RESEARCH ARTICLE

CYTOGENETICAL AND MORPHO-ANATOMICAL STUDIES IN AN IMPROVED MULBERRY CULTIVAR G-2, AND ITS PARENTS (*MORUS* SPP.)

Sheelavathi A.V., Ravi Kumara R.* and Basavaiah

Department of Sericulture Science, Manasagangotri, University of Mysore, Mysuru -570 006

Received-03.09.2022, Revised-15.09.2022, Accepted-28.09.2022

Abstract: Mulberry (*Morus* spp.:Moraceae) breeding was programmed in a systematic way in India and progress was made in different aspects considering the overall needs of the sericulture industry. Mulberry varieties with higher levels of succulence and nutrition are suitable for healthy and robust chawki worms (young-age silkworms). The mulberry variety S-36 was recommended for chawki rearing. However, Victory-1 is the most exploited mulberry variety for chawki gardens. Further, a new mulberry variety, G-2 (Genotype-2), is being popularized currently for chawki rearing centers. The present study was conducted on the mulberry cultivar G-2 and its parents (*Morus multicaulis* Perr and S-34) for comparative studies with respect to cytological, morphological, and anatomical features. The results revealed that *Morus multicaulis*, S-34, and G-2 varieties are diploid in nature, with the chromosome number of 2n=28. Normal meiosis was observed in the male parent (S-34) of the G-2 variety. The morphometric traits of the hybrid variety, G-2, are exhibited in an intermediate nature between the two parents. The new combinations in the important yield-contributing parameters clearly indicated the improved vigor of this hybrid.

Keywords: Chawki worms, G-2, *Morus multicaulis* Perr, S-34

INTRODUCTION

Mulberry (2n=2x=28) is a high-biomass-producing foliage plant cultivated as a small to medium bush through repeated pruning for the rearing of the monophagous silkworm, *Bombyx mori* L. Indian sericulture, being essentially mulberry silk production, is responsible for substantial foreign exchange. To increase silk production, not only the silkworm but the host plant (mulberry) needs constant improvement by both conventional and non-conventional approaches. Rearing of young silkworms up to their second moult is called "chawki rearing," which usually lasts for 7 to 8 days. Chawki silkworm rearing plays a vital role in the mulberry sericulture industry by providing robust chawki worms for the production of quality cocoons with improved yields. Rearing of young silkworms under ideal environmental conditions, feeding succulent, nutritious (protein 25%, carbohydrate 14%, and moisture 80%) and tender leaves makes the larvae robust and more tolerant to stress during late-age rearing. In this regard, the Central Sericulture Research and Training Institute (CSRTI), Mysore, developed an improved mulberry hybrid variety, G-2 (Genotype-2) in 2003, for raising mulberry gardens exclusively for the rearing of young silkworms. The variety is a hybrid of *Morus multicaulis* and S-34 and is characterized by erect branches, thick dark-green leaves, high moisture content (80.30%), high moisture retention (83.40%), high sprouting (96%), deep rooting (94%) and moderately resistant to leaf spot and leaf rust diseases. Under assured irrigation and a recommended package of practices, the G-2 variety has a leaf yield potential of 38 mt/ha/year in an 8-crop schedule of alternate leaf picking and shoot harvest and yields 33% more chawki leaves than S-36 and 20% more than Victory-1. In this context, the present investigation was undertaken to study and compare the nature and behavior of the chromosomes of a recently developed high-yielding mulberry hybrid G-2 with its parents (*Morus multicaulis* Perr and S-34). Furthermore, an attempt has been made to understand the extent of variation in some selected morphological and anatomical features between the hybrid and its parents.

MATERIALS AND METHODS

The improved variety of mulberry G-2 and its female parent, *Morus multicaulis* Perr (Acc. No. ME 0006), and male parent, S-34 (Acc. No. MI 0160), were used in the present study. The studies were conducted with the plants maintained in the Department of Studies in Sericulture Science, University of Mysore, Manasagangotri, Mysuru. The observations on morphological features were recorded by adopting the standard procedure described by Sahay *et al.* (2016) and DUS test guideline. Leaf anatomy and stomatal studies were carried out following the method described by Mallikarjunappa *et al.* (2007). The chromosome numbers were confirmed from root and shoot tip squash by the 1% aceto-orcein method. Meiotic studies were made from anther smears in 1% aceto-carmine stain (Sharma and Sharma, 1980). The chromosomes were screened under a microscope and photomicrographed. The standard deviation (±) method was used to analyze the data, and mean values were presented.

RESULTS AND DISCUSSION

Plant Morphology

*Corresponding Author

Morphological characterization of mulberry has been used as a tool to examine possible genetic relationships and this information has been used in its improvement (Tikader, 1997; Adolkar *et al.*, 2007). In the present investigation, we assessed the variations in morphological characteristics among three mulberry genotypes belonging to *Morus indica* and *Morus multicaulis* species (Table-1). The hybrid variety G-2 (Fig.1B) resembled its female parent *M. multicaulis* (Fig.1A) only in terms of plant vigor, mature bud shape; leaf surface, leaf base, leaf width, leaf hairiness (Fig.1D), sex, and stigma nature and its male parent S-34 (Fig.1C) only in terms of leaf texture (Fig.1F) and petiole length. These morphological characters are strongly heritable in nature (Dandin and Kumar, 1989). Further, the hybrid showed differences with both parents having a new combination of traits in growth nature (erect), branching nature (straight), bud attachment (tilting to one side), leaf color (dark green), leaf shape (wide ovate), and stigma type (spreading). The smooth surface with succulent leaves of the hybrid (Fig.1E) is more palatable for the young-age silkworms. The inter-nodal length of the hybrid is 4.5 cm. It is a genetic characteristic of the variety which determines the total amount of foliage produced by the unit length of shoot. The shorter the nodal distance, the more leaves per unit area can be accommodated. In mulberry, the optimum inter-nodal length is 4.5 cm for selecting superior varieties. The higher leaf area of the hybrid is one of the important higher chawki leaf yield-contributing traits. These characters have greater significance because broad leaves in hybrids usually grow quickly and are very efficient in photosynthesis due to their large surface area (Fei *et al.*, 2009). As a result, G-2 has a higher chawki leaf-yielding potential than both Victory-1 and S-36 varieties.

Figure 1: Plant morphology - a) *M. multicaulis*, b) G-2, c) S-34; leaf morphology - d) *M multicaulis*, e) G-2, f) S-34.

Table 1: Comparative account of morphological characterization of the mulberry hybrid, G-2, and its parents.

Characteristic	***Morus multicaulis***	**G - 2**	**S - 34**
Plant vigor	High	High	Medium
Growth nature	Semi-erect	Erect	Semi-erect
Branch nature	Slightly curved	Straight	Slightly curved
Color of young shoot	Green	Green	Green
Color of mature shoot	Yellowish Green	Greyish Brown	Greyish Green
Phyllotaxy	Mixed type	Mixed type	Mixed type
Stipule nature	Free-lateral	Free-lateral	Free-lateral

Stipule duration	Caducous	Caducous	Caducous
Leaf lobation type	Unlobed	Unlobed	Unlobed
Bud attachment	Slanting outward	Tilting to one side	Adhering to branch
Mature bud shape	Long triangle	Long triangle	Acute triangle
Leaf nature	Homophyllus	Homophyllus	Homophyllus
Leaf color	Light Green	Dark Green	Green
Leaf surface	Smooth	Smooth	Slightly Rough
Leaf texture	Membranous	Charataceous	Charataceous
Leaf apex	Acuminate	Acuminate	Acuminate
Leaf margin	Serrate	Serrate	Serrate
Leaf base	Cordate	Cordate	Trunctae
Leaf shape	Ovate	Wide ovate	Ovate
Leaf length	Medium (20.1 cm)	Medium (19.5 cm)	Medium (15.8 cm)
Leaf width	Broad (18.3 cm)	Broad (20.4 cm)	Medium (12.5 cm)
Petiole length	Long (6.2 cm)	Medium (4.3 cm)	Medium (3.6 cm)
Petiole width	Medium (0.4 cm)	Medium (0.3 cm)	Medium (0.3 cm)
Inter-nodal distance	Medium (6.0 cm)	Medium (4.5 cm)	Medium (4.3 cm)
Leaf hairiness	Glabrous	Glabrous	Sparsely Hairy
Sex	Gynoecious	Gynoecious	Androecious
Stigma nature	Pubescent	Pubescent	NA
Stigma type	Erect	Spreading	NA
Fruit color	Black	Black	NA

Leaf Anatomy

Anatomical characteristics of the mulberry leaves are found to be of diagnostic importance, such as the ratio of the density of the palisade parenchyma and collenchyma layers in the midrib region, type and density of trichomes, and length and width of stomata (Erarslan *et al.,* 2021). Leaf anatomical features are associated with many physiological functions, which in turn influence the quality of the leaf (Laltanmawii and Roychowdhuri, 2010). The increased leaf cuticular thickness in the hybrid variety G-2 is directly correlated with the higher leaf moisture content and its retention that facilitates absolute consumption of leaf by the larvae, leading to increased larval growth, which is an important parameter of young-age silkworm rearing (Paul *et al.,* 1992). The leaf thickness (142.25 µm) of hybrid G-2 (Fig.2B) is in between its parents and quite less (148.35 µm) than the female parent (Fig.2C). The cystolith and stomata are two epidermal structures that play a prominent role in determining the yield and quality of mulberry leaves. The hybrid displayed significant anatomical variation between the two parents, having a higher stomata frequency (Fig.2E) and cystolith frequency (Fig.2H) (Table-2) than both parents and being closer to the male parent S-34, which is a drought-resistant variety (Venkatesh *et al.,* 2014). The higher ratio of chlorophyllous palisade tissue, leaf thickness, and stomatal

frequency, all of which are directly correlated with high leaf yield in mulberry (Kumar *et al.*, 2012).

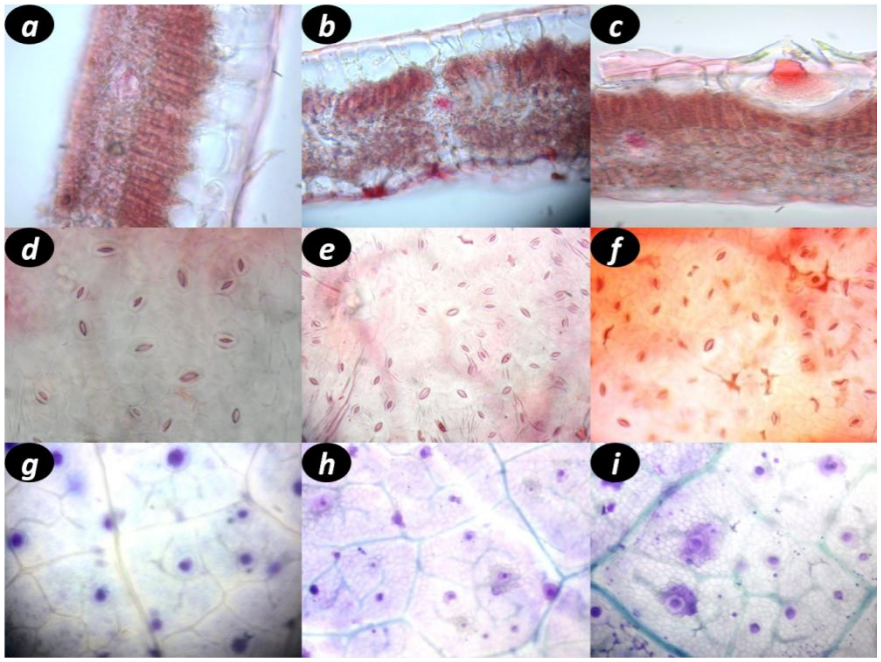

Figure 2: Cross section of leaf blade - a) *M. multicaulis*, b) G-2, c) S-34; Stomata - d) *M. multicaulis*, e) G-2, f) S-34; Cystolith g) *M. multicaulis*, h) G-2, i) S-34.

Table 2. Comparative account of leaf anatomical features of the mulberry hybrid, G-2, and its parents.

Parameters	***Morus maulticaulis***	**G - 2**	**S - 34**
Leaf thickness (µm)	133.91±1.6	142.25±1.4	148.35±1.7
Palisade thickness (µm)	38.35±1.4	41.30±1.8	46.78±1.3
Spongy thickness (µm)	51.5±2.3	53.28±1.3	61.3±1.6
Upper cuticle thickness (µm)	7.72±0.5	8.11±1.4	7.19±2.3
Lower cuticle thickness (µm)	3.85±1.5	4.31±1.1	3.11±1.9
Upper epidermis thickness (µm)	22.83±1.7	25.06±1.7	27.91±1.4
Lower epidermis thickness (µm)	12.11±1.6	12.69±1.4	13.31±1.7
Cystolith size (µm)	15.87±0.9	51.98±1.6	55.90±1.8
Cystolith frequency (no./sq.mm)	320.14±3.7	472.17±3.3	460.33±2.8
Stomatal size (sq. µm)	335.69±2.5	333.35±1.4	320.36±2.4
Stomatal frequency (no./sq.mm)	550.38±3.3	620.64±4.5	517.78±3.1
Idioblast length (µm)	12.59±0.8	11.33±1.8	8.11±2.1
Idioblast width (µm)	2.93±0.7	3.56±1.9	3.25±1.4
No. of chloroplast/ stomata	11.33±0.1	10.16±0.2	13.27±0.4

Cytogenetics

The *Morus* species have a complex ploidy ranging from haploid (2n=14) to docosaploid (2n=22x=308). However, the optimum level of ploidy considered from a biological point of view is triploidy, but the leaf surface becomes rougher as the ploidy level increases, making the foliage unsuitable for silkworm rearing (Laltanmawii and Roychowdhuri, 2010). As a consequence, only diploid and triploid species of mulberry are commonly used for cultivation, and the foliage is used as a sole food source for the silkworm *Bombyx mori* L. Moreover, the majority of the mulberry varieties available in nature are diploids with 2n=2x=28 chromosomes. The mitotic chromosomes in the root and shoot meristematic cells of *Morus multicaulis* chromosomes showed a varied degree of condensation at prophase. By counting the chromosomes in 5–6 well-spread metaphase plates, the somatic chromosome number was confirmed to be the diploid nature of the variety, with 2n=28 chromosomes (Fig.3A). The observed chromosome number 2n=28 is in conformity with the observation of Kundu and Sharma (1976). There are no cells that show the chromosome number of 2n=30 as reported by Venkatesh and Munirajappa (2015).

In the cultivar S-34, which also possesses a diploid number of 2n=28 chromosomes (Fig.3C), are recorded. Also, there were no mitotic abnormalities or variation in chromosome number. The mitotic division was normal with normal nuclei in the daughter cells. Meiosis during microsporogenesis was studied to understand the gametic chromosome number and the behavior of the chromosome in this male cultivar. The pollen mother cells of an anther showed synchronous division. During prophase-I (Fig.3D), one large nucleous with chromatin was observed in various stages of condensation to form bivalents. At metaphase-I (Fig.3E), 14 bivalents were recorded in the majority of the meiocytes. The characteristic feature of meiotic chromosomes in diploid mulberry is the occurrence of secondary associations of bivalents in metaphase-I (Das, 1961). Occasionally, one tetravalent with two bivalents and multivalents is also recorded. During anaphase-I (Fig.3F), the chromosome segregation was almost normal. The meiosis-II also regularly showed two equal sized daughter nuclei at prophase-II and also two equal sized daughter cells at metaphase-II. Furthermore, further stages of division were normal in the majority of the cells, forming normal tetrads (Fig.3H). However, a small percentage of PMCs at meiosis-II showed meiotic irregularities in the distribution of chromatids to opposite poles. This leads to abnormal pollen grains. The tapetal cells are found to be multinucleate. The pollen size found varied between 15.34 µm and 18.72 µm (Fig.3I). The study indicated that the meiosis in cultivar S-34 is almost regular. The nature of normal meiotic division in the diploid mulberry genotypes has also been reported by many investigators (Das *et al.*, 1970; Dwivedi *et al.*, 1988; Susheelamma *et al.*, 1990; Venkatesh and Munirajappa, 2013).

In the hybrid variety G-2, the chromosomes displayed a varied degree of condensation at prophase. In the metaphase stage, chromosome number revealed that the somatic chromosome number is 28 (Fig.3B), which is diploid in nature. Furthermore, mitotic divisions, anaphase and telophase were found to be regular. The regular mitosis found in shoot and root meristematic tissue clearly indicated the stable nature of this hybrid.

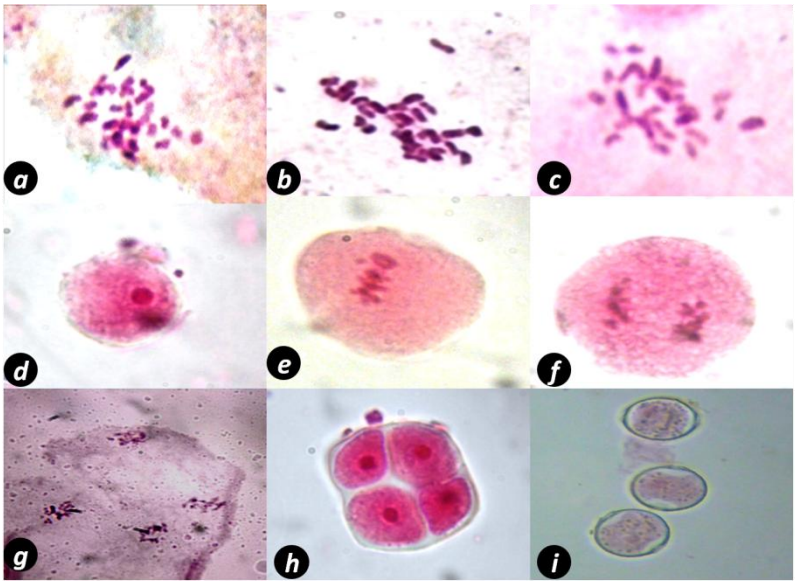

Figure 3: Somatic chromosome spread - a) *M. multicaulis*, b) G-2, c) S-34; Meiotic division stages of S-34 - d) Prophase-I, e) Metaphase-I , f) Anaphase-I, g) Anaphase-II, h) Tetrad, i) pollens.

CONCLUSION

Keeping in view the importance of mulberry leaves in sericulture, both qualitative and quantitative improvement with respect to leaf biomass is desirable so that the cost of cocoon production can be reduced. The present study on mitosis in shoot and root meristematic cells clearly indicated that the hybrid G-2 and its female parent, *Morus multicaulis*, as well as its male parent, S-34, are diploid in nature with the somatic chromosome number of 2n=2x=28. The new combinations in the important yield-contributing traits clearly indicated the improved vigor of this hybrid G-2. Moreover, the leaf morphological and anatomical traits of the hybrid make it more palatable to the young-age silkworms.

REFERENCES

Adolkar, V.V., Raina, S.K. and Kimbu, D.M. (2007). Evaluation of various mulberry *Morus* spp. (Moraceae) cultivars for the rearing of the bivoltine hybrid race Shaanshi BV-333 of the silkworm *Bombyx mori* (Lepidoptera: Bombycidae). *Int. J. Trop. Insect Sci.*, **27**:6-14.

Google Scholar

Dandin, S. B. and Kumar, R. (1989). Evaluation of mulberry genotypes for different growth and yield

parameters," In: K. Sengupta and S. B. Dandin, Eds., Genetic Resources of Mulberry and Utilization. CSRTI, CSB, Srirampura, Mysore. pp.143-151.
Google Scholar

Das, B.C. (1961). Cytological studies on *Morus indica* L. and *Morus laevigata* Wall. *Caryologia,* **14**: 159-162.
Google Scholar

Das, B.C., Prasad, D.N. and Sikdar, A.K. (1970). Colchicine induced tetraploids of mulberry. *Caryologia.*, **23**: 283-293.
Google Scholar

Dwivedi, N.K., Sikdar, A.K., Jolly, M.S., Susheelamma, B.N. and Suryanarayanan, N. (1988). Induction of tetraploidy in Colchicine induced mutant of mulberry I. Morphological and cytological studies in cultivar Kanva-2. *Ind. J. Genet.*, **48**: 305-311.
Google Scholar

Erarslan, Z.B., Karagoz, S. and Kultur, S. (2021). Comparative morphological and anatomical studies on *Morus* species (Moraceae) in Turkey. *Turk. J. Pharm. Sci.*, **18**:157-166.
Google Scholar

Fei, X., Weihua, G., Weihong, X., Yinghua,W. and Renqing, W. (2009). Leaf morphology correlates with water and light availability: What consequences for simple and compound leaves?, *Prog. Nat. Sci.*, **19**: 1789-1798.
Google Scholar

Kumar, V., Ramaia, J.K. and Rajan, M.V. (2012). Leaf and anatomical traits in relation to physiological characteristics in mulberry (*Morus* sp.) cultivars. *Turk. J. Bot.*, **36**:683-689.
Google Scholar

Kundu, D. and Sharma, A. (1976). Chromosome studies in some Indian moraceae. In: Recent Advances in Botany, (Eds. P. Karchroo, Bishen Singh, Mahendra Pal Singh) Dehradun. pp.348-365.
Google Scholar

Laltanmawii and Roychowdhuri, S. (2010). Effects of chromosomal variations on morphology and leaf anatomical behaviors in mulberry (*Morus* spp.). *J. crop weed.*, **6**:35-39.
Google Scholar

Mallikarjunappa, R.S., Venkateshaiah, H.V., Bongale, U.D. and Eswar Rao, M.S. (2007). Leaf anatomical variations in *Morus laevigata* (Moraceae) complex. *Indian J. Seric.*, **46**: 92-95.
Google Scholar

Paul, D.C., Subba Rao, G. and Deb, D. C. (1992). Impact of dietary moisture on nutritional indices and growth of *Bombyx mori* and concommitant larval duration. *J. Insect Physiol.*, **38**: 229-235.
Google Scholar

Sharma, A.K. and Sharma, A. (1980). Chromosome Techniques: Theory and Practice. Third edition, Butterworths, London.
Google Scholar

Susheelamma, B.N., Jalaja, S.K., Dandin, S.B., Jolly, M.S., Sengupta, K. and Raju, R. (1990). Karyomorphological studies in a few exotic varieties of genus *Morus* L. *Cytologia*, **55**: 107-114.
Google Scholar

Sahay, A., Jhansi Lakshmi, K., Saraswathi, P. and Sekar, S. (2016). Manual of mulberry gene bank operations and procedures. *Technical Manual. CSGRC (CSB), Hosur*.
Google Scholar

Tikader, A. (1997). Studies on heritabilities, genetic parameters and response to selection in mulberry. *Bulletin Sericulture Resources*, **8**:19-22.
Google Scholar

Venkatesh, K.H and Munirajappa (2015). Comparative micro-morphological and karyomorphological studies in three mulberry varieties (*Morus* spp.). *Cytologia,*, **80**:31-35.
Google Scholar

Venkatesh, K.H. and Munirajappa (2013). Cytogenetical studies in two tetraploid mulberry varieties (Moraceae). *Int. Soc. Chromosome Bot.*, **8**: 65-69.
Google Scholar

Venkatesh, K.H., Shivaswamy, S. and Munirajappa (2014). Cytomorphological studies of two mulberry varieties (Moraceae). *Afr. J. Biotechnol.*, **13**: 2203-2207.
Google Scholar