Karyomorphological Studies in a Few Exotic Varieties of Genus *Morus* L.

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Among the genera of complex species which have defied the efforts of taxonomists and remained critical and confused, the genus *Morus* L. seems to have a place of its own owing to free cross fertilization among the species. The available population is highly heterogeneous, hence the classification on gross morphological characters alone has become difficult. The information in the field of cytology, karyomorphology, reproductive biology etc. is inadequate to seek the answer for some of the problems faced by the classical taxonomists. Reports of Tahara (1909), Osawa (1920) and Janaki Ammal (1948) confine only to the chromosome counts. Meiotic behaviour of a few cultivars have been reported by Datta (1954), Seki (1952), Das (1961) and Gill and Gupta (1974). The only report of karyomorphological studies is that of Kundu and Sharma (1976) in a few Indian cultivars. For most of the remaining species there is no information on karyomorphology. In the present studies an attempt has been made to elucidate the karyomorphological details in some important mulberry cultivars of exotic origin.

Materials and methods

Ten exotic varieties, namely Kosen, Kokuso-13, Kokuso-21, Kokuso-27, Roso and Rokokuyoso belonging to *Morus latifolia* Poir., Kenmochi, Ichihei and Mizusawa belonging to *M. bombycis* Koidz. and Chinapeking belonging to *M. alba* maintained in the mulberry germplasm of the Institute, were taken for the present study. Cuttings of these varieties were planted in pots for experimental use. Somatic preparations were made from excised root tips. Root tips were pretreated with aqueous solution of 0.002 molar 8-hydroxyquinoline for 3 hours at 10°C and fixed in 3:1 acetic acid: alcohol for about 4 hours. After washing in water the root tips were hydrolysed in 1N HCl for seven minutes at 60°C and stained in 1% aceto-orcein. Squash preparations were made in 45% acetic acid. Photomicrographs and drawings of good and clear plates were made immediately. To ascertain the chromosome number and their morphology, a number of preparations were examined in each variety. Camera lucida drawings were made at table level and idiograms were drawn using suitable scale. Karyotype classification was made according to Leven *et al.* (1964) by taking arm ratio into account.

Observations

Details of the somatic chromosome number, ploidy level chromatin length and karyotype formula are presented in Table 1. Of the cultivars studied, five are diploids ($2n=28$), three are aneuploids ($2n=30$) and two are triploids ($2n=42$).

Table 1. Karyomorphological details of 10 exotic varieties of mulberry

Species/ variety	Somatic chromosome no. (2n)	Ploidy level	Chromatin length (μ)	No. of sat- chromosomes	Range of chromosome length (μ)	Karyotype formulae	Relative chromosome length (μ)
<i>Morus latifolia</i>							
Kosen	28	2x	14.14	—	0.84-1.24	2n=28=13M+1m	100.17
Kokuso-13	30	2x+2	18.07	—	0.60-1.48	2n=30=5M+10m	120.47
Kokuso-21	28	2x	18.34	—	0.94-1.70	2n=28=9M+5m	131.00
Kokuso-27	42	3x	20.27	—	0.62-1.48	2n=42=17M+4m	96.52
Rokokuyoso	30	2x+2	15.25	—	0.84-1.26	2n=30=10M+5m	101.67
Roso	28	2x	14.78	—	0.84-1.48	2n=28=6M+8m	105.14
<i>Morus bombycis</i>							
Kenmochi	28	2x	14.50	1	0.60-1.69	2n=28=10M+4m	103.57
Ichihai	42	3x	25.45	—	0.95-1.48	2n=42=7M+13m+1sm	121.19
Mizusawa	28	2x	16.35	1	0.34-1.48	2n=28=7M+7m	116.43
<i>Morus alba</i>							
Chinapeking	30	2x+2	16.64	—	0.73-1.48	2n=30=6M+8m+1sm	110.93

M. latifolia Poir. Cv. Kosen is one of the popular strains which was introduced to India from Japan in 1930's and propagated through grafts. Leaves are entire, large with good feeding quality. This is a diploid cultivar with $2n=28=13M+1m$. Somatic chromosomes and idiograms are shown in Figs. 1-3 ($2n=28=13M+1m$).

M. latifolia Poir. Cv. Kokuso-13 is a hybrid variety evolved by crossing Murasakiwase

Figs. 1-12. 1-3, somatic chromosomes, camera lucida drawings and idiograms of Kosen ($2n=28$). 4-6, somatic chromosomes, camera lucida drawings and idiograms of Kokuso-13 ($2n=30$). 7-9, somatic chromosomes, camera lucida drawings and idiograms of Kokuso-21 ($2n=28$). 10-12, somatic chromosomes, camera lucida drawings and idiograms of Kokuso-27 ($2n=42$).

(*M. bombycis*) and Tsukasaguawa (*M. latifolia*). This bears lobed leaves with short internodes. Presence of $2n=30$ chromosomes indicates the aneuploid nature of the cultivar ($2n=30=5M+10m$). Somatic chromosomes and karyotype are shown in Figs. 4-6.

Figs. 13-23. 13-15, somatic chromosomes, camera lucida drawings and idiograms of Roso ($2n=28$). 16-18, somatic chromosomes, camera lucida drawings and idiograms of Rokokuyso ($2n=30$). 19-21, somatic chromosomes, camera lucida drawings and idiograms of Kenmochi ($2n=28$). 22-23, somatic chromosomes and camera lucida drawings of Ichihei ($2n=42$).

M. latifolia Poir. Cv. Kokuso-21 is a diploid variety evolved by hybridization between Naganuma and Tsukasaguwa varieties of *M. latifolia*. This is recognised as the most popular

Figs. 24-31. 24, idiograms of Ichihiei ($2n=42$). 25-27, somatic chromosomes, camera lucida drawings and idiograms of Mizusawa ($2n=28$). 28-30, somatic chromosomes, camera lucida drawings and idiograms of Chinapeking ($2n=30$). 31, relative chromatin length of some exotic mulberry varieties.

male component for interspecific crosses in Japan. Leaves are entire, glossy, deep green with smooth surface. Unlike the other two strains, the complement consists of two pairs of large chromosomes. Somatic chromosomes, camera lucida drawing and karyotypes are shown in Figs. 7–9 ($2n=28=9M+5m$).

M. latifolia Poir. Cv. Kokuso-27 is a triploid cultivar ($2n=42=17M+4m$). This is a hybrid between unreduced gamete and reduced pollen of Naganuma (*M. latifolia*) and Kairyonezumigaeki (*M. alba*) which was released in the year 1948 (Seki 1952). Somatic chromosomes and idiograms are shown in Figs. 10–12.

M. latifolia Poir. Cv. Roso is a diploid variety of Japan ($2n=28=6M+8m$). This variety is characterised by large unlobed glossy leaves with high moisture content. Somatic chromosomes and idiograms are shown in Figs. 13–15.

M. latifolia Poir. Cv. Rokokuyso is also a Japanese strain bearing unlobed, large succulent and lustrous green leaves. This strain shows aneuploid chromosome number ($2n=30=10M+5m$). Karyotype and idiogram are shown in Figs. 16–18.

M. bombycis Koidz. Cv. Kenmochi is a open pollinated hybrid selection. This is one of the good rooting and frost resistant varieties of Japan and popular among the sericulturists of Tohoku and Hokuriku prefectures. Leaves are perfectly five lobed, medium sized, glossy and dark green in colour. This is a diploid strain ($2n=28=10M+4m$). Somatic chromosomes and idiograms are shown in Figs. 19–21. Chromosomes are small with a narrow length range. Longest chromosome pair bears a satellite on short arm.

M. bombycis Koidz. Cv. Ichihei is also a frost resistant variety with lobed dark green leaves. Unlike Kenmochi, leaves are more coarse in texture. This is more popular in Iwate, Miyagi, Yamagata and Fukushima prefectures (because of its quick sprouting nature). This is a triploid cultivar ($2n=42=7M+13m+1Sm$). Somatic chromosomes, camera lucida sketch and idiograms are shown in Figs. 22–24.

M. bombycis Koidz. Cv. Mizusawa is a cultivar with large unlobed, dark green, thin leaves having longest petiole and is native of Japan. This is a diploid with 28 somatic chromosomes ($2n=28=7M+7m$). One pair is larger in size and the second largest pair bears satellite in short arm. (Figs. 25–27).

M. alba L. Cv. Chinapeking is a strain with variously lobed large leaves. Leaves are thin and characterised by round apices and deeply cut incisions. This is also an aneuploid strain ($2n=30=6M+8m+1Sm$). Karyotype and idiograms are shown in Figs. 28–30.

Comparative chromatin length of all ten cultivars is shown in Fig. 31.

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 crossing. To evolve dependable system of classification, a study of all the four types of relationships viz. Phylogenetic, Phenotypic, Geotropic and Tropic (interdependent) is imperative as stated by Chennaveeraiah (1983). Clausen *et al.* (1936) considered that morphology, cytology and ecology of the taxa furnish critical data for studying phylogenetic evolution. Baker (1970) quotes Mayer (1942) that for determination of biological species in cultivated plants the observational bases are full description of morphological variation, physiological and biochemical information, chromosome number and karyotype analysis and evidence of natural hybridisation. Nakajima (1982, unpublished) supported this view and pointed out the paucity of information with regard to mulberry.

Koidzumi (1917) and Hotta (1953) have classified the genus *Morus* based on the morphological characters of female flower. Katsumata (1972) observed some anatomical char-

acters in addition to morphological features. The above systems are considered to be incomplete as data from cytology, embryology etc. are lacking. Kundu and Sharma (1976) have analysed the karyotypes of a few mulberry cultivars belonging to *M. alba* L., *M. mungolika* Hort., *M. multicaulis* Perr. and *M. nigra* L. The other reports confine only to chromosome number (Tahara 1909, Sinoto 1929, Janaki Ammal 1948, Katsumata 1979). Gill and Gupta (1978) while studying cytology of sex types of *Morus* species reported the presence of large sized chromosomes. Datta (1954) and Das (1961) have studied meiosis in a few cultivars of *M. alba*, *M. latifolia* and *M. indica*.

In the present investigation, Kosen, Kokuso-13, Kokuso-21, Kokuso-27, Roso and Rokokuyso belonging to *M. latifolia* Poir. are morphologically distinct. Varieties, Roso, Kosen and Rokokuyso, show some similarities in their thick glossy leaves with identical leaf margin. Cytologically also they show similar karyotype with only two types of chromosomes, equal chromatin length and also length range. Despite of common origin, Kokuso-13 is an aneuploid, Kokuso-21 is a diploid and Kokuso-27 is a triploid. This different chromosome number has reflected on their morphology.

Cultivars Kenmochi and Ichihei belonging to *M. bombycis* Koidz. have similarity in their adaptation i. e. frost and die-back resistance, lobed and dark green leaves, good rooting, etc. However, leaves of Ichihei are coarse in texture. Induction of triploidy has resulted in variation of chromosome shape and size. Mizusawa, another cultivar of *M. bombycis*, has large thin leaves with long petiole unlike the other two. Cytologically also it is different in having Sat chromosomes. Chinapeking which is aneuploid is the only cultivar of *M. alba* L. included in the present study. Unlike other cultivars, there are three types of chromosomes in the complement. Morphologically also it stands apart in having highly unlobed large leaves and long petiole.

Previous and present reports on chromosome number and cytology show the presence of $2n=28$ chromosomes in most of the cultivars in this genus. Polyploidy, especially aneuploidy seems to have played a major role in the evolution of various taxa as evidenced by the occurrence of aneuploids with $2n=26, 27, 30$ chromosomes (Sinoto 1929, Das 1961, Kundu and Sharma 1976). The reason attributed by Kundu and Sharma (1976) for such an aneuploid occurrence is probably due to extensive vegetative propagation. No doubt, vegetative propagation has helped for the perpetuation of aneuploids rather than their origin. From the present work it may be concluded that outbreeding has played an important role in the origin of aneuploids due to the formation of gamete with unbalanced chromosome numbers. Occurrence of good number of natural triploids in Japan has been attributed to the process of fertilization between an unreduced female gamete and reduced pollen by Seki (1961). In all the above cases the cytological identity of each cultivar has been represented in phenotypic variation. However, extensive karyomorphological investigations coupled with meiotic behaviour, biochemical and physiological information seems to be imperative in drawing a conclusion about the taxonomical position of the taxa and the work in this direction has been in progress in this Institute.

Summary

Karyotype studies of ten exotic cultivars namely, Kosen, Kokuso-13, Kokuso-21, Kokuso-27, Roso and Rokokuyso belonging to *Morus latifolia* Poir., Kenmochi, Mizusawa and Ichihei belonging to *Morus bombycis* Koidz. and Chinapeking belonging to *M. alba* L. were reported for the first time. Of the ten cultivars, five are diploids ($2n=28$), two are triploids ($2n=3x=42$) and remaining three are aneuploids ($2n=28+2$). Chromosomes are generally small with an average length of 1.16μ and length ranges from 0.62μ to 1.70μ in all the cultivars

studied. Relative length ranges from 96 to 131 μ . In most of the cases, complement consists of only two types of chromosomes with median and submedian centromeres resulting in bimodal karyotypes. As a whole, frequency of median chromosomes is more than submedian ones. Of the ten strains, two strains namely Kokuso-13 and Mizusawa bear satellite chromosomes. Morphological variation and evolution of taxa were attributed to structural changes in the karyotype and an attempt has been made to study the phylogenetic relationship among the varieties of each species based on their karyotype. Vegetative propagation seems to have played a major role in maintaining the taxa at different ploidy level.

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