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COMPARATIVE MICRO-MORPHOLOGY AND REPRODUCTIVE STUDIES IN THREE MULBERRY VARIETIES (MORACEAE)

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ABSTRACT
Three evolved mulberry verities, viz., RFS_{175}, S_{41} and *Morus cathyana* were selected for the micro morphology and reproductive studies. Height, internodal distance, colour of leaves, stem, stomatal frequency, length and number of inflorescence and pollen fertility were studied for these three varieties. Mulberry varieties studied exhibited considerable variations in height, internodal distance, leaf texture, stomatal frequency and pollen stain ability. Stomatal frequency and pollen stain ability was higher in diploids when compared to triploid and tetraploid variety. Tetraploid showed reduction in height, number of branches and internodal distance followed by diploid and triploid varieties.

KEY WORDS: Mulberry (*Morus spp*.). Diploid, Triploid, Tetraploid, Micro morphology.

INTRODUCTION

Mulberry (*Morus spp*.) is a multipurpose, predominantly dioecious, heterozygous and out breeding tree. The foliage of the plant is used mainly as a unique source of silkworm (*Bombyx mori* L.) feed and cultivated in over 40 countries (Machi and Katagiri, 1991). Most of the cultivated varieties of mulberry are diploid with 2n=28 chromosomes, a few are polyploids (Datta, 1954, Das 1961, Gill and Gupta 1979). Triploid varieties have higher leaf yield as well as better nutritive qualities from the point of silkworm rearing when compared to diploid varieties (Seki and Oshikane 1959, Sugiyama 1959 and Alekperova 1978). Hamada (1963) also reported about the cold and disease resistance of triploids. Generally triploids are obtained by crossing tetraploids with diploids and for these tetraploids are first induced and then crossed with diploids. In the present study an attempt has been made to present a comparative account of micro morphological and reproductive details in three important mulberry cultivars.

MATERIALS AND METHODS

Three exotic mulberry varieties, namely RFS_{175}, S_{41} and *Morus cathyana* which are maintained in the mulberry germplasm bank of Department of Sericulture, Bangalore University, Bangalore, India, were taken for present study. Cuttings of these varieties were planted in pots for experimental use. Morphological characters, stomatal frequency and size was calculated by using the formula and expressed as number of stomata/mm^2 (Sikdar *et al*., 1986; Aneja, 2001), and pollen fertility was also assessed by staining pollen grains with 2% aceto- carmine. Photo-micrographs were taken using labomed microscope fitted with Nikon cool fix digital camera.

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

OBSERVATIONS

Comparative morphological and reproductive data on three varieties, some variations recorded with respect to ploidy level, stem colour, stomatal frequency, pollen stain ability, etc. are summarized (Table. 1).

RFS_{175}. This variety has been recommended for cultivation under rain fed condition. Stem is purple green to brown in colour. Leaves are light green chordate, unlobed serrate and acuminate. This is diploid cultivar with 2n=28 chromosomes (Venkatesh, 2007). The diploids exhibited medium height, reduction in internodal distance and increase in number of stomata per unit area. Leaves, stomatal frequency and pollen stain ability are shown in Figs. 1-3. Stomatal frequency and pollen stain ability was found to be 181.81/mm^2 and 96.23% respectively.

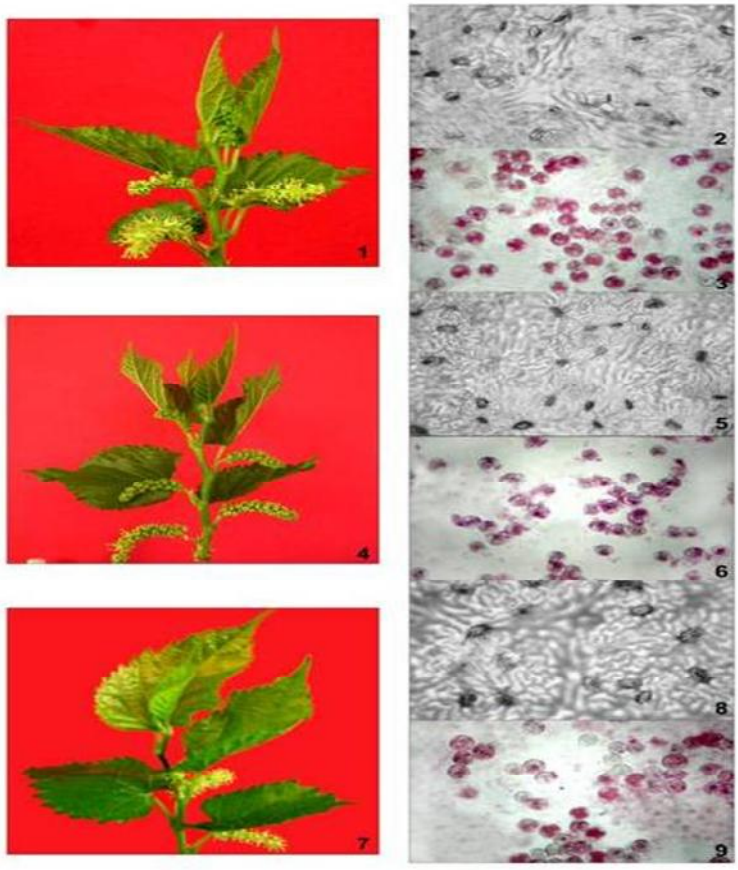
S_{41}. It is a male variety, evolved through mutation breeding and selection. Stem is light green to brown in colour. Leaves are green, unlobed, chordate, dentate and acuminate. This is triploid cultivar with 2n=42 chromosomes (Venkatesh 2007). The triploids exhibited increase in height and rooting habit, internodal distance and number of stomata per unit area in comparison to diploids. Leaves, stomatal frequency and pollen stain ability are shown in figs. 4-6. Stomatal frequency and pollen stain ability was found to be 121.21/mm^2 and 90.61 respectively.

TABLE 1. Comparison of morphological characters in diploid, triploid and tetraploid

Characters	Diploid	Triploid	Tetraploid
Growth habit			
Height	219	222	197
Number of branches	08	06	05
Intermodal distance (cm)	4.0	3.8	3.5
Leaf			
Leaf size	177.35	182.76	169.45
Length of petiole (cm)	3.5	3.5	3.8
Width of petiole	0.32	0.34	0.391
No. of stomata per unit area (mm^2)	181.81	121.21	105.76
Width of stomata (µm)	13.9	15.8	17.6
Leaf texture	Thin, Green, Chartaceous	Thin, Green, Chartaceous	Thick, Green, Coriaceous
Flower			
Length of inflorescence (cm)	2.8	3.3	3.5
Diameter of inflorescence (cm)	1.0	1.2	1.2
No. of flowers per inflorescence (cm)	30	37	37
Length of flower (cm)	0.60	0.67	0.69
Pollen stain ability (%)	96.23	90.61	90.34

Morus cathyana

It is a male variety. Stem is purple green to grey brown in colour. The leaves, upper surface is dark green and lustrous with a pale green under surface, unlobed, margin is crenate-dentate, acuminate and having long internodes. This is tetraploid cultivar with 2n=56 chromosomes (Venkatesh 2007). The tetraploids exhibited reduction in height, number of branches and internodal distance. Leaves, stomatal frequency and pollen stain ability are shown in Figs. 7-9. Stomatal frequency and pollen stain ability was found to be 105.76/mm^2 and 90.34% respectively.



FIGURES 1-9: 1-3, Twig, stomatal frequency and pollen stain ability of RFS $_{175}$ (2x)
4-6, Twig, stomatal frequaency and pollen stain ability of S_{41} (3x)
4-6, Twig, stomatal frequency and pollen stain ability of *Morus cathyana* (4x)

DISCUSSION

Koidzumi (1917) and Hotta (1953) have classified the Genus *Morus* based on the morphological characters of the female flower. Kastumata (1972) observed some morphological features. Comparative morphological and reproductive data on three varieties, some variations were recorded with respect to ploidy level, stem colour, stomatal frequency, pollen stain ability, etc. In the present investigation, RFS_{175}, S_{41} and *Morus cathyana* belonging to *Morus alba* are morphologically distinct and some similarities in their leaves with identical leaf margin and dissimilarities in their, leaves texture, height, internodal distance, stem colour, inflorescence, pollen stain ability and stomatal frequency were recorded. Cytologically RFS_{175}, S_{41} and *Morus cathyana* showed 2n=28, 2n=42 and 2n=56 chromosomes respectively. These different chromosome number has reflected on their micro morphology and reproductive data of diploid, triploid and tetraploid varieties or different ploidy level of same mulberry varieties are scanty (Khurana *et al*. 2003).Cultivars RFS_{175} and S_{41} have similarity in their adaptation *i.e.* unlobed and light green leaves, good rooting, etc. However, leaves of *Morus cathyana* are dark green, coarse in texture. Triploid forms are better rooting, grow more quickly and posses larger leaves when compared to diploids and tetraploids (Eswar Rao *et al*. 2000). The frequency of stomata per unit area is significantly less in triploid and tetraploid 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 per unit area is more suitable for rain fed conditions. However, reduction in the internodal and number of stomata per unit area indicates that the increased dosage of genes does not always increase in size but may also reduce it (Dwivedi *et al*. 1986). Diploid variety showed higher pollen fertility when compared to triploid and tetraploid variety. The reduced pollen fertility in triploid and tetraploid can be attributed to various meiotic anomalies which invariably result in loss of chromatin materials (Darlington 1965, Gottschalk 1978). 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 ploidy level, micro morphology and reproductive data.

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