Original Article

Studies on Micromorphology and Karyotype Analysis of Three Mulberry Genotypes (*Morus spp.*)

K.H. Venkatesh*

Department of Sericulture/Life Science, Bangalore University, Bangalore-56006, Karnataka, India

Address for Correspondence

Department of Sericulture/Life Science, Bangalore University, Bangalore-56006, Karnataka, India.
E-mail: cytogenetics1@gmail.com

ABSTRACT

The study of karyotype is of great value in modern taxonomy for evolving progeny of different ploidy levels of hybridization. Stomatal frequencies are important parameters while selecting drought resistant genotypes as being correlated with drought and disease resistant. Micro-morphology, chromosome numbers and characters of three indigenous mulberry cultivars were studied. S_{13} and V_1 are diploid with 2n=28 and Tr_{-8} is triploid with 2n=42 somatic chromosomes numbers respectively. Somatic chromosome length ranges from 1.29µm to 2.59µm where as an arm ratio ranges from 0.49 to 0.97µm. Their karyotypes were commonly bi-modal, decreasing in length from the longest to the shortest chromosomes. Experimental results have confirmed that, out of three varieties studied two are diploids with 2n=28 and one variety showed 2n=42 chromosomes. Lesser frequency of stomata in triploid than diploid varieties. Stomatal frequency and size decrease with increase in ploidy level. It can be suggested that triploid with lesser stomatal frequency are suitable for breeding triploids resistant to drought conditions.

Keywords: Mulberry (Moraceae), Stomatal frequency, Mitosis, karyotype analysis.

INTRODUCTION

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. The study of karyotype which deals with not only the number but also shape and size of chromosomes is of great value in modern taxonomy. Knowledge of chromosome structure has played a crucial role on the improvement of high yielding mulberry varieties. Karyotypic differences are often great value in understanding the nature of plant variations particularly from the level of population to

genus. Most of the cultivated varieties of mulberry are diploids with 2n=28 chromosomes, but a few are polyploids[8,14]. Micro morphology and reproductive characteristics of different ploidy level of the mulberry varieties studied[15] and are considered diploid parents are superior to triploid and tetraploid. Stomatal frequency and karyotypic studies have been reported by[15,17]. Karyotypes of these taxa are symmetric, only metacentric and submetacentric chromosomes are found in the somatic complement. These different chromosomes numbers has reflected on their micromorphology and karyomorphology of different ploidy level of mulberry varieties. In the present study, we report stomatal frequency and karyotype of three indigenous mulberry cultivars.

MATERIALS AND METHODS

Root tips are collected from potted plants of S_{13}, V_1 and Tr-$_8$ between 9.45 to 10.30 a.m. and pre-treated in saturated solution of 0.002 M 8 - hydroxyquinoline at 10^0C for 3 hours and then fixed in 1:3 glacial acetic acid: alcohol. Root tips were transferred 45% acetic acid for 15 minutes stained with mixture of 2% aceto-orcein: 1N HCl (9:1) for seven minutes and squash preparations were made in 45% of acetic acid. Photomicrographs and drawings were made on the same day of preparation[11]. 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[10] method.

Stomatal frequency

Stomatal frequency was determined by nail polish impression[12] method. Stomatal frequency was calculated by using the formula and expressed as number of stomata/mm[1,2].

Stomatal frequency = Number of Stomata / Area of microscopic field x mm^2.

RESULTS

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.

Variety S_{13}

It is an open pollinated hybrid (OPH). This variety is best suited for rainfed condition and yields 18,000 to 20,000 Kgs of leaves/hec/year. The stomatal frequency was found to be 196.00/mm^2 (Fig. A). Chromosomes are very small (1.29 to 2.31µm) in size. This taxon revealed diploid chromosome number of 2n=28 (Fig. B) with four medium chromosomes with median primary constriction, two medium chromosomes with sub median primary constriction, 18 short chromosomes with median region primary constriction and four short chromosomes with sub-median primary constriction. The karyotype formula of this taxon is $2n=28=4B^{m}+2B^{sm}+18C^{m}+4C^{sm}$ (Fig. G). The karyotype is symmetrical with an arm ratio ranging from 0.71 to 0.98 (µm). The total chromatin length of haploid complement was 25.38µm.

Variety V_1

This variety is best suited for irrigated conditions. Under ideal agro-climatic conditions this genotype yields 68 tonnes of leaf yield/hec/year. It is a fast growing taxon exhibits good rooting and sprouting ability. The stomatal frequency was found to be 206.44/mm^2 (Fig. C). Chromosomes are small (1.59 to 2.86µm) in size. This taxon also revealed diploid chromosomes number of 2n=28 (Fig. D) with 18 medium chromosomes with median primary constriction, 8 medium chromosomes with sub median primary constriction and two

short chromosomes with median region primary constriction. Only metacentric and sub metacentric chromosomes are found in the somatic complement. The karyotype formula of this taxon is $2n=28=18B^{m}+8B^{sm}+2C^{m}$ (Fig. H). The karyotype is symmetrical with an arm ratio ranging from 0.43 to 1.63 (µm). The total chromatin length of haploid complement was 32.43µm.

Variety Tr-8

It is triploid mulberry variety, evolved through polyploidy breeding technique. It is being cultivated as perennial bush especially in hilly tract. The stomatal frequency was found to be 170.65/mm^2 (Fig. E). Chromosomes are very small (1.49 to 2.59 µm) in size. This taxon revealed triploid chromosomes number of 2n=42 (Fig. F) with 18 medium chromosomes with median primary constriction, 12 medium chromosomes with sub-median primary constriction, six short chromosomes with median region primary constriction and six short chromosomes with sub-terminal primary constriction. Only metacentric and sub metacentric chromosomes are found in the somatic complement. The karyotype formula of this taxon is $2n=42=18B^{m}+12B^{sm}+6C^{m}+6C^{sm}$ (Fig. I). The karyotype is symmetrical with an arm ratio ranging from 0.49 to 1.00 (µm). The total chromatin length of haploid complement was 45.65µm.

DISCUSSION

To classify existing mulberry populations objectively as taxa of different magnitude is rather difficult owing to the continuous variations as a result of out breeding. To evolve dependable system of classification, a study of all the three types of relationship viz., Phylogenetic, Phenotypic and Geotropic is imperative as stated by Chennaveeraiah[4]. Determination of biological species in cultivated plants stated by Barker[2] and the observational bases are full description of chromosome number and karyotype analysis and evidence of natural hybridization.

Stomatal frequency and size are considered as two important parameters in characterization of mulberry genotypes. These two characters are having positive correlation with drought resistance. The observed small size and lesser frequency of stomata in triploids than diploid variety. Stomatal frequency and size decrease with increase in ploidy level was established Tikader[13]. The observed genotypic level differences in stomatal frequency are in agreement with various other reports of [7, 6, 18]. The present findings also clearly shows frequency of stomata per unit area is significantly less in triploid compared to diploid. Moisture retention capacity will be higher in those mulberry varieties possessing smaller and lower stomatal frequency reported by[3].

Basic chromosome number of the genus *Morus* L., has x=14 for majority of the species[5,9] have been reported. In the present study the three mulberry varieties belong to *Morus alba*. Out of three varieties studied two are diploid with 2n=28 and one variety showed 2n=42 chromosomes. In general chromosomes are smaller with a close range of length variation. Confirming the earlier reports, cytologically they showed similar karyotype with only two types of chromosomes, equal chromatin length and also length range. These different chromosome numbers has reflected on their morphology. Previous and present reports on chromosome number and cytology showed the presence of 2n=28 chromosomes in most of the cultivars in this *genus*. This information may be considered as an essential contribution to the chromosomal database repository in relation to indigenous mulberry cultivars which will be useful for the researchers working either on the improvement of mulberry varieties having

significant agronomical and commercial traits or on the phylogeny and evolutionary study of the taxa in wider perspectives.

CONCLUSION

These micro morphological and karyomorphological investigations will be made use of while selecting the parent's plants for evolving progeny of different ploidy level of both by hybridization and colchicine treatment. In addition, this information will be of much use in establishing a phylogenetic relationship and evolution of mulberry and will also help in selecting mother plant for hybridization based on chromosome number.

REFERENCES

1. Aneja KR. Experiments in microbiology plant pathology tissue culture and mushroom production technology (3rd edition); 2001; New age international publishers New Delhi.
2. Barker HC. Taxonomy and biological species concept in cultivated plants. In Genetic Resources in Plants, their Exploration Conservation. Eds. O. H. Frankel and E. Bennet. Blackwell Scientific Publications, Oxford and Edinburgh, 1970; 49:68.
3. Basavaiah, Murthy T. C. S. Relation between anatomical features, moisture content and Retention capacity of leaf in some mulberry (*Morus spp*.) genotypes of different ploidy. *Natl. Semi. Mulb. Seri. Res.* November, 2001; 26-28, KSSR&DI, Bangalore India 98.
4. Chennaveeraiah MS. Biosystematics-A Presidential Address. 70th Indian Science Congress, 1983; Section of botany.
5. Das BC. Cytological studies on *Morus indica* L and *M laevigata Wall Caryologia*, 1961; 14: 159-162.
6. Dwivedi MK, Sikdar AK, Jolly MS, Susheelamma BN, Suryanarayana N. Induction of tetraploidy in colchicine induced mutant of mulberry. I Morphological and cytological studies in cultivar Kanva-2. *Indian J. Genet.* 1988; 48(3):305-311.
7. Eswar Rao MS. Improvement of mulberry through ploypoid breeding. Ph. D. Thesis, 1996. Bangalore University, Bangalore.
8. Gill BS, Gupta RC. Cytological studies in the sex types of *Morus alba* L. Moraceae, *Cur. Sci.* 1979; Vol. 48 (1):35-36.
9. Kundu D, Sharma A. Chromosome studies in some Indian Moraceae In P. Kachroo (ed) Recent advances in Botany Bishen Singh Mahedra Pal Singh Dehradum, 1976; 348-369.
10. Levan AK, Fredga, Sandberg AA. Nomenclature for centromeric position on chromosome. *Hereditas,* 1964; 52: 201-220.
11. Sharma AK, Sharma. Chromosome techniques. Theory and practice (II edition). Butterworth and Co., London and University Park Press, Baltimore.
12. Sikdar AK, Jolly MS, Susheelamma BN, Giridhar K. Stomatal chloroplast count technique as a tool to ascertain different ploidy level in mulberry *Indian J. Seric.,* 1986; 25 (2): 88-90.
13. Tikader A. Hierarchical cluster analysis of mulberry germplasm (*Morus spp*.) based on phenological traits *Indian J. Forestry,* 2001; 24(3):344-350.
14. Venkatesh KH. "Cytogenetical investigation in the Genus *Morus* L." Ph. D., Thesis, 2007 Bangalore University, Bangalore.
15. Venkatesh KH, Venu N, Dinesh B, Munirajappa Stomatal frequency and karyotypic studies in three varieties of mulberry *J Cytol Gen.* 2013; 14 (NS): 101-107.
16. Venkatesh KH, Shivaswamy S, Munirajappa. Comparative micro morphology and reproductive studies in three mulberry varieties (Moraceae). *International Journal of Science and Nature,* 2014; 4 (4): 608-610.
17. Venkatesh KH, Munirajappa, Shivaswamy S. Studies on Cytomorphology of two mulberry varieties (Moraceae). *Indian Journal of Applied Research*, 2014; 4(6): 35-37.
18. Vijayan K, Sahu PK, Chakraborti SP, Roy BN. Evaluation of tetraploid for triploids

breeding in mulberry. *Indian J. Genet*. 1999; 59(14): 512-522.

Table 1. Karyotype analysis in S_{13}, V_1 and Tr-$_8$ mulberry varieties

M. varieties	Stomatal frequency/ mm^2	2n chromosome number	Ploidy level	Karyotype	Chromosome size range (µm)	Arm ratio (µm)	Haploid chromatin Length (µm)
S_{13}	196.00	28	Diploid	$2n=28=4B^{m}+2B^{sm}+18C^{m}+4C^{sm}$	1.29 - 2.31	0.71- 0.98	25.38
V_1	206.44	28	Diploid	$2n=28=18B^{m}+8B^{sm}+2C^{m}$	1.59 - 2.86	0.43-1.63	32.43
Tr-$_8$	170.65	42	Triploid	$2n=42=18B^{m}+12B^{sm}+6C^{m}+6C^{sm}$	1.49 -2.59	0.49 -1.00	45.65

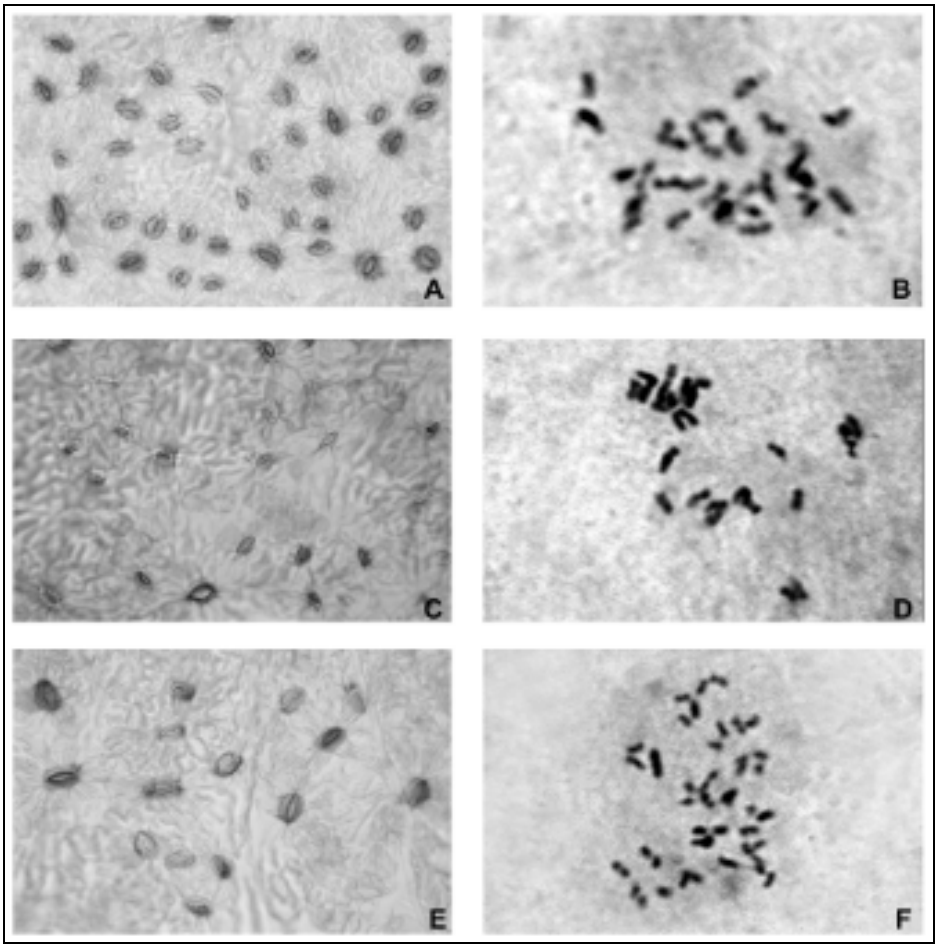

Figure 1. A & B, Stomatal frequency and somatic chromosomes of variety S_{13}. C & D, Stomatal frequency and somatic chromosomes of variety V_1. E & F, Stomatal frequency and somatic chromosomes of variety Tr-$_8$

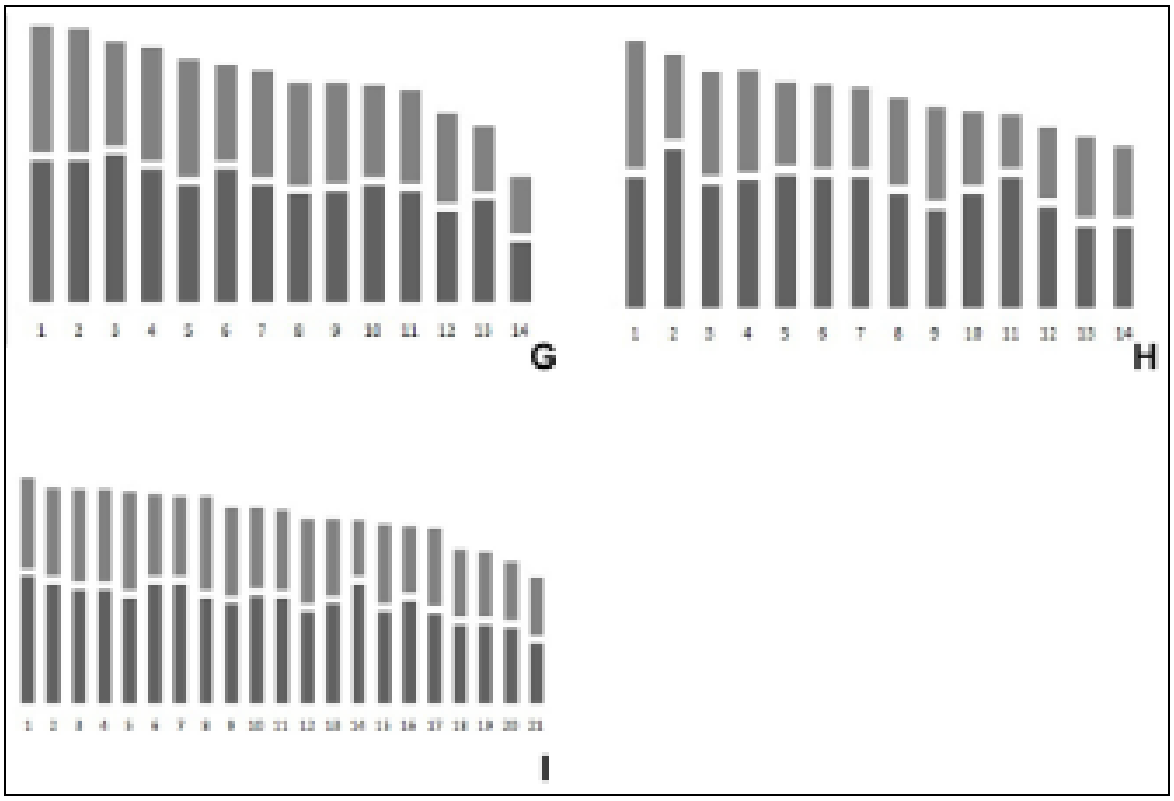

Figure 2. G, H & I, Ideograms of varieties S_{13}, V_1 and Tr-8 respectively