Interspecific Hybridizations of Tomatoes by the Use of Gamma Radiations and Breeding of Disease Resistant Tomato Lines

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Lycopersicon peruvianum is one of the most promising species as a gene source for disease-resistant tomato breeding. It has been reported by several researchers to have resistance against tomato mosaic virus, curly top, spotted wilt, leaf-mold, Fusarium wilt, Septoria and Alternia leaf blight, root-knot nematode and potato-cyst-eelworm.

The hybridization between *L. peruvianum* and *L. esculentum* was attempted as early as 1915. When *L. esculentum* is used as the pistillate parent, fruits set easily but embryos-abort at such early stages that the mature fruit contains only small aborted ovules. This interspecific sterility was partially overcome by the application of the embyo-culture technique by Smith (1944[7]) and others. Embryos of sufficient size for culturing are remarkably rare in this material, success being achieved only after a search of hundreds of fruit.

In contrast, when *L. esculentum* is used as the pollen parent, the pollen tubes stop elongating in the style of *L. peruvianum* resulting in no fertilization. This inhibition of pollen-tube growth is not observed in a cross when *L. esculentum* is used as a pistillate parent. Hogenboom (1972[1]) broke this unilateral incompatibility by inbreeding and selection in *L. peruvianum*. The parental genotypes of *L. peruvianum*, however, are rather limited by this method.

There have been several reports in breaking both cross sterility and cross incompatibility by means of ionizing radiations (see Yamakawa 1971[10]). In most of studies, however, short-term irradiation was used on mature seed or pollen and during specific periods of gametogenesis. The effects of long-term chronic irradiation remained to be clarified.

The experiments concerned with the use of radiation in interspecific hybridization were conducted at the Institute of Radiation Breeding (IRB) and those concerned with disease resistance were conducted at both the IRB and the Vegetable and Ornamental Crops Research Station.

Interspecific hybridization

1) *Effects of chronic gamma radiation on the cross fertility between L. esculentum and L. peruvianum*

A series of experiments were started in 1966 and continued in 1967, 1969 and 1970 (Yamakawa, 1971[10]). In all of the experiments, *L. esculentum* was used as the pistillate parent. In 1966 and 1967, gamma-induced malesterile mutants of the *L. esculentum*, variety Shugyoku (Yamakawa, 1973[9]) were used in order to eliminate the need for emasculation and to prevent self pollination. Plants of Okitsu Nos. 9, 10 and 11 were used in 1969 and 1970 because of their combined resistance to leaf mold and Fusarium wilt (Sugahara and others, 1971[8]). As the staminate parent, an accession of *L. peruvianum*, P.I. 126944, was used throughout the project. Gamma irradiation was applied only to the staminate parent and not to the pistillate one.

In 1966, pollen from freshly dehisced flowers which had been irradiated with 5 kR of gamma rays for 20 hours was applied to the *L. esculentum* stigma. The fruit set was slightly affected, while the number of enlarged seeds (larger than 1 mm in length) per fruit was markedly decreased by irradiation. No adult hybrids were obtained from a total of 300 pollinations.

In 1967, 1969 and 1970, plants of *L. peruvianum* were irradiated chronically before samplings of the pollen. In 1967, pollen samples were taken 4 days after the beginning of irradiation and lasted for more than one month. Pollen abortion was mainly induced by irradiation in the period from the differentiation of pollen mother cells to the end of meiosis, 18 days to 8 days before anthesis respectively; earlier or later irradiation had almost no influence. When this 'pollen sensitive' period was fully irradiated, the exposure rate reducing normal pollen grains by 50% was approximately 170 R/day.

Irradiation for more than 11 days (including at least meiosis and most cases premeiosis) markedly decreased the number of enlarged seeds per fruit, while that of 4–8 days (irradiated only after meiosis) did not. This result was similar to that for pollen abortion mentioned above. The germination (shoot or root emergence) percentage of enlarged seeds was little affected by the treatments. The shoot was often retarded or deformed. Growth of germinated seeds, however, were better at treatments of longer exposures at lower rates, namely 60–130 R/day for more than 11 days. At this treatment 13 adult hybrids from 72 germinations were obtained from 733 fruits, in comparison to 2 from 195 germinations in 1065 fruits of unirradiated controls. No adult hybrids were obtained from the short-term exposure or higher exposure rates.

In 1969 *L. peruvianum* plants were irradiated at 70 R/day for more than 20 days because the experimental results in 1967 suggested that longer exposures at lower rates will be favorable in hybrid survival. The cross fertility between *L. esculentum* and *L. peruvianum* varied depending on which of the three varieties of *L. esculentum* and which of the three vegetative clones of *L. peruvianum* were used. The effects of irradiation were not clear this year. This was considered mainly due to the high ratio of hybrid survival in the untreated control, namely 78 adult hybrids were obtained from 896 fruits in contrast to 2 from 1065 in 1967. The cause of this difference was not clear. That the effect of radiation will be deleterious in a cross with farely high fertility can be well understood since deleterious effects of radiation still exist even at a lower exposure and the beneficial effect of radiation would be negligible in a highly fertile cross.

In 1970 experiments were conducted using F_1 seedlings between two vegetable clones of *L. peruvianum* and Okitsu No. 9 as the staminate and pistillate parents respectively. Exposure rate and duration were the same as those in 1969. This year radiation treatments consistently surpassed the unirradiated control both in germinations per 10,000 ovules (49.7 for irradiated and 15.6 for unirradiated) and adult hybrids per 100 germinations (28.5 and 18.7 respectively). In this experiment germinations and adult hybrids were carefully grouped according to the individual fruit from which they were derived. The number of germination or viable adult hybrids formed from a single fruit was compared between fruits pollinated with pollen after long (32–51 days) and short (20–31 days) term irradiation and also with unirradiated pollen. Long irradiated pollen produced more germinations or viable adult hybrids from a single fruit than short irradiated or unirradiated pollen. The figures for viable adult hybrids were 7, 3 and 3 respectively. This indicates that radiation produced a compatible germ-line cell at an early stage and it multiplied by cell division, resulting in compatible pollen grains. This suggests that the radiation effect is genetic rather than physiological. To expect a compatible mutation in the interspecific cross seems reasonable because significant variations of interspecific compatibility were found between varieties and clones in both *L. esculentum* and *L. peruvianum*.

As the conclusion of four years experiments it can be said that radiation had both deleterious and beneficial effects. Irradiation after meiosis including mature pollen irradiation had only deleterious effects. Long term chronic irradiation had a beneficial effect when the exposure rate was suitable, but the effect was deleterious when the rate was too high. The effect of radiation was presumed to be genetic rather than physiolosical.

2) *Effect of chronic gamma radiation on the unilateral incompatibility between L. esculentum and L. peruvianum*

In this experiment *L. peruvianum* (P.I. 126944) was used as the pistillate parent. As the staminate parent, F_1 hybrids between *L. esculentum* and *L. peruvianum* obtained in 1967 (see 1.) were used instead of *L. esculentum* itself in order to reduce the presumed cross sterility after fertilization. Incompatibility between the hybrids pollen and *L. peruvianum* style has been reported (McGuire & Rick, 1954[6]). Both staminate and pistillate parents were irradiated chronically in a gamma field and gamma greenhouse respectively. Exposure rate ranged from 50 to 150 R/day and the duration extended more than twenty days before pollination. Acute irradiation of the detached style was also administered in the gamma room at a rate of 4 kR/hr providing total exposures of 10, 20, 30, 60 and 100 kR. Pollen tubes in the style were observed by means of fluorescence (Kho and Baër, 1968[2]).

Chronic irradiation of *L. peruvianum* styles and/or *L. esculentum* pollen before anthesis markedly stimulated the elongation of the pollen tubes, and when both pollen and style were irradiated the effects seemed additive. However, the elongation was not enough to make fertilization possible. Acute irradiation of mature styles also stimulated the growth of pollen tubes when the exposures were suitable (10, 20 and 30 kR) but the effect was rather small compared to chronic irradiation. No hybrid was obtained in this direction of the cross.

Screening of progenies of the interspecific hybrids for diseases resistance

The appearance of the hybrids plant habit and fruit was closer to *L. peruvianum*. Compatibility tests and back crossing to *L. esculentum* were applied to fifteen hybrid plants obtained in 1967 (see A-1). The fifteen plants were all self incompatible and divided into four groups, cross incompatible within group and cross compatible between groups. Three plants chosen at random were back crossed to *L. esculentum*. Unilateral incompatibility again occured in this cross; *L. esculentum* (a male sterile line of Shugyoku) had to be used as the pistillate parent. In this case no effect of radiation was observed. From 3301 fruits 84 back crossed hybrids were obtained. In this generation male sterile plants segregated because the male sterile line which had been used as a parent of original interspecific cross was used again as a recurrent parent in the back cross. Self incompatible plants also segregated in the back cross hybrids in the ratio of 53 self compatible to 20 self incompatible. When pollen of *L. esculentum* was applied to the stigma of these plants, all 20 self incompatible plants failed to set fruit, whereas self compatible plants set fruit easily. Thus, self incompatibility and unilateral incompatibility completely agreed. It has been known that the self-incompatibility in *L. peruvianum* follows the Nicotiana or gametophytic system controlled by multiple alleles (S-alleles) at one locus. McGuire and Rick (1954[6]) explained compatibility relationships in F_1 and F_2 plants by assuming that a plant with Si/Se (self incompatible gene from *L. peruvianum*/self compatible gene from *L. esculentum*) inhibits growth of pollen tubes not only with the same Si but also with the Se allele. Martin (1961[5]) demonstrated in back cross generations of interspecific hybrids between *L. esculentum* and *L. chilense* that in addition to an S allele, dominant gene(s) from *L. chilense* are necessary for the expression of self-incompatibility.

Table 1. Segregation of disease resistance in BCF_2 generation (selfed progenies of back cross hybrids)

Plant No.		Diseases tested				
F_1	BCF_1	TMV	Leaf mold	Fusarium wilt (J-1)	Fusarium wilt (J-3)	Bacterial canker
1	1					
1	2			R		
1	5			R	R	
1	23	*R*	R	R	—	—
1	27				R	R
9	9			R		
9	11		*R*	R	R	*R*
9	12			R	R	*R*
9	13		*R*	R	—	
9	15	*R*	R		—	—
9	17	*R*	R	R	R	R
9	18			R	—	
9	22	*R*	R		R	
9	25		R	R		
9	26			R	R	R
9	29		*R*		—	
9	30			R	—	—
14	4	*R*		R	R	*R*
14	6		*R*	R		*R*
14	10				—	*R*
14	14		R	R		*R*
14	16	*R*	*R*	*R*	—	
14	19		*R*		R	*R*
14	20		R	R	R	
14	21					*R*
14	24		*R*		—	—

R—Segregated of resistant plants; resistance was not confirmed by progeny tests

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Incompatibility relationships found in F_1 and back cross generations of the present experiments conform to these hypotheses.

Selfed progenies were raised from 33 back cross hybrids which were male fertile and self-compatible, and tested for their resistance to various diseases. The results are presented in Table 1. Because the resistant back cross hybrids were heterozygous for the resistance gene(s), the selfed progenies segregated for resistance.

All tests were by artificial inoculation of seedlings. Experiments were conducted in 1969 for tobacco mosaic virus(TMV), Fusarium wilt (race J-1) and leaf mold by Yamakawa and Kuniyasu, in 1971 for bacterial canker by Yamakawa and Kuniyasu, and in 1974 for Fusarium wilt (race J-3) by Yamakawa and others[11]. For the latter two, several progenies were not available for the tests because of lack of seed.

Of five diseases (Fusarium wilts listed here are caused by different races, J-1 and J-3, of *Fusarium oxysporum* f. *lycopersici*, but they can be treated as different diseases rather than as physiological races of the same disease for the reason mentioned later), TMV, leaf mold and Fusarium wilt (J-3) were chosen for resistance-breeding in this project. There are many resistant cultivars available against Fusarium wilt (J-1). The breeding of resistant varieties to bacterial canker was accomplished by using the another wild species, *L. hirsutum* var. *glabratum* (Kuriyama and Kuniyasu, 1974[4]).

1) TMV

From four BC_1 plant-progenies (Plant Nos. 4, 16, 17 and 22) back cross breeding was conducted. In 1972 three resistant lines were released as the resistant breeding stocks from the Institute of Radiation Breeding; IRB 301-30 and -31 were from BC_2F_6 generation and IRB 301-32 was from BC_2F_4 generation. Inoculation tests of these lines and F_2 plants from crosses between the lines and GCR lines (Glasshouse Crops Research Institute, England) possessing known alleles of TMV-resistant genes were carried out using the four Pelham's and the five Alexander's strains of TMV. The results showed that the lines possessed Tm-2 gene (Yamakawa & Nagata, 1975[12]). Since this Tm-2 gene has no linkage with the undesirable gene nv, the lines are being used as the breeding stocks by breeders of regional experiment stations and seed companies.

2) Leaf mold

From three BC_1 plant-progenies (Plant Nos. 6, 24 and 29) back cross breeding is now being conducted. Although the lines have not yet been released, they are resistant not only to Japanese races (three pathogenic races have been identified using Japanese differential varieties, Kishi & Abiko 1975[3]) but also to races 1.2.3, 4, 1.2.4 and 2.3.4 as tested at the Institute for Horticultural Plant Breeding the Netherlands (Ir. I. W. Boukema, personal communication, 1973).

3) Fusarium wilt (race J-3)

This disease has increased as tomato growing under glass or plastic has been extended to the cooler season. Although the causal agent was identified by Yamamoto and others (1974[13]) as *F. oxysporum* f. *lycopersici*, the symptoms are quite different from those of the two known races in Japan (race J-1 and J-2). J-3 causes root- and crown-rot but not much browning of vascular bundles of the stem while J-1 or J-2 cause dark browing of the vascular bundles but not the root-rot. The optimum temperatures for disease development are also different. J-3 is more severe in cooler seasons; J-1 and J-2 in warmer seasons. Komada found resistant plants in *L. peruvianum* P.I. 126944, the same one used in the present interspecific hybridization. Yamakawa and others (1975[11]) reported the segregation of resistance in the BCF_2 generation as can be seen in Table 1. IRB 301-30 and -31, which had been released as the breeding stock for TMV resistance, were also found to possess the resistance to this disease. They also have been shown to be resistant to the Fusarium root-rot disease which is increasing in Ohio, U.S.A. and Canadian tomato gasshouses (Dr. James D. Farley, personal communication). Back cross breeding is now rapidly proceeding in order to meet urgent demands for the cultivars resistant to this disease.

The author is very grateful to Dr. James D. Farley of Ohio State University for critical reading and correction of the English text.

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