Research of Polyploidy and Its Application in *Morus*

By ISAO TOJYO*

Tohoku Branch, Sericultural Experiment Station
(Iizaka, Fukushima, 960-02 Japan)

In the first decade of 1900s, when the sericultural industry of Japan began to prosper, the existence of a large number of natural triploid was already found out among mulberry cultivars.[5,6] This finding was a pioneering study on crop polyploidy, not only in Japan, but also in the world. Of these triploid plants, not a few constituted superior varieties with a long history of cultivation and wide cultivation areas. Particularly in cold regions, for example in Tohoku, the northern region of Japan, such cultivars as Ichihei, Fukushima-oha, Shimanouchi, Akagi, etc., were distributed widely. They were cultivated for many years as the varieties for feeding young silkworms or cold-resistant varieties throughout Japan,[14] although their acreage has decreased due to recent changes in silkworm rearing method. This fact seems to verify the superiority of the triploid and highly evaluate the significance of breeding for polyploidy.

Furthermore, because of the easiness of vegetative propagation and high hybridity of *Morus*, the crossing at a polyploidy level such as triploidy crossing can accumulate more number of target genes and get higher heterosis than usual varietal crossings. Geographical distribution of *Morus* shows that an extremely large number of triploid plants are found in the high latitudes or in high elevation mountaineous areas. In view of the high possibility of expanding plant adaptability,[1] increasing plant resistance, and of effective breeding for chemical components of plant,[4] it was considered that the polyploidy breeding of *Morus* is highly significant.[20] However, in spite of the fact that the colchicine method was discovered by Blakeslee and Avery in 1937, the efficient studies on polyploidy breeding of *Morus* were initiated only since 1950s,[12,20] i.e. more than 10 years later for colchicine. In the genus *Morus*, *M. tiliaefolia* M. is known as hexaploid (2n = 84), and *M. nigra* L. as docosaploid (2n = 308). It is said that *M. cathayana* H. contains tetraploid, hexaploid, and octaploid.[9]

Mechanism of natural triploid occurrence

It was made clear that natural triploid mulberry varieties of more than 120 kinds exist in Japan.[9,10,13] Existence of such a large number of triploid in one crop can not be observed in other crops. Existence of such a large number of triploid cultivars may be attributable to the combination of (1) many chances of triploid occurrence, (2) a high possibility of selection of superior triploid mutants by naked eyes, and (3) many chances of selection in sericultural areas and mulberry saplings producing districts.[17]

Osawa[7] postulated the following three mechanisms for natural occurrence of triploid: (1) crossings between diploid and tetraploid, (2) formation of diploid gamete cells in diploid plants, caused by either the hybridity of *Morus* or rapid lowering of temperature at the time of meiosis, and (3) originating from triploid varieties. Later,

* Present address: Upland Farming and Sericulture Division, Tohoku National Agricultural Experiment Station (Iizaka, Fukushima, 960-02 Japan)

Seki reported that the main cause for natural triploid formation was the crossings between diploidized gamete induced by low temperature of late frost and normal haploid gamete.[9] And he recognized that Shinso No. 1 and Shinso No. 2 were triploid varieties produced by the diploidy crossings.[8] Tojyo observed abnormal nuclear divisions and diploidized gametes caused by late frost, and confirmed the occurrence of triploid.[17] Furthermore, he observed that diploidized gametes are rather easily produced even under natural conditions without late frost, and discussed the mechanism of triploid formation.[14]

Polyploid formation

The colchicine treatment is generally employed to induce polyploids. The main procedures are as follows: (1) Air-dry seeds are soaked in 0.1–0.4% colchicine aqueous solution for 24 hr, and then sown in Petri dishes after a light washing by water. The germinating seedlings showing a thickening are planted to soil. (2) One drop of 0.05–0.2% aqueous solution of colchicine is applied to the growing portion of a seedling (at the cotyledon expanding stage) once a day for several days. (3) After removing all buds except the terminal one on a shoot, only the apical bud is immersed in 0.2–0.4% colchicine aqueous solution for 24 hr before planting the shoot. In order to protect the shoot from dryness paraffin is applied to the removed bud marks. (4) Cutting is made in a room, and one drop of 0.1–0.4% aqueous solution of colchicine is applied to the growing point of the apical bud of the rooted cuttings 1–2 times a day for several days. (5) By bending young shoots, the apical portion of the shoots are dipped into 0.01–0.1% colchicine solution (aqueous) for 24–48 hr.

All the procedures listed above are the methods to double the genome of each variety used, i.e. the methods to produce autopolyploid.[16] The treatments given to winter buds or axillary buds may be expected to be of unsuccessful value. To produce triploid, crosses of 4x × 2x or 2x × 4x are made. The latter combination gives higher fertility and the resulting seeds show higher germination rate.[15] Another method is to make crosses between normal haploid gamete (pollen) and diploidized female gamate, caused by colchicine treatment.[10,11]

Characteristics of polyploids

Newly derived tetraploids are generally characterized by enlarged organs such as large guard cells, thickening of epidermis, palisade parenchyma, spongy parenchyma, and thick and large leaves with thick petioles and nerves. They appear to be rough and sturdy. Although the number of florets per cluster is less, ovaries and seeds are big.[15] Male flowers show no change in appearance, but their pollens are big. Branches of tetraploid mulberry show thick phloem, thin xylem, and big pith. In summer–autumn season, the first and second layers of phloem and primary cortex show big cells, and both the depth from epidermis to cork cortex and the thickness of primary cortex are large. Vascular bundles are more or less thicker at the upper portion of branches but thinner at the lower portion in tetraploid than in diploid. Vessels are big, but less in number in tetraploid. Root tissues also showed a similar tendency to branches having the above characters.[22]

In triploid and tetraploid, the number of chloroplasts, content of chlorophyll, and dry matter production are greater than in diploid.[16,20] The respective water content percentages of leaves, petioles, and young shoots in tetraploid are higher than those of diploid by 2–3%, irrespective of different seasons.[18] Water content percentages of branch is higher in tetraploid than in diploid by 1–10%, although it varies with seasons or pruning methods.[20] Fresh leaf weight per 100 cm^2 increases by 20–40%, and leaf wilting occurs slowly in tetraploid,[16] while hardness of branches declines.[22]

Sprouting in spring season generally delays in tetraploid as compared with its original diploid, but that of triploid occurs earlier.[19] Elongation of branches and roots of tetraploid is inferior to that of diploid, but there is no difference with rooting ratio of cuttings, and tetraploid shows high survival ratio of graftings.[20]

Morphological characters of triploid produced from the crosses between diploid and tetraploid are similar to tetraploid which has more genomes, but the other characters are generally intermediate between diploid and tetraploid. The typical characters of triploid are as follows: leaves rather greater than parents, 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 the highest for tetraploid, followed by triploid and diploid in that order.[20] From the standpoint of breeding material for heteroploid, it is interesting to note that relatively fertile triploids existed among the triploids produced by diploidizing gamate cells.[21]

Breeding of new polyploid varieties

Shinkenmochi (Kuwa-Norin No. 5)[2] belongs to *Morus bombycis* K., and is a new triploid variety (2n = 42), composed of two genomes of Kenmochi and one genome of Kokuso No. 21. It has a slightly expanding plant type with many branches, which elongate well and uniformly, and slightly longer internodes. Brown winter buds and purplish

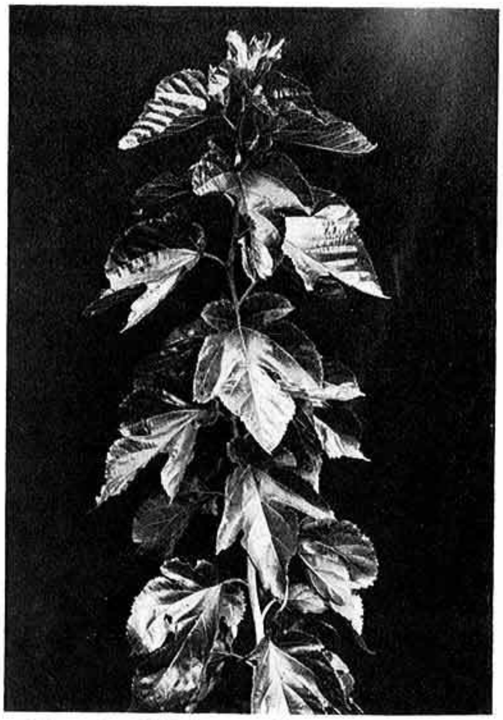

Plate 1. Shinkenmochi (triploid), a new mulberry variety

Table 1. Results of cultivation of Shinkenmochi (Kuwa-Norin No. 5), a new variety

Locality	Mulberry variety	Condition of shoot (Middle of September)				Yearly leaf yields (kg/10a)	
		Summer pruning		Spring pruning			
		Length of the longest shoot	Number of shoot	Length of the longest shoot	Number of shoot	Summer pruning (Index)	Spring pruning (Index)
		cm		cm		kg	kg
Matsumoto	Shinkenmochi	146	20.7	217	14.9	1,643(169)	1,523(154)*
(Nagano Pref.)	Ichinose	123	13.6	197	10.7	971(100)	990(100)*
Kobuchizawa	Shinkenmochi	130	18.9	—	—	1,337(106)	—
(Yamanashi Pref.)	Ichinose	138	15.1	—	—	1,263(100)	—
Mizusawa	Shinkenmochi	167	21.0	204	23.7	2,038(121)	906(119)
(Iwate Pref.)	Kairyo-nezumigaeshi	169	22.6	193	20.2	1,680(100)	759(100)
Komoro	Shinkenmochi	146	11.9	202	10.4	1,690(123)	928(125)
(Nagano Pref.)	Ichinose	152	9.1	209	8.3	1,370(100)	745(100)

* alternate pruning

Table 2. Cocoon production of silkworms fed on leaves of Shinkenmochi, a new variety

Rearing station	Rearing season	Mulberry variety	Cocoon quality				
			Cocoon weight	Cocoon shell weight	Cocoon shell percentage	Survival rate of pupae	Amount of cocoon crop per 10,000 silkworms
			g	cg	%	%	kg
Iwate	Spring	Shinkenmochi	1.66	37.8	22.8	99.1	15.3
		Kairyo-nezumigaeshi	1.60	36.5	22.8	97.9	14.8
	Late autumn	Shinkenmochi	1.79	42.5	23.7	98.0	16.5
		Kairyo-nezumigaeshi	1.81	43.5	24.0	97.0	16.4
Nagano	Spring	Shinkenmochi	1.81	44.6	24.6	98.0	16.2
		Ichinose	1.68	40.4	24.0	97.0	14.6
	Late autumn	Shinkenmochi	2.15	53.1	24.7	97.0	18.7
		Ichinose	1.95	47.8	24.5	98.5	17.2

brown trees are similar to Kenmochi, but they are slightly pale with smooth tree surface. The slightly large and wide 4-lobed leaves are thick, very elastic, smooth, glossy, and dark green (Plate 1). Hardening of leaves in late autumn is nearly the same as that of Kairyo-nezumigaeshi and Ichinose. Time of spring sprouting is the same as Kairyo-nezumigaeshi, and several days earlier than Ichinose. The subsequent growth is active, resulting in long young shoots at the beginning time and grown silkworm stages of spring silkworm rearing. It has the same number of leaves as Kairyo-nezumigaeshi. The total leaf yield per year for the grown silkworms in spring and summer-autumn rearing is fairly high as compared with Kairyo-nezumigaeshi and Ichinose (Table 1). Furthermore, it has cold-resistance, moderate resistance to dwarf disease and die-back, and high resistance to bacterial blight and powdery mildew. Damage by mulberry shoot gall midge is small. This variety shows an extremely good rooting of hard-wood cuttings, and is suitable for the use to establish quick-yielding, dense-planted mulberry fields. Silkworm-rearing test of the variety showed better results than Kairyo-nezumigaeshi and Ichinose in spring or summer–autumn season, because of good leaf quality in both seasons (Table 2). This variety is adaptable to cold areas with little snow in Tohoku and Chubu regions, and can be used for both spring and summer–autumn rearing or only summer–autumn rearing, or for quick-yielding, dense-planted mulberry fields. And it may take the place of Kanmasari, Kairyo-nezumigaeshi, Ichinose, etc. However, due to moderate resistance to dwarf, the extension in the seriously infested areas should be avoided.[2,23]

Aobanezumi (Kuwa-Norin No. 8)[2] is a new triploid variety developed from a cross between tetraploid (♀) obtained by colchicine treatment of Kenmochi seeds, produced by natural pollination, and Kairyo-nezumigaeshi (♂). It belongs to *M. alba* L. It shows an erect type, with many upright branches which have short internodes, and elongate actively and uniformly (Plate 2). It has deep brown winter buds on ash-colored trees with smooth surface, showing a close resemblance to Kairyo-nezumigaeshi. Leaves are slightly large, wide, and 4-lobed, with somewhat rough and less glossy surface. Their green color of leaves is slightly darker than that of Kairyo-nezumigaeshi. Leaf-hardening in late autumn is the same as that of Kairyo-nezumigaeshi. Time of spring sprouting is almost the same as Kairyo-nezumigaeshi, but several days earlier than Ichinose. The subsequent growth

Table 3. Results of cultivation of Aobanezumi (Kuwa-Norin No. 8), a new variety

Locality	Mulberry variety	Condition of shoot (Middle of September)				Yearly leaf yields (kg/10a)	
		Summer pruning		Spring pruning			
		Length of the longest shoot	Number of shoot	Length of the longest shoot	Number of shoot	Summer pruning (Index)	Spring pruning (Index)
		cm		cm		kg	kg
Mizusawa (Iwate Pref.)	Aobanezumi	151	19.0	225	20.6	2,266(115)	1,352(123)
	Kairyo-nezumigaeshi	159	21.9	233	18.2	1,979(100)	1,102(100)
Komoro (Nagano Pref.)	Aobanezumi	144	11.2	194	10.4	1,659(112)	868(138)
	Ichinose	155	9.3	202	8.5	1,478(100)	629(100)

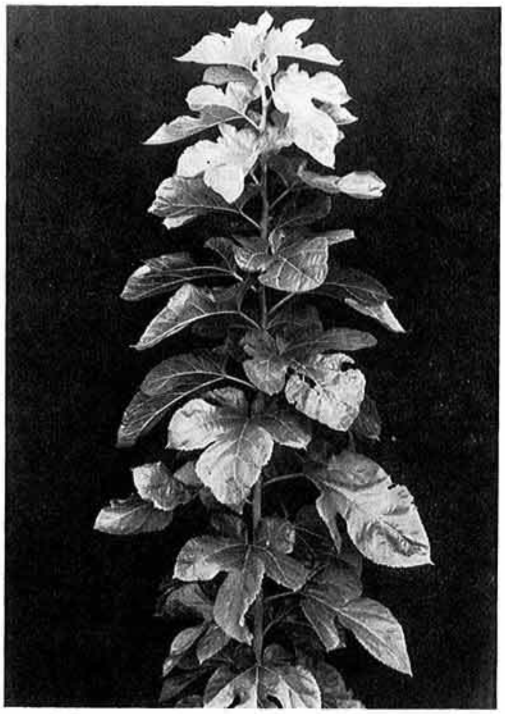

Plate 2. Aobanezumi (triploid), a new mulberry variety

is vigorous: length of young shoots, and the number of leaves at the grown silkworm stage in spring rearing season are similar to those of Kairyo-nezumigaeshi.

The total leaf yield per year for the grown silkworms in spring and summer–autumn rearing is fairly high as compared with Kairyo-nezumigaeshi and Ichinose (Table 3). It is cold resistant, moderately resistant to dwarf disease and powdery mildew, and resistant to bacterial blight and mulberry shoot gall midge. Rooting of hard-wood cuttings is better than Ichinose, and bud regeneration is also good. The silkworm rearing test showed good results, as it is applied to Kairyo-nezumigaeshi (Table 4).

This variety is adaptable to cold areas with little snow, particularly to Yamase area* of Tohoku district. As a mulberry variety to feed grown silkworms in both spring and summer–autumn rearing season or only summer–autumn rearing season, including the use of quick-yielding, dense-planted mulberry fields, this variety seems to take the place of Kairyo-nezumigaeshi or Ichinose. As the dwarf-resistance is moderate, planting to severely infested areas should be avoided.[2]

As the tetraploid is generally characterized by poor shoot elongation, its yield is slightly lower than diploid, but the feeding value of its leaves is very high. Therefore, it is desirable to use the tetraploid as a feed for silkworm breeding (rearing of parent silkworms for hybridization). The tetraploid is also quite valuable as parents as well as stocks to be used for hybridization. As the triploid is excellent in shoot elongation, rooting, and regeneration and it gives very high yield with good leaf quality, it may be effectively used

* Pacific side of Tohoku district, where north-east wind of low temperature frequently blows in summer season.

Table 4. Cocoon production of silkworms fed on leaves of Aobanezumi, a new variety

Rearing station	Rearing season	Mulberry variety	Cocoon quality				
			Cocoon weight	Cocoon shell weight	Cocoon shell percentage	Survival rate of pupae	Amount of cocoon crop per 10,000 silkworms
			g	cg	%	%	kg
Iwate	Spring	Aobanezumi	1.84	43.6	23.7	99.0	15.8
		Kairyo-nezumigaeshi	1.84	43.9	23.9	100.0	15.2
	Late autumn	Aobanezumi	1.64	38.0	23.2	97.5	14.6
		Kairyo-nezumigaeshi	1.73	40.8	23.6	99.3	15.8
Nagano	Spring	Aobanezumi	1.66	40.0	24.1	97.0	14.5
		Ichinose	1.70	40.4	23.8	97.0	14.5
	Late autumn	Aobanezumi	1.77	45.4	25.7	99.0	15.6
		Ichinose	1.82	46.3	25.4	97.5	16.1

for silkworm rearing of general cocoon production for reeling.[20]

Recently, *M. nigra* L., which is known to have the largest number of chromosomes among Phanerogamae, was introduced into Japan from Lebanon, and by crossing it with Japanese cultivars, many kinds of hybrids, such as diploid, triploid, tetraploid, pentaploid, heptaploid, octaploid, dodecaploid, tridecaploid, tetradecaploid, heptadecaploid, docosaploid, mixoploid, etc. were produced. Cytological, and thremmatological investigations, particularly research on practical utilization of hyperploid, are in progress.[24]

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