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Research Article

Cytogenetic characterization of a triploid mulberry (*Morus* spp.) cultivar Suvarna-2

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Abstract: Mulberry exhibits a high degree of polyploidy ranging from haploidy to docosaploidy and its various species are being cultivated for foliage to practice sericulture and edible fruits. Triploid mulberries for sericulture porpuse and higher polyploids are proved to be superior. The cultivar Suvarna-2 is an improved high leaf yielding triploid in south India. The meiotic behavior of this cultivar has been presented here. Meiosis was highly irregular. Various anomalies such as univalent, bivalents, trivalent, hexavalent, loose association, unequal separation and precocious movement of chromosomes and laggards have been observed in pollen mother cells. These irregularities prevented the formation of viable gamete that leads to pollen sterility.

Key words: Associations, Chromosome, Meiotic behavior, Microsprogenesis, Ploidy.

Introduction

Mulberry the common name for all the species belomg to genus *Morus* L. under the family of Moraceae, includes a group of prennieal and deciduous tree species. It is the sole food plant of silkworm, *Bombyx mori* L. and its various species are being cultivated for their foliage to practice sericulture. Polyploidy has played a major role in the evolution of its various species. Polyploidy ranging from haploidy to docosaploidy has benn reported in its various species. It is imperative that higher production of foliage will increase the volume of rearing and in turn the production of silk per unit area (Datta, 2001). Genetic improvement in a crop is considered as permanent one and essential to increase productivity. Among the various polyploid mulberries, triploids are reported to be superior in high yield of good quality leaf (Das and Prasad, 1974; Dzhafarov *et al.,* 1985; Tojyo, 1985; Sikdar, 1990; Tikader *et al.,* 1996). In mulberry, both natural (Osawa 1916, 1920, Seki 1959) and developed triploids have been reported (Das *et al.,* 1970, Dwivedi *et al.,* 1989, Basavaiah and Shafiei, 2017). Generally, triploids are obtained by controlled hybridization between tetraploids and diploid parents (Sarkar, 2009; Shafiei and Basavaiah, 2017). The polyploidy breeding programs of mulberry are aimed at the evolution of superior triploids.

Cytological confirmation of ploidy and chromosome behavior during meiosis of genotypes is very much essential in polyploidy breeding. In mulberry, the ploidy status, size, nature, pairing behavior and segregation of chromosomes during meiosis have been studied by various workers (Osawa, 1920; Sinoto, 1929; Kundu and Sharma, 1976; Gill and Gupta, 1979; Katsumata, 1979; Ogurtsov, 1979; Dandin *et al.,* 1989; Basavaiah *et al.,* 1990; Chakraborti *et al.,* 1999; Venkatesh and Munirajappa, 2013 and Venkatesh et al., 2014). In the present study, an attempt has been made to understand the meiotic behavior in a triploid mulberry cultivar Suvarna-2 which is recommended for cultivation in south india (Anonymous, 2015).

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Materials and Methods

The meiosis was studied during microsprogenesis in pollen mother cells (PMCs) employing standard cytological techniques (Sharma and Sharma, 1980). The Young male inflorescences of appropriate stages of development were picked from plants grown in evaluation plot at 8:30 - 9.30 a.m. during sunny days and fixed in 6:3:1- Methanol: Chloroform: Propionic acid for 24 hours and preserved in 70 % alcohol in refrigerator. Young anthers were squashed in 2% aceto-carmine stain on a clean slide. The slides were observed under microscope (Lietz) and various stages of meiosis were recorded. The frequency of various types of chromosomal associations at metaphase-I, and meiotic abnormalities were scored observing 30 meiocytes.

Results and Discussion

The genotype investigated in the present study displayed a triploid chromosome number of n=21.Young PMCs often showed large cytoplasmic channels between them indicating the possibility of cytomixis in varied environmental conditions in the triploid as it is recorded by Verma *et al.,* (1984) in one evolved male triploid of mulberry. In this genotype, different types of chromosomal associations are recorded in varied frequencies viz.,

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0.2 hexavalents + 12.6 trivalents + 1.2 bivalents + 0.6 univalents in different cells. (Figures 1a-e) and this corroborated with the findings of Das *et al.,* (1984) and Basavaiah *et al.,* (1990).

A high percentage of trivalent occurrence in metaphase I is suggestive of a fair degree of homology between the constituent of triploids and also the auto triploid nature of this cultivar. This is in agreement with the observations by Seki (1959) and Das *et al.,* (1984) in some natural triploid varieties of mulberry.

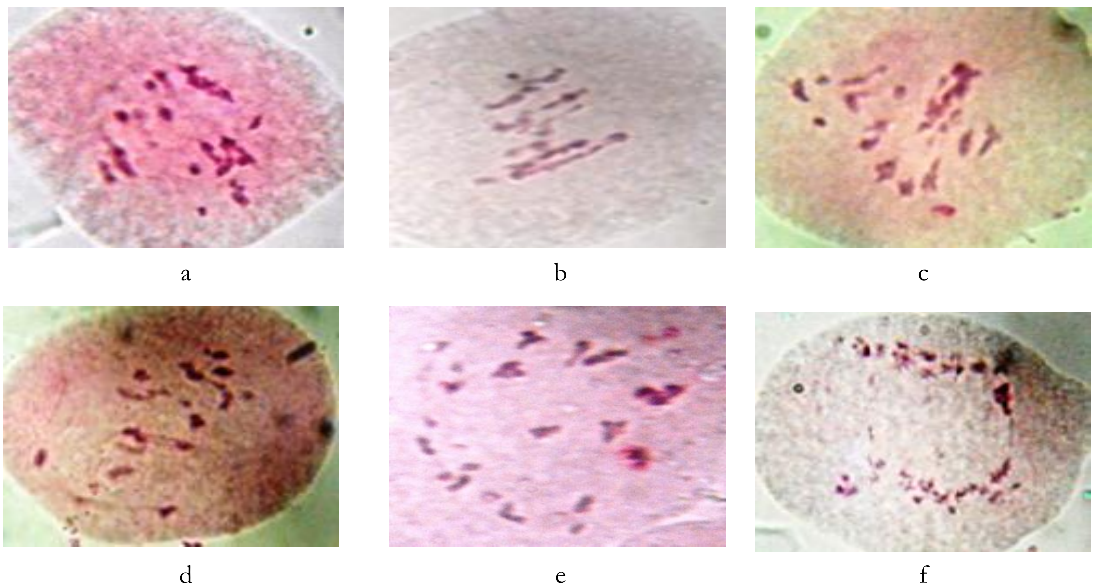
Depending on the pairing of the chromosomes the multivalents showed different configurational shapes and majorities of PMCs exhibit regular alignment of chromosomes at metaphase plate. The mega chromosomes occurred as trivalents and very rarely as hexavalents associating themselves with another trivalents. Occasional occurrence of one hexavalent (Figure 1b) may be attributed to the difference in parentage. Its parents (M-5(4x) × Viswa) have a difference in their geographical origin. M-5 being Open Pollinated Hybrid originated in Karnataka and its tetraploid is developed at KSSRDI, Bengaluru. But, the cultivar Viswa is the clonal selection made in the collection from Dehradun. (Eshwar Rao, 1996). In a RAPD marker assisted evaluation of cultivated and local mulberry genotypes, Keshava Murty *et al.,* (2009) have recorded wider genetic difference between M-5 and Viswa.

During anaphase-I, majority of meiocytes showed unequal separation of chromosomes (Figure 1g) and a few cells exhibited chromatin bridges (Figure 1f). The disturbance in meiosis-I has an adverse effect on the subsequent stages of meiosis II in this genotype. Also, due to unequal separations the subsequent stages of meiosis-II were irregular and a large number of degenerating meiocytes and non-synchronous divisional stages were recorded frequently (Figure 1h). Metaphase-II showed many chromosomes scattered in the cytoplasm without alignment in the equatorial plate (Figure 1i). Large number of cells at telophase-II showed grouping of chromosomes at more than four poles (Figure 1j). Occasionally polyads occurred with regular tetrads (Figure 1k). Pollen grains were observed unequal size (Figure 1l) and their size varied between 12.6 and 30.2 µ. Due to irregularities in first and second meiotic divisions, the resulting tetrads are aberrant and unbalanced and hence the pollen fertility is accordingly much reduced and this results in loss of chromatin materials (Darlington, 1965 and Gottschalk, 1978). The same condition is observed in some natural triploid varieties of mulberry by Seki (1959) and Das *et al.,* (1984).

Table 1. Chromosomal associations and percentage of meiocytes showing irregular meiosis in a cultivar Suvarna-2

Meiotic stages	Suvarna-2
Chromosomal associations in meiocytes at metaphase-I (mean and range)	
Hexavalent	0.2 (0 - 1)
Tetravalent	---
Trivalent	12.6 (12 – 14)
Bivalent	1.2 (0 - 2)
Univalent	0.6 (0 - 2)
Percentage of meiocytes at anaphase-1 showing	
Unequal separation	100
Laggard	6.1
Chromosomal bridge	1.2
Percentage of meiocytes at anaphase-II showing	
Unequal separation	100
Laggard	2.6
Pollen grains	
Pollen size range (µ)	12.6 - 30.2

Figure 1. Chromosomes in meiocytes during microsporogenesis in triploid cultivar Suvarna-2 of mulberry.



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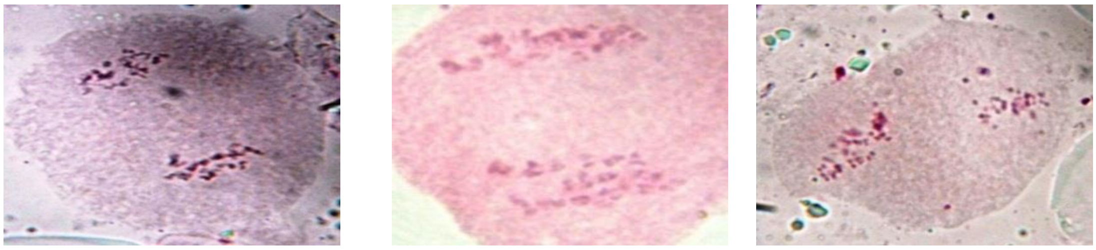

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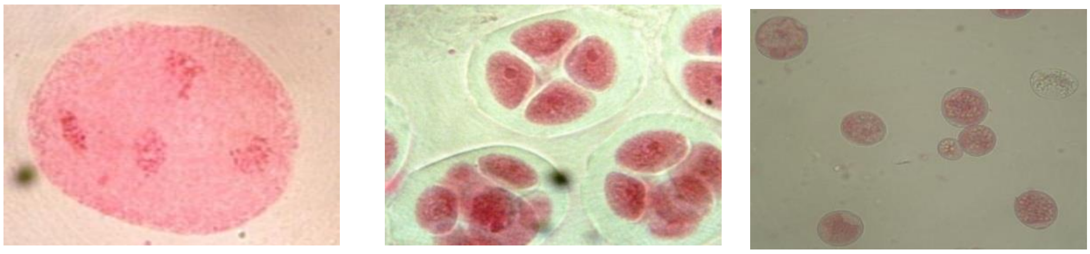

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a) Tri-, bi-and univalent at metaphase-I; b) Hexa-, tri-, bi- and univalent at metaphase-I; c) Tri-, bi- and univalent at metaphase-I.; d) Tri-, bi- and univalent at metaphase-I; e) Tri-, bi- and univalent at metaphase-I; f) chromosome bridges and laggards at metaphase-I; g) : irregular disjunction of chromosomes at anaphse-I; h) Meiocyte showing sign of degeneration; i) Meiocyte showing sign of degeneration; j) unequal sized daughter nucleiat telophase-II; k) Tetrad and polyad; l) Pollen grains of varied size and stainability
Magnifications: Figures a, b, c, d & g × 3000; Figurs h, i & j × 1500; Figures k × 1000 and Figure l × 500

Conclusion

The meiotic behavior in Suvarna-2 was highly irregular and confirmed its gametic chromosome number as 2n=21. this triploid showed a very high percentage of trivalents and a less percentage of tetra-, bi- and uni-valents, indicating its auto-triploid nature. Due irregularities in first and second meiotic divisions viable gamete formation was prevented and this leads to sterility.

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