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STUDIES ON MORPHO-CRITERIA AND KARYOTYPE ANALYSIS IN TWO MULBERRY CULTIVARS (*MORUS SPP.*)

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ABSTRACT
Morpho-criteria and karyotype analysis of two mulberry verities, namely, Kollegal and *Morus cathyana* were selected. Morpho-criteria, stomatal frequency, somatic chromosome number, ploidy level, karyotype analysis, arm ratio and total haploid chromatin length were studied. Kollegal is diploid with 2n=28 and *Morus cathyana* is tetraploid with 2n=56. Somatic chromosome length ranges from 1.26 μm to 3.22 μm where as arm ratio ranges from 0.62 to 0.97 μm. Stomatal frequency is lesser in diploid when compared to tetraploid variety. In both the varieties 3 to 4 types of chromosomes have been observed. Chromosomes are small sized with a narrow range of variation in length.

KEY WORDS: Mulberry (*Morus spp*.). Morphology, Diploid, Tetraploid, Mitosis, Karyotype analysis.

INTRODUCTION

The leaves of mulberry (*Morus alba* L.) from the basic food of silkworm (*Bombyx mori* L.). Therefore, the quality and quantity of mulberry leaf has an intimate relation with the health of silkworm and quality silk production. *Morus* L. is an important genus of the family Moraceae. The information in the field of cytology, genetics and karyomorphology is inadequate to seek the answer for some of the problems faced by the classical taxonomists. Most of the cultivated varieties of mulberry are diploids with 2n = 28 chromosomes, but a few are polyploids (Gill & Gupta 1979, Venkatesh 2007). Venkatesh *et al*., (2012 & 2013) 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. 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 micro-morphology and reproductive characteristics of different ploidy levels of the mulberry varieties and are considered diploid parents are superior to triploid and tetraploid. 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 morphology and reproductive characters of diploid and tetraploid varieties. In the present study, is focused on the Morpho-criteria and karyotype analysis in two mulberry genotypes.

MATERIALS & METHODS

Morphology

Mulberry varieties used in the present study are Kollegal and *Morus cathyana* 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 & Jolly 1986).

Mitosis

Somatic preparations were made from excised root tips of potted plants. Root tips were collected between 9.45 to 10.30AM 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 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/mm^2 (Aneja, 2001; Sikdar *et al*., 1986).

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

OBSERVATIONS

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 tetraploid (2n = 56).

TABLE 1: Karyomorphological details of two mulberry genotypes

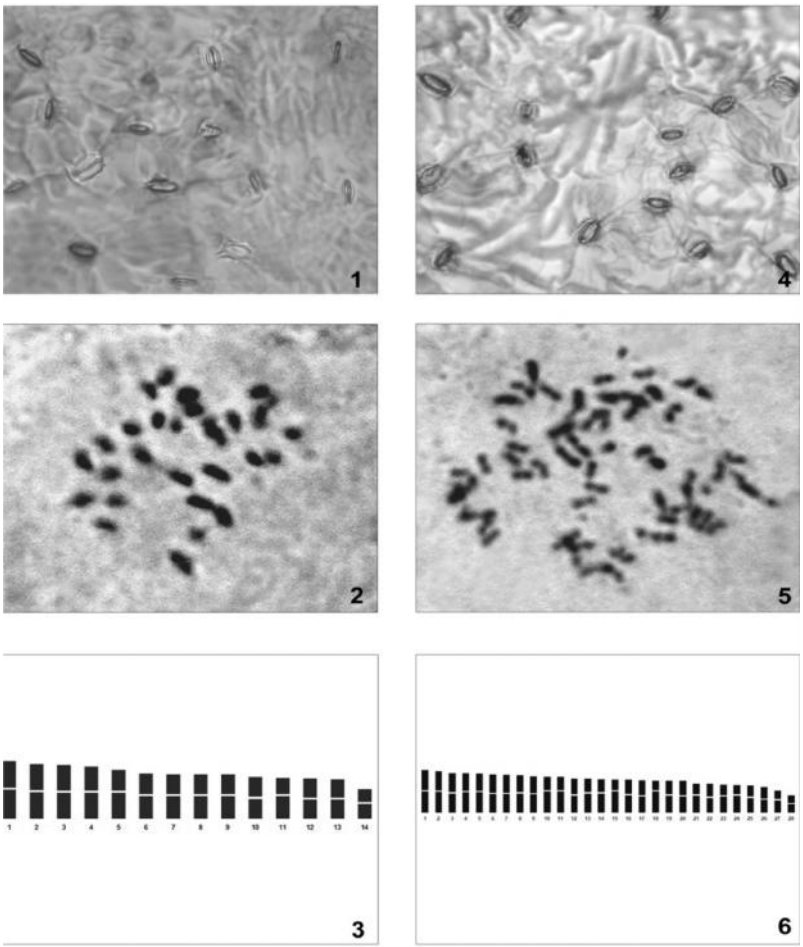
Mulberry varieties	Stomatal frequency/ mm^2	2n chromosome number	Ploidy level	Karyotype formulae	Range of chromosome length(μm)	Arm ratio ($\frac{m}{\mu m}$)	Haploid chromatin length(μm)
Kollegal	192.42	28	Diploid	$2n=28=18B^{m}+2B^{sm}+6C^{m}+2C^{sm}$	1.42-2.83	0.71-0.97	31.07
Morus cathyana	152.40	56	Tetraploid	$2n=56=28B^{m}+22B^{sm}+4C^{m}+2C^{sm}$	1.26-3.22	0.43-0.93	69.52

Variety Kollegal

It is male variety, grown in tropical region and it is a good rooter and cultivated as perennial bush. Stem is greenish brown in colour. Leaves are entire, lobed, light green with smooth texture. The stomatal frequency was found to be 192.42/.mm^2 (Fig. 1). This taxon is revealed diploid chromosome number of 2n=28 (Fig.2). The longest chromosome measured 2.83 μm., while the shortest measured 1.42 μm. 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 of this genotype is $2n=28=18B^{m}+2B^{sm}+6C^{m}+2C^{sm}$ (Fig. 3). The total chromatin length of the haploid set is 31.07 μm.

Morus cathyan

It is also male variety, evolved through colchicine treatment. It is cultivated as perennial bush especially in hilly tracts. Stem is green to brownish in colour. The leaves' upper surface is dark green and lustrous and pale green in under surface, lobed, margin is crenate-dentate, acuminate and having long internodes. Stomatal frequency was found to be 152.40/mm^2.(Fig.4). It is tetraploid cultivar with 2n=56 chromosomes (Fig. 5). Somatic Chromosomes are small measuring from 1.26 μm to 3.22 μm in length. Metacentric and sub-metacentric chromosomes are found in the somatic complement. The karyotype formula of this genotype is $2n=56=28B^{m}+22B^{sm}+4C^{m}+2C^{sm}$ (Fig. 6). The total chromatin length of the haploid set is 69.52 μm.



FIGURES 1-**6**. 1, 2, & 3, Stomatal frequency, somatic chromosomes and ideograms of variety Kollegal (2n=28).
4, 5, & 6, Stomatal frequency, somatic chromosomes and ideograms of *Morus cathyana* (2n=56).

DISCUSSION

Basic chromosome number of the genus *Morus* L. as x = 14 for majority of the species have been reported by Das (1961) and Kundu and Sharma (1976). Hotta (1953) have classified the Genus *Morus* based on the morphological characters of the female flower. The above systems are

considered to be incomplete as data from cytology, embryology etc. are lacking. The other reports confined only to chromosome number (Tahara 1909, Sinoto 1929, Katsumata 1979). Gill and Gupta (1978) while studying cytology of sex types of *Morus* species reported the presence of large sized chromosomes.

Variety Kollegal and *Morus cathyana* belonging to *Morus alba* are morphologically distinct and some similarities in their adaptation, good rooting and with identical leaf margin and dissimilarities in their, stem colour, leaf texture and stomatal frequency were observed. Leaves of *Morus cathyana* are light green, coarse in texture. Diploid forms are grow more quickly and posses larger dark green leaves when compared to tetraploids (Eswar Rao *et al*., 2000).Higher stomatal frequency was recorded in diploid variety Kollegal (192.42/mm^2) when compared to tetraploid mulberry variety *Morus cathyana* (152.40/mm^2). Stomatal frequency is important parameter in selecting drought resistant genotype and correlated with drought and disease resistance (Hatalli *et al*., 1993; Nautiyal *et al*., 1994). Further lesser frequency per unit area is more suitable for rain fed conditions. 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.

Cytologically, varieties Kollegal and *Morus cathyana* showed 2n=28 and 2n=56 chromosomes respectively. Confirming the earlier reports for few of them and also they show similar karyotype with only two types of chromosomes, equal chromatin length and arm ratio. Despite of common origin, Kollegal is diploid and *Morus cathyana* is tetraploid. These different chromosome numbers has reflected on their morphology and different ploidy level of same mulberry varieties are scanty (Khurana *et al*., 2003). Previous and present reports on chromosome number and cytology show the presence of 2n = 28 chromosomes in most of the cultivars in this genus. In all the above cases the cytological identity of each cultivar has been represented in phenotypic variation. 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. Although gross similarities among the karyotypes suggest their homogenous assemblage, yet 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 the chromosomal repatterning is involved in speciation.

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