Chapter

Studies on Basic Chromosome Number, Ploidy Level, Chromosomal Association and Configuration and Meiotic Behavior in Mulberry (*Morus* Spp.)

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Abstract

Mulberry leaves are primary food for silkworm, *Bombyx mori* L. to feed silkworms and harvest quality silk cocoons. Mulberry belongs to family Moraceae and includes 60 species found distributed in both Hemisphere. In mulberry, chromosome numbers are varies from 2n = 28 to 22n = 308 (Diploid to Decosoploid) with ploidy level x to 22x. Based on chromosome numbers and meiotic behaviors x = 14 has been considered as basic chromosome numbers of the genus. In the present study, two diploids, two uneuploids, two triploids and two teteraploids mulberry varieties were selected for detailed chromosomal numbers and meiotic behaviors belongs to three species, namely *Morus indica, Morus alba* and *Morus latifolia.* Varieties, Vishaala and Kosen were diploids with 2n = 2x = 28 chromosomes and varieties Ber-S_1 and S_{13} were uneuploids with 2n = 30 chromosomes belongs *Morus indica.* Varieties NAO Khurkul and KPG-1 were triploids with 2n = 3x = 42 chromosomes belongs to *Moru alba* and varieties Kokuso and Icheihei were tetraploids with 2n = 4x = 56 chromosomes. Diploids and uneuploids were showed normal meiosis with high pollen fertility and triploids and teteraploids were showed abnormal meiosis with low pollen fertility, due to virtue of higher ploidy level have been discussed in this chapter.

Keywords: Mulberry, chromosomes, polyploids meiotic behavior

1. Introduction

Sericulture is as an important agriculture-based, labor intensive, export-oriented cottage industry, introduced more than 200 years ago in India. This industry consists of several sectors or processes that are linked to one another like a chain. They are mulberry cultivation, silkworms egg production, silkworm rearing, harvesting of cocoons, silk reeling, twisting and weaving and manufacturing silk fabrics. Mulberry silk is produced from silkworms (*Bombyx mori* L.), which form the base of silk production. Mulberry is a fast growing plant and hence farmers can harvest 5–6 silkworm crops in a year at an interval of 26–28 days. The marginal and small farmers opted for sericulture since it was a remunerative crop as compared to other competing crops like Raagi, Jowar, Paddy, Potato and other vegetable crops.

Morus L. is an important genus of the family Moraceae under the order Urticales [1, 2] established the genus *Morus* with seven species viz., *Morus. alba, M. nigra, M. rubra, M. indica. M. tartarica, M. papyrifera* and *M. tinctoria.* Later, a number of species have been discovered by various workers from different parts of the world. Mulberry is a dicot, mesophytic, heterozygous and cross pollinated plant.

Importance of cytogenetical studies is very well understood in all most all agricultural crops. Even in mulberry breeding emphasis has been laid to understand the cytology of genotypes used as parents in breeding, in all sericultural advanced countries. In various mulberry genotypes, basic gametic and somatic chromosome numbers suggesting the ploidy level [3, 4]. These Cytogenetical data are useful to mulberry breeders in identifying and evolving promising genotypes and selecting the suitable species/varieties for commercial exploitation. Keeping this in view the identifying proper representation of genotypes of three species of mulberry has been discussed in this chapter.

2. Chromosome numbers

It is well established fact that cytological features are employed in differentiating and tracing the phylogeny of organisms. Cytotaxonomy based on chromosomal characteristics was most popular in plant taxonomy between 1930 and 1960 [5]. In such taxonomic studies chromosome number, chromosome morphology, chromosomal association, chromosome behavior and cytochemistry, etc., have yielded valuable results and revolutionized the phylogenetic interpretations.

Cytological studies in mulberry have been carried out in all sericulturally advanced countries. In view of their importance in breeding programs, chromosome numbers, chromosomal association and meiotic behaviors of various diploid, triploid, tetraploid and uneuploid genotypes of mulberry have been studied.

2.1 Polyploidy

The organisms having more than two genomes or two sets of chromosome in their somatic cells are called as polyploids. Among plants and animals, the polyploidy occurs in a multiple series of 3, 4, 5, 6, 7, 8, etc., of the basic chromosome numbers or genomes number and thus it causing triploidy, tetraploidy, pentaploidy, hexaploidy, heptaploidy, octaploidy, respectively. Polyploidy is most common among angiosperms.

The phenomenon of polyploidy is one of the widespread and distinctive features, which has played a major role in the evolution of higher plants. It plays important role in the natural selection and better adaptability of species in the new ecological niches.

In mulberry, polyploidy breeding techniques are found to be more suitable than mutation breeding techniques. A number of varieties have been developed in sericulturally advanced countries like Japan [6], China [7] and also in India [8]. In India, triploid mulberry varieties like TR_8 and TR_{10} have been recommended in hilly areas of eastern states.

Polyploidy may arise by several ways. (1) The egg may be fertilized by more than one sperm. If normal haploid egg is fertilized by two haploid sperms a triploid will result. (2) There may be failure of mitosis. (3) Triploids may arise as a result of fertilization of unreduced gametes. Diploid gametes arise because of failure in meiosis. If these gametes are fertilized by haploid sperms, triploids are formed.

(4) An autotetraploids may arise either by the doubling of chromosomes or by fertilization between two diploid gametes.

2.2 *Morus indica*

2.2.1 Variety Vishala

Vishala is developed by Central Sericultural Research Institute in Mysuru. It is fast growing variety, under ideal agro-climatic conditions (**Figure 1**). This variety yields 34,000 to 60,000 kgs and 14, 000 to 20,000 kgs of leaves/hector/year under irrigated and rain fed conditions respectively. Leaves are larger, dark green in color, unlobed and retain high moisture content. Stomatal frequency was found to be 260. 51/mm^2 & size 138.30μm^2.

2.2.2 Variety Kosen

This variety is evolved from cross pollinated hybrids. It is medium branching and fast growing in tropical conditions and it is good rooter (**Figure 2**). It possesses wide acclimatization in different agro-climatic conditions. This variety yields 26,000 to 40,000 kgs and 13, 000 to 18,000 kgs of leaves /hector/year under irrigated and rain fed conditions respectively. Leaves are larger, dark green in color, unlobed and retain high moisture content. Stomatal frequency was found to be 234. 60/mm^2 & size 126.40μm^2.

Each species of plants and animals is characterized by a particular chromosome complement or a set of genome, represented once in gametic haploid cell and twice in somatic diploid cells.

Genus *morus* exhibits a high degree of polyploidy ranging from diploid (2n = 2x = 28) to Decosoploid (2n = 22x = 308). Accordingly, its various species show chromosome numbers ranging 28 to 308. Majority of the species are diploids and polyploidy complexes were reported in many species.

Figure 1.
Variety Vishala.

Figure 2.
Variety Kosen.

Varieties, Vishala and S_{13} are belonging to Indian species and many cultivars belong to this species. Both the varieties were found to be 28 chromosomes (2n = 2x = 28) in their shoot somatic cells, during mitotic division, thereby confirming their diploid status. The diploid nature is related with fertility, normal growth, great vigorosity, adoptability and survivality of the diploid species. The typical characters of diploid are good elongation of branches and roots, good root initiation ability, good regenerating ability of buds, high yielding potential and easiness of raising saplings. Feeding value of leaves is highest for diploid, followed by triploid and tetraploid.

2.2.3 Variety Ber-S_1

It is an evolved variety from Berhampore Institute and showed uneuploid nature with 2n = 30 chromosomes (**Figure 3**). Morphologically uneuploid varieties are almost similar to the diploids in all parameters except minor variations. The uneuploid nature is related with fertility, normal growth, great vigorosity, adoptability and survivality of the uneuploids varieties. It possesses wide acclimatization in different agro-climatic conditions. This variety yield 24, 000 to 38, 000 kgs and 11, 000 to 16, 000 kgs of leaf/hector/year under and rainfed conditions respectively. Leaves are larger, dark green, unlobed and retain high moisture content. Stomatal frequency was found to be 238.20/mm^2 & size 116.00μm^2.

2.2.4 Variety S_{13}

It is selected from open pollinated hybrid (OPH) of $Kanva_2$ during 1986. This variety is characterized by short internodes and having a capacity of produces large numbers of branches. Leaves are thick and dark green unlobed with smooth surface. This variety best suited for rainfed condition and yield 16,000 to 18,000 kgs of leaf /hector/year (**Figure 4**). Leaves of diploids and uneuploids varieties are found to be

Figure 3.
Variety Ber-S_1.

Figure 4.
Variety S_{13}.

succulent, rich in moisture and nutrient contents when compared to triploids and tetraploid varieties and are suitable to silkworm larvae [9]. Stomatal frequency was found to be 210.00/mm^2 & size 128.00μm^2.

Varieties, Ber-S_1 and S_{13} are belonging to Indian species and many cultivars belong to this species. Both the varieties were found to be 30 chromosomes

(2n = 30) in their shoot somatic cells, during mitotic division, thereby confirming their uneuploid nature. Uneuploids plants have incomplete genomes. Individual chromosomes may either be less than diploid number (monosomic and nullisomic), or more than the diploid number (polysomic). Uneuploid chromosome number recorded in present work as well as reported by others are mainly due to extensive vegetative propagation followed for the multiplication of *Morus* spp. Therefore, the genus *Morus* has monobasic number x = 14. The polyploid numbers found in this taxa must have derived from this base number (x = 14) an account of auto and allopolyploidizaton. No doubt, vegetative propagation has helped for the perpetuation of uneuploids rather than their origin. Out breeding has played an important role in the origin of uneuploids due to the formation of gametes with unbalanced chromosome numbers.

2.3 *Morus alba*

2.3.1 Varieties: NAO Khurkul and KPG_1

These are exotic varieties grown in both tropical and temperate conditions. It possesses wide acclimatization in different agro-climatic conditions (**Figures 5** and **6**). These varieties yields 30,000 to 35,000 kgs of leaves/hector/year under rain fed land. Leaves are medium, unlobed, light green in color and high moisture retention capacity. Stomatal frequency was found to be 290.80/mm^2 & size 190.80µm^2 and 262.44/mm^2 & size 198.30 µm^2 respectively.

Both the varieties were found to be 42 chromosomes (2n = 3x = 42) in their shoot somatic cells, during mitotic division, thereby confirming their triploid status. Triploids produced from the cross between diploids and tetraploids. Triploid is the characterized by three genomes or three sets of chromosomes in each somatic cells. Thus, a triploid originates by the fusion of haploid gamete (n) with a diploid gamete (2n), the later of which may be originated by irregularities during meiosis. Most of the naturally occurring polyploids are either allo-polyploids or complexes

Figure 5.
Variety NAO Khurkul.

Figure 6.
Variety KPG_1.

between allopolyploids and auto-polyploids. Agronomic ally, the triploid mulberry varieties are known to be superior to other ploidy levels in rooting, chemical components and silkworm rearing performance. They also posses resistance to cold and high temperature.

2.4 *Morus latifolia*

2.4.1 Varieties Kokuso and Icheihei

Morphological characters of these varieties are entirely different from other varieties (**Figures 7** and **8**). *Morus latifolia is e*asily distinguishable from *M. alba* and *M. indica* by cylindrical fruits and hairy leaves. Leaves of tetraploid varieties are smaller, thin, rough, lobed, highly dissected, less moisture content and coriaceous due to the presence of many cystoliths, on the leaf surface, the calcium oxalate deposit and have a very detrimental effect on the palatability to silkworm larvae. Hence, these leaves are considered as inferior quality and are not suitable to silkworm larvae. The leaves' upper surface is dark green and lustrous with a pale green under surface. Stomatal frequency was found to be 310.22/mm^2 & size 238.10μm^2 and 340.40/mm^2 & size 260.30μm^2 respectively.

The above description suggests that diploid and triploid plants of this genus may exist anywhere in the world and that the morphological characteristics of diploid and triploid plants will be different to a certain extent from those of the polyploid ones.

The number of chromosomes of both the varieties at metaphase of somatic division were 2n = 4x = 56, which means both are teteraploids. Tetraploid may be originated by the somatic doubling of the chromosome numbers or by union of unreduced gametes.

Figure 7.
Variety Kokuso.

Figure 8.
Variety Icheichei.

3. Micro-morphological features

Stomata are the important anatomical and physiological characteristics of a leaf which control the rate of CO_2 movement from air to the chlorophyllus tissue within the leaf and thereby influence the rate of CO_2 exchange [10]. Stomatal size and frequency are important parameters in selecting drought resistant genotypes and these are also believed to regulate the leaf yield. As such, the Stomatal frequency has been studied in these taxa to correlate with cytological information recorded in the present investigation. In general, Stomatal frequency and size was found to be high in tetraploid when compared to triploid and diploid varieties.

Stomatal frequency was correlated with drought resistance and disease resistance [11]. Further, the Stomatal frequency was found to be related to the dry weight of the aerial parts of the plants and diffusive resistance [12]. Smaller stomata with lesser frequency per unit area make mulberry leaves become more succulency, palatable and are suitable for silkworms. [13] reported that, moisture retention capacity will be higher in those mulberry varieties possessing smaller stomata and lower stomatal frequency. Similarly we found average more number of chloroplasts in the guard cells were found be 10–12 in diploids and uneuploids compared to the triploids and tetraploids 6–8. In diploids and uneuploids number chloroplast were almost double than triploids and uneuploid.

As mulberry is a heterogeneous, predominantly unisexual, cross-pollinated and as well as vegetatively propagated perennial plant, it exhibits a wide range of variations in each and every phenotypic characteristic. Often the same genotype reveals the presence of both and unlobed leaves.

The genotypic differences in trichome density observed in the present study provide evidence to such observations in the field. The dense cover of trichomes in shoot tip and leaves of diploids may pose resistance to thrips attack in diploid and uneuploid mulberry varieties. On the other hand shoot tip and leaves of triploid and tetraploid mulberry varieties are less protected with sparse trichome density and hence may become susceptible to the thrips attack. It is also reported that diploids has high drought tolerance capacity than triploids [14]. So the dense trichomes may provide a better barrier to excessive water loss than sparse trichomes.

Trichomes are epidermal structures occur on stem, petioles & leaves. Diploid and uneuploid varieties showed highest trichomes density in both stem and leaves. On the other hand triploid and tetraploid varieties showed sparsh trichome density in both stem and leaves. Similar to various other anatomical feature cystolith frequency was lower in diploids and uneuploid compared to triploid and tetraploids. Triploids and Tetraploids showed distinctly high frequency and large sized cystolith, similar to stomatal frequency and size. In the evaluation of mulberry varieties the frequency and size of the idioblast has been taken as a parameter instead of frequency and size of cystolith [15].

3.1 Chromosomal association and configuration

Varied chromosomal associations and configurations recorded in the present study indicate closer homology between associated chromosomes. Depending on the ploidy level and pairing of chromosomes bi-, tri- and quadrivalents showed in different cofigurational shape. In both diploid and uneuploid varieties the bivalents frequency was high and the regular univalent's appears to be a mere matter of chance. Most of the cultivated diploid mulberry varieties showed 14 bivalents at diakinesis and metaphase-I. Theoretically diploids should forms more bivalents in meiosis due to presence of two homologous chromosomes. Bivalents and univalents were observed ‘ - - ’ or “‿” and “ -” types.

In triploid varieties the trivalent frequency was high and low frequency of bi and univalent's and no higher chromosome configuration was found. On the contrary some univalent's and bivalents were observed.

The alignment of centromeres of trivalents and univalents appears to be random and hence the assortment of chromosomes was also random. Hence majority of the cells showed unequal separation of chromosome at anaphase-I. Trivalents were observed “- - -” or “>-’ types.

In tetraploid varieties various types of chromosomal associations ranging from uni- to quadrivalents are observed during diakinesis and metaphase-I. Frequencies of quadrivalents are more than that of uni-, bi- and trivalents. However, the occurrence of multivalent such as tri- and quadrivalents indicates its polyploidy nature. Quadrivalents were observed “‿‿” or “- - - -” types.

Among different chromosomal associations, the bivalents in diploid and uneuploids, trivalents in triploid and quadrivalents in tetraploid were frequent. Majorities of quadrivalents were of ring type and other were chain types.

The concept of assessing the nature of polyploidy based on the sole criterion of multivalent frequency appears to have a limited value. Several workers have shown that, pairing of chromosomes is mainly governed by genes.

4. Meiotic behavior

4.1 *Morus indica* variety Vishaala

Meiotic behaviors in the present study revealed that microsporogenesis was normal in diploid varieties. These varieties showed regular pairing of chromosomes in majority of PMCs similar to many other diploid mulberry varieties [16] and 14 bivalents. The present investigation also showed there were no secondary association of chromosomes and multivalents in diakinesis and metaphase- I. The behavior of chromosome in metaphase is an index of fertility. Occurrence of normal bivalents results in the regular separation at anaphase and regular meiosis leading to high percentage of pollen fertility.

4.2 *Morus indica* variety S_{13}

Uneuploid varieties have incomplete genomes. Individual chromosome may either be less than the diploid number (*monosomics* and *nullisomic*) or more than the diploid number (polysomic). These varieties revealed marginal irregular meiosis and pollen fertility was also slightly less in these varieties.

4.3 *Morus alba* varieties NAO Khurkul and KPG_1

Triploid varieties are characterized by highly irregular meiosis and very low pollen fertility. Irregular meiosis is the almost common feature of triploids in all plants. These varieties showed high frequency of trivalents which suggests the fair homology between its constituent genome and autotriploid nature of these varieties [17, 18]. Such irregular meiosis has been reported in other natural triploid mulberry varieties also [19, 20]. The most common aberrations observed includes the occurrence of univalent’s, laggards, stickiness, precocious movement and in anaphase unequal number of chromosome segregate to the poles which again attribute to irregularities in chromosome pairing and their alignment on equatorial plate. These irregular meiosis leads to formation of aberrant, unbalanced microspore and finally resulted in the reduction of pollen fertility. Formation of such aberrant sporads in some mulberry triploids has been recorded by [21].

4.4 *Morus latifolia*

4.4.1 Varieties Kokuso and Icheihei

Meiotic behavior was irregular in these varieties. Theoretically tetraploid forms revealed more quadrivalents in meiosis due to the presence of four homologous chromosomes. Occurrence of high frequency of tetravalent in both the varieties indicates their relatively more stable autotetraploids nature. The presence of low frequency of trivalents and univalent's along with bivalents also indicates segmental homology of chromosomes and the allotetraploid nature of these varieties have been observed by [22]. Both the varieties showed low pollen fertility. The reduced pollen fertility of tetraploids could be attributed to association of chromosomes into multivalent during synopsis and other meiotic abnormalities which invariably results in loss of chromatin material [23–25]. Chromosome segregates unequally during anaphase leading to imbalanced chromosome complement in microspores and also leads to pollen sterility.

During the study of meiosis, nature of pollen mother cells (PMCs) at pre-meiotic interphase, nature of chromosomes at early stages of prophase- I, pairing behavior of chromosomes at diakinesis and metaphase-I, segregation of chromosomes at anaphase-I, separation of chromatids at anaphase-II, type and variations in tetrad, pollen size and pollen stainability were studied. The frequency of different types of chromosomal associations scored in 50 meiotic cells at metaphase. The bivalents showed 1 or 2 chaisma could not be distinguished, as the chromosomes were shorter.

Basic chromosome numbers, ploidy level, chromosomal association and configurations and meiotic behaviors of eight mulberry varieties were studied. The present research work revealed the diploid chromosome number of 2n = 2x = 28 (Vishaala & Kosen), uneuploid chromosome number of 2n = 30 (Ber-S_1 & S_{13}), triploid chromosome number of 2n = 3x = 42 (NAO Khurkul & KPG_1) and tetraploid chromosome number of 2n = 4x = 56 (Kokuso & Icheihei). High percentage of pollen fertility in diploid plants with 2n = 28 chromosomes indicate the dibasic nature of these taxa.

Variation in the chromosome pairing, assortment and pollen stainability in different varieties of mulberry indicate that they vary in genetic status. The basic number x = 14 appears to deep sited in the genus *Morus* and other numbers might have derived through secondary polyploidy, hybridization followed doubling of chromosome and uneuploid alteration.

5. Conclusion

The present investigation was carried out with objectives of generating useful information on Studies on basic chromosome number, ploidy level, chromosomal association and configuration and meiotic behaviors of eight mulberry varieties (Genotypes).

The mulberry varieties studied (Genotypes) have unraveled diversity with respect of branching pattern, leaf yield, leaf color, size, lobation, shape, stomatal frequency and size, chloroplasts in the guard cells and trichomes density. The taxa studied were taxonomically distinct. The interaction between genotype and the environment is largely responsible for the diversity observed.

Based on the present findings diploids have 2n = 28, uneuploids have 2n = 30, triploids have 3n = 42 and teteraploids have 4n = 56 chromosomes. Meiosis has been found to be regular with high frequency of bivalents, high pollen stainability and development normal pollen grain in diploids compare to irregular meiosis characterized by the presence of high frequency of trivalents and univalent's, low pollen stainability and aberrant pollen formation in triploids and teteraploids. The reduced pollen fertility has been attributed to various meiotic anomalies. The role of chromosome repatterning coupled with polyploidy (both euploids and uneuploids) structural alternation of chromosomes and mutations in the evolution of the genus *Morus* is suggested.

It may be concluded that more intensive biosystematics studies involving a large numbers of varieties /genotypes of a species definitely throw more light on the phylogeny and systematics of mulberry species. Such information's are highly essential and useful in mulberry improvement programmes.

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