Induction of Male-Sterile Tomato Mutants by Gamma Radiation

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More than 95 per cent of commercial tomato seeds in Japan today are of F_1 hybrid. They have been produced by hand pollination, and the castration is said to occupy approximately 30 per cent of the total labor needed for F_1 seeds production.

It will be, therefore, of much economical value if male-sterile plants can be used instead of the female parent of a cross. It will also be effective in improving seed quality by eliminating contaminated, self-fed pollen.

Male-sterile tomato plants are not of rare occurrence. Rick[2),3)], for instance, has found many ms-lines governed by single recessive genes at various loci.

However, back crossings are necessary to transfer such existing ms-genes into the parental genotype of an economical variety, and much space and labor will be needed. Therefore, a ms-mutation free from hampering the agronomic characters of a parental variety would be of much use for production of F_1 seeds.

Induction methods

For treatments of M_1 generation, seeds or growing plants were irradiated. Two exposures, 15 and 35 kR, used with seed irradiation in a preliminary experiment, permitted about 75 and 60 per cent of irradiated seeds to grow up to flowers, respectively.

In the case of plant irradiation, seedlings were raised in pots and 10 kR of gamma rays were administered at an exposure rate of 1–2 kR per day shortly before blooming of the first inflorescence.

For some time after the exposure, several distorted leaves continued to expand, then new leaves completely stopped being produced both on the main shoot and on the lateral shoots. About a month later, however, new shoots appeared on the axillary region and developed rapidly into flowers.

The process is typical of the phenomenon "radiation-induced internal disbudding" which was disclosed by Yamakawa and Sekiguchi[6)] and Sekiguchi, Yamakawa and Yamaguchi[4)]. They made histological studies of radiation damage and recovery in various meristems of *Antirrhinum majus* and scoring of somatic mutations which appeared on regenerated shoots, and found that destruction of existing meristems by sublethal irradiation and the following reorganization of a new meristem from fewer number of cells are very effective to enlarge mutation sectors.

Yamakawa and Sekiguchi[6)], also applying this technique to tomato, enlarged successfully the mutation sector of sporocyte tissue to enable a screening of recessive mutants in the next generation following the irradiation.

Only one fruit was harvested for the seeds of next generation from each plant or regenerated shoot with seed or plant irradiation respectively, in order to avoid a chance of repeated occurrence of the same mutants.

For treatments of M_2 and later generations, plants of M_2 generation were raised as M_1-fruit progenies. The number of plants within a progeny was from 10 to 20. Fruit setting was checked wih the first one or two trusses, and plants having no fruits were studied for their pollen, i.e., size, shape and stainability with cotton blue.

When a plant had no normal pollen-grain, it was pollinated with viable pollen collected from pollen-fertile plants within the same progeny, part of which should be heterozygous for the locus if the pollen sterility was governed by a recessive gene. This made it possible to get the ms-plants segregated again in the following progenies.

A plant which did not have any normal pollen-grain but set fruits easily when pollinated with normal pollen was further investigated for its progeny, and was finally determined as ms-mutant when the pollen sterility proved hereditary.

Frequency of ms-mutations

About one-third of the progenies of irradiated plants segregated for unfruitfulness, and roughly half of them were presumed to be governed by single recessive genes as the segregation ratio agreed with a 3:1, while another half appeared chromosome aberrations. About 20 per cent of these apparent single-recessive unfruitful plants proved ms-mutants.

The proportion of ms-plants to unfruitful ones agreed with Rick's observation[1]; 3 out of 14 unfruitful plants found in a commercial farm were ms-plants. This suggests that the ratio of ms-genes to total recessive genes causing unfruitfulness is not greatly different regardless of whether they are of natural occurrence or induced by mutations.

The ms-mutants so far obtained were listed in Table 1 together with the methods and frequencies of the induction. From the table, it can clearly be said that plant irradiation is much more effective in producing ms-mutants than seed irradiation, because 13 ms-lines have been obtained from 505 treated lines in terms of the former irradiation while none from 313 lines in terms of the latter.

Table 1. Varieties, irradiation methods and induced male-sterile mutants

Variety	Year	Irradiation method			Number of	
		Object	Exposure (kR)	Rate (kR/hr)	Lines tested[1]	Ms. segregating lines
Shugyoku	1962	plant	10	0.1	5	1
Okitsu No. 3	1966	plant	10	0.1	61	1
	1967	plant	10	0.05 and 0.1	51	1
	1967	seed	15	1.0	136	0
Okitsu No. 5	1967	plant	10	0.1	62	4[2]
Okitsu No. 6	1966	plant	10	0.1	42	1
	1967	plant	10	0.05 and 0.1	22	0
	1967	seed	15	1.0	113	0
	1967	seed	35	1.0	64	0
Delicious	1969	plant	10	0.1	62	1[2]
Unknown	1967	plant	10	0.1	200	4[2]
		Plant irradiation			505	13
		Seed irradiation			313	0

1) M_1-plant progeny for seed irradiation and M_1-shoot progeny for plant irradiation
2) Irradiations were carried out in the Gamma field of the Institute of Radiation Breeding for commercial seed growers who screened the mutants at their experimental farms

Although it is not the subject of the present paper, anthocyanin mutants which serve as a genetic marker have also been studied by Yamakawa and Choi[5], and in this case too, plant irradiation by means of "internal disbudding" was overwhelmingly superior to seed irradiation; only one anthocyanin mutant was obtained from 494 treated lines in comparison to 9 from 729 lines in terms of plant irradiation.

Since the anthocyanin mutants were also determined by single recessive genes, it is now evident that the internal disbudding with sublethal irradiation is very efficient in increasing a gene-mutation rate as well as enlarging mutation sector. In all the six varieties used, induction of ms-mutants was successful indicating that genetic backgrounds have little effect on the induction of ms-mutants.

Genetic behavior

Progeny tests have shown that all the 13 ms-mutants so far obtained by radiation treatment were governed by single recessive gene. Segregation ratios were normal in most cases indicating that no chromosomal aberrations were involved which may reduce gametic and/or zygotic viability of a mutant. In contrast, most of the semi-sterile plants having more or less normal pollen grains have shown markedly reduced segregation ratio of the sterile plants.

Characters of ms-mutants

It has been said that one of the merits of mutation breeding is the possibility of changing only one genetic character of a parent without changing the genetic background. Some of the ms-mutants obtained in the present experiments, however, were more or less different from the original variety.

Even when the appearance is not different, all agronomic traits of a mutant must be scrutinized. For example, the ms-mutant of Okitsu No. 3 apparently looked normal, but proved to have lost the resistance to Fusarium after an inoculation test.

Since most mutations induced by radiation are supposed to be recessive, they are expected to be masked in the F_1 hybrid by corresponding dominant alleles. This was not checked with all ms-mutants, but two selected by commercial seed growers after the service irradiation in the gamma field were carefully studied for their alteration in combining ability.

The seed growers told me that the F_1 hybrids using the ms-mutants as a parent were almost the same as the original F_1 varieties, but slight differences could be seen in such characters as fruit size, plant habit and time of maturity.

The seed growers could not tell which was better, but they still hesitated to release the ms-hybrid seeds under the same brand as the original variety.

Although it is not known whether these changes other than pollen sterility were due to the concurrent multiple mutations or to the pleiotropic effects of the ms-genes, it is clear that we can hardly expect a ms-mutant with other characters kept intact.

From these results, it will be recommended that a promising breeding line rather than a ready-made variety is used as the material for ms-induction and the combining ability of the ms-mutant will be further studied instead of that of the original line.

Summarized procedures

1) Prepare seedlings of a variety or line which is promising as the female parent of an F_1 hybrid.

2) Irradiate them with ca. 10 kR of gamma rays at an exposure rate of 1-2 kR/day.

3) Regenerated shoot appears about a month after the irradiation. Harvest one fruit from each shoot for the seeds.

4) Raise the plants of the next generation as M_1-fruit progenies. Detect unfruitful plants and check their pollen fertility. Pollinate pollen-sterile plants with normal pollen sampled from fertile plants in the same progeny, and

pick up normal fruiting plants for their seeds.

5) Raise the next generation as male parental progenies and find the ms-segregating progenies. Study carefully the characters of the ms-plants other than pollen sterility, and eliminate any defective plants.

6) Investigate the combining ability of the ms-line.

References

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