Induction of Useful Mutations in Mulberry by Gamma Radiation

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Mutation works in mulberry (*Morus latifolia*, *M. alba* and *M. bombycis*) by use of gamma rays have been carried out since 1957, utilizing radiation facilities such as a gamma room established at the Sericultural Experiment Station, Ministry of Agriculture and Forestry and a gamma field attached to the Institute of Radiation Breeding, M.A.F., both of which contain cobalt-60 as sources.

In this decade, in mulberry, a large amount of basic information was gained regarding irradiation techniques, post-irradiation treatments, materials to be used, etc., while several mutants which proved to be of practical value or were expected to be of potentially practical value were obtained.

The present article reports mainly the origins and characteristics of these useful mutants. Some practical problems encountered in the radiation breeding works of mulberry will be also discussed.

Problems on selection of mulberry mutants

High leaf yield, good leaf quality and resistance to diseases are three principal objectives toward which mulberry breeders' efforts are directed. This may apply to the improvement of mulberry trees by means of artificial mutations and polyploids as well as by the conventional hybridization method.

Mutations with high leaf yield seem possible to detect and select with comparative ease, since leaf yield consists of such visible characters as number of branches per plant, length of a branch or total length of branches per plant, leaf size and length of an internode, that is, number of leaves per branch.

Leaf thickness has also an important effect on leaf yield and the selection of mutants with thick leaves is not much difficult, as an experienced mulberry breeder is able to discriminate a difference of about 10 microns in leaf thickness by the sense of touch when he grasps a leaf in his hand.

Compared to mutants showing high yield, the selection of mutants with good leaf quality is extremely difficult and, in fact, such mutants have not yet been obtained.

Although leaf quality is a general term for various kinds of chemical and physical characters concerning food value of mulberry leaves, it is usually determined by means of bioassay for the practical purpose.

For example, when mulberry leaves of unit weight are fed to silkworms and cocoons of heavy shell weight are produced, these are said to be of high quality. For definite evaluation, however, hundreds of silkworms should be raised by the leaves of several mulberry trees of the same kind.

As mulberry mutants are generally selected in a state of a wholly mutated shoot or a wholly mutated plant at most, the selection of mutants with good leaf quality is impossible due to shortage of leaves to be used. Therefore, special bioassay techniques which bring about good results in spite of a small amount of leaves should be urgently developed.

Hairlessness on the surface of leaves may be a good leaf quality, in a broad sense, since silkworms prefer such leaves to hairy ones. A few mutants with leaves of this kind were obtained in a hairy cultivar.

As to disease resistant mutants, special emphasis is placed on the selection of mutants resistant to die-back or 'Dogare' blight which is caused by *Diaporthe nomurai*. Irradiated materials are planted in a severely infected test field and shoots or plants which exhibit no or rare symptoms are selected. Selection of mutants resistant to dwarf disease and bacterial blight is tested at present.

Cutting-back treatment after irradition

In mutation breeding works of vegetatively propagated plants, the induction of wholly mutated shoots or sports is prerequisite to the selection of useful mutants.

Cutting back of shoots which developed from irradiated materials proved to be effective for that purpose and the techniques have been extensively applied with success to some woody plant species[1),4),8),9),10)].

In mulberry, Katagiri[7)] found an interesting relationship between frequencies of induced sports and pruning positions on a shoot.

In mid-April, grafted plants of cultivar Ichinose which were at the bud swelling stage, were irradiated with 7.5 kR of gamma rays at the exposure rate of 5 kR per hour and were planted in a field.

In general, mulberry shoots developed from irradiated plants are divided into three parts according to radiation injuries which appeared in their leaves; the basal part with about 10 malformed leaves, the middle, leafless part with about 10 buds and the upper part with normal leaves.

In mid-July, the newly-developed shoots of irradiated Ichinose were pruned right above the fifth leaf of the upper part, directly above the leafless part and directly below the leafless part and, as the result, frequencies of the lateral shoots showing complete or nonchimeric mutations in leaf and shoot characteristics in the autumn turned out to be 1.3, 4.3 and 3.8 per cent, respectively. This indicates that cutting back of shoots directly above their leafless parts is most effective in the production of a large number of mulberry sports.

Useful mulberry mutants

1) No. 3183

This is a mutant strain of Kairyo-nezumigaeshi, the second prevailing mulberry cultivar in Japan. The mutant was produced by the gamma irradiation of one-year-old grafts with 5 kR at the exposure rate of 5 kR per hour. It is characterized by a change in leaf shape from five-lobed to entire.

In addition, its leaf thickness shows an increase of about 7 per cent and its internodal length shows a decrease of about 20 per cent over the original cultivar[4),5)].

According to a preliminary test performed at the Hino Mulberry Plantation of the Sericultural Experiment Station, Tokyo, its total yield in the second and third year of cultivation increased by about 10 per cent over the original, as can be seen in Table 1.

Adaptability of No. 3183 to climatically different regions and food value of the leaves have been examined on a seven years' program in Iwate, Fukushima and Nagano prefectures where the original Kairyo-nezumigaeshi is predominantly cultivated.

2) IRB 240–1

This is an entire-leaved mutant strain of a five-lobed cultivar Ichinose, the most leading cultivar in Japan. The mutant originated from a mutant shoot appearing in a plant which had been grown 41 meters distance from the source of a gamme field for three years under the chronic irradiation with an average daily exposure of 8.8 R.

Besides the changes in leaf shape, it shows increases in dry matter weight of unit leaf area and in length of new shoots in spring,

Table 1. Leaf yield per 10 ares of mulberry cultiver Kairyo-nezumigaeshi and its mutant strain No. 3183

	2nd year of cultivation	3rd year of cultivation			Total
	Late autumn rearing season	Spring rearing season	Early autumn rearing season	Late autumn rearing season	
Kairyo-nezumigaeshi	994 kg	819 kg	136 kg	546 kg	2495 kg (100)
No. 3183	1184	821	128	576	2709 (109)
Harvested by	Picking of leaves	Picking of new shoots	Plucking of lower one-half of shoots	Pruning of upper one-third of shoots	

compared to the original cultivar[2] and both of the changes are considered to be favorable for spring silkworm rearing.

IRB 240–1 is at present under a field trial at the Hino Mulberry Plantation and, if good results are obtained there, the examination for its adaptability to various regions is to be extensively conducted.

Re-treating this entire-leaved mutant with 10 kR of gamma rays at the exposure rate of 5 kR or 10 kR per day produced mutant shoots which showed a reversion to the original five-lobed leaf[3]. It is not yet investigated what mechanisms are responsible for the reversion and whether the reversion extends to dry matter weight of leaf and length of new shoots or not.

3) No. 3198

This is a mutant strain of cultivar Ichinose which was produced by the gamma irradiation of one-year-old grafts with 5 kR at the exposure rate of 5 kR per hour.

The mutant is considerably different from the original cultivar with respect to several characters; entire, thicker, deeper green, coarser, more elongated and smaller leaves, increase in number of branches and decrease in length of branches. The changes in leaf thickness, leaf color and number of branches may be promising to leaf yield, but other changes may be not.

According to field trials performed in Gumma, Aichi and Miyazaki prefectures, its leaf yield in a spring rearing season was slightly higher than that of the original cultivar, whereas it showed a relatively poor yield in case of autumn rearing season. This is likely to be due to the decreases in leaf size and length of branches.

The mutant was irradiated again with gamma rays of 10 kR at the exposure rate of 5 kR per day in order to obtain mutants which show smooth leaf surface, increased length of branches and increased leaf size without changing the above-mentioned good traits. The detection of such mutants is now in progress.

Among three types of the desirable mutants, it is expected to obtain those with their leaf surfaces being smooth, based on the fact that mutants of such kind arose from mulberry cultivar Sinso No. 2 which had been chronically exposed to gamma rays.

Induction of mutants with increased length of branches may be possible, since it was observed in the above-described Ichinose mutant, IRB 240–1 that its vigorous shoot growth nature which appeared in spring continued till the end of autumn and, in consequence, their branches turned out to be noticeably longer than those of Ichinose.

Contrary to these two types, mutants with increased leaf size is less likely to be induced, since such a mutant has not so far reported in mulberry, as far as the author is aware.

In other vegetatively propagated plants, however, a few information in this respect are available, as in the case of gamma irradiated plants of tea cultivar Yabukita in which doubling, though in an extreme case, of both leaf length and width occurred.

4) Mutants resistant to die-back or 'Dogare' blight

This mulberry disease is endemic to heavy snowfall regions such as Niigata Prefecture and causes serious damage to the spring leaf yield due to stem blight and resultant leaf wilting which arise after the snow melts.

For the purpose of obtaining mutants resistant to the disease, branches of mulberry plants which had been under chronic irradiation for about three years in a gamma field were propagated by grafting means and afterwards the grafts were planted in a severely infected test field in Ojiya, Niigata Prefecture, where the detection and selection of the mutants were performed for subsequent several years.

The number of the mutant plants so far selected are two from a susceptible cultivar Kairyo-nezumigaeshi (Table 2) and six from an intermediately resistant cultivar Kenmochi[6].

Table 2. Number of plants showing various grades of susceptibility to die-back in the vegetative progenies of mulberry cultivar Kairyo-nezumigaeshi trees which were chronically exposed to gamma rays

Total exposure	Susceptibility rating					Dead or dying
	−2.0	−2.5	−3.0	−3.5	−4.0	
ca. 5 kR		1	6	2	1	83
ca.15			8	10	4	96
ca.25			2	1	5	27
ca.35	2		5	5	10	25
ca.45						11
ca.55					1	11

Remarks '62–65: Grown in a gamma field
'66: Propagated by grafting
'67–'71: Grown in an infected field
Susceptibility rating: 1, 2, 3 and 4 are graded by decreases in leaf yield on shoots by 0, 1–30, 31–70 and over 71%, respectively. In the table, average values from '69 to '71 are shown

All the mutant plants presented no or little symptoms in the third to fifth year of cultivation when the disease usually made its apparent appearance. These mutants are to be re-assessed concerning resistance to die-back in their vegetatively propagated progenies.

As in the above, we adopt a relatively time and labor-consuming method for the selection of die-back resistant mutants. Therefore, some special techniques, for example, the selection of the mutant in early growth stages of irradiated materials should be devised.

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