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Pollen irradiation in tomato: minor effects on enzymic gene transfer

D. Zamir

Department of Vegetable and Field Crops, Hebrew University of Jerusalem, Faculty of Agriculture, P.O. Box 12, Rehovot, Israel

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Summary. Pollen irradiation was tested as a possible means of expediting gene transfer in tomato breeding. The experiment was also designed to examine the possibility that irradiation effects which have been previously reported for *Nicotiana* were due to gametophytic selection against mutated male and female gametes.

Pollen from the wild tomato species *Solanum pennellii* was irradiated with γ-rays and used in crosses with *Lycopersicon esculentum*. Pollen from five M1 hybrid plants and five control hybrids was used in backcrosses to an *esculentum* variety and the same plant also functioned as female parents in crosses with normal *S. pennellii* pollen. Seven enzymic gene markers which differ between the species were assayed in the backcross populations in the two directions. Allele frequencies did not differ between the populations that were derived from the M1 or F1 hybrids except for one marker, *Pgm-2*. Elimination of male and female gametes containing the irradiated parent allele of *Pgm-2* was observed in a single population. This gene is located on chromosome 4 close to the gamete eliminator (*Ge*) locus. A review of some of the main characteristics of *Ge* raises the possibility of the existence of a controlling element activity.

Key words: *Lycopersicon* – Pollen irradiation – Gametophytic selection – Gamete elimination – Isozymes

Introduction

Pollen irradiation in *Nicotiana* crosses can facilitate more rapid gene transfer than is achieved by conventional plant breeding methods. Jinks et al. (1981) irradiated pollen from one *Nicotiana* variety using a γ source and used it to pollinate a second variety. The M1 generation, particularly the plants produced from the 20 Krad treatment, was variable in its phenotype (Caligari et al. 1981) and its progeny showed a great resemblance to the female parent. Some of the M2 progenies produced lines which were nearly identical to the female parent with the exception of single characters transferred from the irradiated parent. Two of the characteristics monitored in these populations were controlled by major interacting genes and three characteristics were of a quantitative nature. Using much higher irradiation doses (100 Krad), Pandey (1975) reported similar results and termed the phenomenon "egg transformation". The mechanism involved in the egg transformation process is not clear. It has been suggested that part of the paternal DNA transforms the egg cells which double their chromosome number and give rise to parthenogenetic progeny with the transferred traits (Pandey 1980). The implications of these results on plant improvement are intriguing: using this scheme it may be possible to dramatically expedite breeding programs.

This study was designed to examine an alternate mechanism for the irradiation induced gene transfer (Fig. 1). Assume we are investigating an organism with a diploid number $2n=4$. Pollen grains from one parent are irradiated from a γ source (20 Krad) causing chromosomal or gene mutations only on one chromosome. This treatment is known not to impair fertilization ability of pollen (Cresti et al. 1977). The M1 plants are more variable in their phenotype than the F1 due to the different mutations induced by the γ-rays. During meiosis the chromosomes assort independently producing four types of microspores two of which

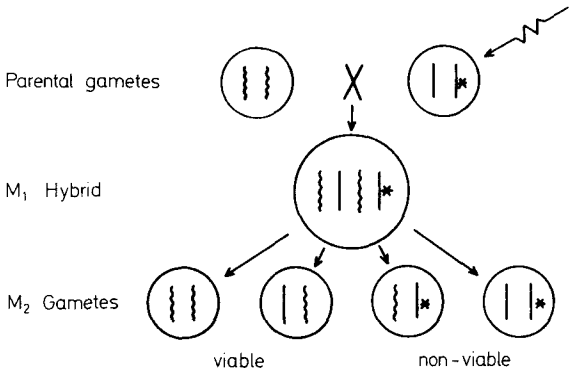

Fig. 1. A proposed mechanism for the irradiation induced gene transfer via gametophytic selection

contain the mutated chromosome (intrachromosomal recombination is not considered in this model). The hypothesis suggests that some of the mutated haploid cells do not complete microsporogenesis; only half the gametes, those containing the normal chromosomes, will produce progeny in the next generation. This suggestion is based on experiments reviewed recently (Zamir 1983) which demonstrate that the haploid pollen genome determines in part its fertilization ability by directing the synthesis of some of the cell components during microsporogenesis. Another possibility is that mutated haploid cells complete microsporogenesis but are less competitive during pollen germination, tube growth through the stylar tissue and fertilization than normal cells (Zamir et al. 1982). Both explanations are supported by results obtained by Rick and Khush (1969) on the transmission of irradiation induced deficiencies and would account for reduced frequencies of the irradiated parent genes in the progeny of the M1. With minor exceptions none of the deficiencies were transmitted either by male or female gametes of the M1 generation. This indicates that the same reasoning developed for the male gametes may hold true for the female gametes.

The tomato provides a good model system for accurately examining the nature of the irradiation effect on allele frequencies in segregating generations. A number of codominant isozyme markers have been located on the genetic map and inbred genotypes are available that differ for well defined alleles (Tanksley and Rick 1980). By irradiating pollen of one genotype and using it in crosses with another it was possible to determine the frequencies of parental alleles in advanced segregating generations. In this study seven independent markers were monitored in segregating generations produced by the M1 hybrids. To determine the radiation effect on male and female gametes the hybrids functioned both as male and female parents in backcrosses.

Materials and methods

Pollen from a single plant of the wild tomato species *Solanum pennellii* (LA 716) was irradiated with γ-rays at doses of 5, 10, 15, 20, 25, 30 and 35 Krad. The treated pollen and the non-irradiated control was applied to stigmas (on the same morning it was harvested) of a male sterile variety (ms 10) of *Lycopersicon esculentum*. The rate of seed set and the number of seed per fruit in each of the crosses were determined. Pollen viability of hybrids was estimated on three different dates using acetocarmine staining and counting 300 grains each time.

Pollen samples from five hybrids produced from the 20 Krad irradiation treatment and pollen from five control hybrids was used to pollinate the male sterile variety. These plants also functioned as female parents in crosses with normal *S. pennellii* pollen. The directions of the crosses were dictated by the unilateral incompatibility of the species (Rick 1979). From every hybrid plant backcross seed produced by the two direction crosses was planted in vermiculite filled speedlings. The number of progeny analysed from each hybrid appears in Table 1. Plant number 4 from the 20 Krad treatment did not survive long enough to produce mature fruit. Backcross plants with 4–5 true leaves were assayed using starch gel electrophoresis to determine their genotype with respect to the following enzyme loci: *Prx-1* maps to chromosome 1, *Prx-2* chrom. 2, *Pgm-2* chrom. 4, *Aps-1* chrom. 6, *Got-2* chrom. 7, *Aps-2* chrom 8 and *Pgi-1* which is unmapped but segregates independently of the other loci. Some seed of plant number 5 from the irradiation treatment was assayed for *Adh-1* and *Pgm-2* which are approximately 4 map units apart. Methods for starch gel electrophoresis, enzyme extraction, activity staining and map positions can be found in Tanksley and Rick (1980).

Results and discussion

Pollen irradiation had little effect on the proportion of flowers that set fruit; even at the highest dose fruit set was observed though no seed was present. The number of seed per fruit decreased sharply with the increased dosage. The following are the mean number of seed from about 10 fruit per treatment: control 129 ± 31; 5 Krad 81 ± 37; 10 Krad 28 ± 17; 15 Krad 9 ± 6; 20 Krad 1 ± 2 with no seed at the higher doses. These observations are consistent with results of Monaco (1967) and indicate the effectiveness of the mutagen treatment.

The isozymic genotype of 112 M1 plants from the various treatments was determined for all the seven loci. Except for one plant from the 15 Krad treatment, which was hemizygous for the *esculentum* allele of *Pgm-2*, all the rest were normal heterozygotes. The two species also differ for a number of metric traits which are inherited in a quantitative fashion (Tanksley et al. 1982). No difference was detected between the treatments for the mean values and variances of leaf ratio, stigma exertion and fruit weight. However, the proportion of aborted pollen grains produced by the hybrids that were used in the crosses was higher for the M1 plants ($\bar{x} = 57\%$) than for the controls ($\bar{x} = 11\%$)

Table 1. Genotype of backcross populations produced by pollinating *L. esculentum* stigmas with irradiated (20 Krad) and control (0) hybrid pollen. Numbers represent heterozygotes/homozygotes for the *esculentum* alleles at each locus. Percent pollen abortion for each hybrid is indicated

Plant no.	Pollen abortion	*Prx-1*	*Prx-2*	*Pgm-2*	*Aps-1*	*Got-2*	*Aps-2*	*Pgi-1*	
20-1	60.3	41/34	30/44	49/55	28/46	44/60	36/38	65/39	
20-2	39.1	40/34	33/40	54/45	41/32	40/59	42/57	59/40	
20-3	74.2	45/56	55/50	53/43	50/52	48/48	41/56	56/41	
20-4	56.0	–	–	52/49	44/58	46/56	44/58	61/61	
20-5	54.8	54/41	44/53	0/97	38/59	47/42	41/54	50/47	
Total	$\bar{x}=56.9$	180/165	162/187	208/289	201/247	225/265	204/263	291/228	$\frac{1471}{1644}$
Heterogeneity χ^2		NS	NS	88.4*	NS	NS	NS	NS	
0-1	13.4	44/56	48/52	50/50	45/53	46/54	39/61	60/40	
0-2	15.6	57/43	48/52	45/55	44/56	49/51	27/73	58/42	
0-3	8.5	53/43	46/46	48/45	42/53	41/52	33/67	55/38	
0-4	9.2	53/44	39/57	47/53	43/57	48/49	24/76	60/40	
0-5	5.7	38/31	32/37	45/59	33/34	63/41	36/69	57/47	
Total	$\bar{x}=10.5$	245/217	213/244	235/262	207/253	247/247	159/346	290/207	$\frac{1596}{1776}$
Heterogeneity χ^2		NS	NS	NS	NS	NS	NS	NS	

* Significant deviation at the 0.1% level

(Table 1). This irradiation effect is consistent with the proposed explanation for the gene transfer phenomenon.

The genotype of the plants in the backcross populations produced by pollinating *esculentum* stigmas with hybrid pollen is presented in Table 1. For each of the five hybrids from the 20 Krad treatment and the five controls, the number of backcross plants heterozygous for the *pennellii* alleles relative to the number of plants homozygous for the *esculentum* alleles at each of the loci is shown. Had there been substantial elimination of the irradiated parent genome we would expect a reduced transmission of the *pennellii* alleles by the affected hybrid pollen relative to the non-irradiated hybrids or the non-affected M1 hybrids. This would be expressed by fewer heterozygotes relative to homozygotes as shown in Fig. 2.

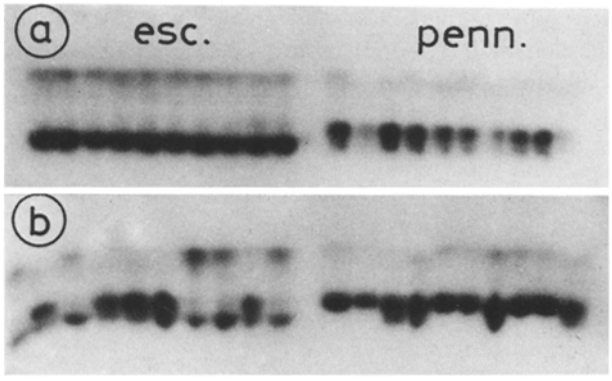

Fig. 2a, b. Starch gels showing backcross segregation of *Pgm-2*. **a** M1 hybrid number 5 crossed to *L. esculentum* (esc.) and *S. pennellii* (penn.), **b** control hybrid crossed to same species

The only case in which the irradiation effect was detected in the expected direction was for M1 hybrid number 5, which did not transmit the irradiated parent allele of *Pgm-2* to its progeny. This result is also reflected by the high heterogeneity χ^2 value obtained for this locus in the irradiated population. The other major effect was detected at the *Aps-2* locus. No significant difference was observed within either the irradiated populations or the controls. However, 43.7% of the plants were heterozygous at this locus in the pooled irradiated population compared to 31.5% in the control ($x=15.4$ 1 df); an unexpected type of response. The pooled values for all the loci in the two groups show a frequency of 47.2% of the irradiated parent alleles in the M1 progenies compared to 47.3% in the F1 progenies. The overall picture demonstrates that pollen irradiation has only a minor influence on gene transfer at the tested loci.

This conclusion is also valid for the second backcross in which the hybrids were female parents in crosses with *pennellii* pollen (Table 2). A comparison of the number of plants homozygous for the *pennellii* alleles relative to the heterozygotes for each of the tested hybrid progenies indicates the effect of the treatment on the female gametes (Fig. 2). The only case in which differences were observed was for *Pgm-2*. The irradiat-

Table 2. Genotype of backcross populations produced by pollinating irradiated and control hybrids with *S. pennellii* pollen. Numbers represent homozygotes for *pennellii* alleles/heterozygotes at each locus

Plant no.	*Prx-1*	*Prx-2*	*Pgm-2*	*Aps-1*	*Got-2*	*Aps-2*	*Pgi-1*	
20 – 1	54/58	38/37	41/56	31/43	44/43	39/35	41/66	
20 – 2	20/24	23/25	20/18	17/31	22/22	22/26	26/20	
20 – 3	38/27	–	27/29	35/28	29/37	40/26	35/31	
20 – 5	33/35	20/42	0/99	43/32	47/52	51/46	43/55	
Total	145/144	81/104	88/202	126/134	142/154	152/133	145/172	$\frac{879}{1043}$
Heterogeneity χ^2	NS	NS	67.0**	8.2*	NS	NS	NS	
0 – 1	29/31	25/35	50/21	25/33	35/36	40/31	22/49	
0 – 2	44/34	32/46	54/44	34/42	34/64	56/42	48/48	
0 – 3	33/18	18/33	36/28	24/27	36/28	30/34	26/38	
0 – 4	25/19	20/24	29/22	24/20	23/28	26/25	26/25	
0 – 5	30/32	28/32	29/21	31/33	23/30	30/32	22/37	
Total	161/134	123/170	198/136	138/155	151/186	182/164	144/197	$\frac{1097}{1142}$
Heterogeneity χ^2	NS	NS	NS	NS	NS	NS	NS	

* Significant deviation at the 5% level
** Significant deviation at the 0.1% level

ed parent allele of *Pgm-2* was not transmitted by the egg cells of the M1 hybrids. The pooled value for all the loci in the two groups shows a frequency of 45.7% of the irradiated *pennellii* parent alleles compared to 49.0% of the *pennellii* alleles in the control populations. This difference is largely due to the *Pgm-2* effect.

Elimination of male and female gametes carrying certain marker alleles on chromosome 4 of the tomato is not a new observation. Rick (1966) identified the gamete eliminator locus (*Ge*) which in certain allelic combinations (Ge^e/Ge^p) is responsible for extreme departures from Mendelian ratios. This gene is located at map position 27 in the proximal heterochromatin, very close to the centromere (Rick 1971) while *Pgm-2* maps to position 30. The isozyme marker *Adh-1* which is expressed in dry seed maps to position 34. When seed of the backcross population derived from plant number 5 was assayed electrophoretically for *Adh-1* and *Pgm-2* 7 recombinants from 179 were counted, which confirms the map distance between *Pgm-2* and *Adh-1* and shows that the induced mutation occurred at a locus closer to *Pgm-2*. No recombination was observed between *Pgm-2* and the mutated allele of *Ge* when 312 plants and seed from backcrosses in the two directions were assayed. Although this relationship deviates significantly from the expected recombination frequency between *Pgm-2* and *Ge* ($\chi^2 = 8.6$ 1 df) it seems likely that the mutation was induced at the *Ge* locus.

It is interesting to note a few of the peculiarities of the *Ge* locus. The only case in which hemizygosity in the M1 generation was observed was at the *Pgm-2* locus. In a different study (Zamir and Vallejos 1983) pollen collected from four cloned cuttings of the interspecific hybrid of *L. esculentum* and *L. hirsutum* was used in backcrosses to an *esculentum* variety. In the four BC1 populations significant differences were detected in the frequencies of the wild parent *Pgm-2* allele, a result that indicates variable expressivity of *Ge*. Several observations support the possible activity of a transposable element controlling gamete elimination in tomato: the apparent high mutation rates in proximity to the *Ge* locus, the variable expressivity, penetrance (Rick 1971) and the linkage changes.

Results presented in this study demonstrate that no irradiation induced enzymic gene transfer occurred in tomato except at the linked loci *Pgm-2* and *Adh-1*. Only the effects at these two loci are consistent with the suggestion that the cause of the egg transformation phenomenon is selection at the gametophytic level (Fig. 1). Since the treatment effects in this experiment were only minor it is not possible to resolve the question of mechanism as it relates to the successful results in *Nicotiana*.

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