotype and phylogenetic relationship among the varieties of each species based on their karyotype.

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Table 1. Comparison of morphological characters in diploids and triploid varieties of mulberries

Characters	Mysore local	RFS ₁₇₅	BC2-59
Height (cm)	198	220	231
Number of branches	07	08	09
Intermodal distance (cm)	4.4	3.9	3.8
Leaf Size(cm ²)	149.00	161.24	172.42
Leaf texture	Light green, thin	Light green, thin	Dark green thick
No. of stomata per unit area (mm ²)	174.24	181.81	133.33
Length of stomata (µm)	12.6	13.9	13.00
Width of stomata (µm)	10.22	11.00	10.98
Length of petiole (cm)	13.9	15.8	12.60
Width of petiole (cm)	0.9	1.1	1.5
Length of inflorescence (cm)	3.1	3.4	3.8
Width of inflorescence (cm)	2.2	2.7	3.1
Diameter of inflorescence (cm)	1.1	1.3	1.6
No. of flowers per inflorescence	22	27	31
Length of flower (cm)	0.60	0.68	0.72
Pollen stain ability (%)	96.32	96.18	91.42

Table 2. Karyomorphological details among three mulberry varieties

Mulberry varieties	Stomatal frequency/mm ²	2n chromosome number	Ploidy level	Karyotype formulae	Range of chromosome Length(μ m)	Arm ratio (μ m)	Haploid chromatin Length(μ m)
Mysore local	174.24	28	Diploid	$2n=28=22B^m+6B^{sm}$	2.20-3.29	0.62-1.00	38.26
RFS ₁₇₅	181.81	28	Diploid	$2n=28=2B^m+8B^{sm}+14C^m+4C^{sm}$	1.32-2.26	0.73-1.00	26.20
BC2 ₋₅₉	133.33	42	Triploid	$2n=42=6B^m+26B^{sm}+6C^m+4C^{sm}$	1.29-3.19	0.55-0.92	47.55

Figs. 1-6. Differences in stomatal frequencies and somatic chromosome numbers among three different varieties of mulberries. 1 and 2. Stomatal frequency and somatic chromosomes ($2n=28$) of variety Mysore local. 3 and 4. Stomatal frequency and somatic chromosomes ($2n=28$) of variety RFS₁₇₅. 5 and 6. Stomatal frequency and somatic chromosomes ($2n=42$) of variety BC2₋₅₉.

Figs. 7-9. Ideograms of karyotypes in different varieties of mulberry 7. Variety Mysore local. 8. RFS175. 9. BC2-59.