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Pollen irradiation and gene transfer in *Capsicum*

S. Daskalov
FAO/IAEA Agricultural Biotechnology Laboratory, A-2444 Seibersdorf, Austria

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Summary. The aim of the experiment was to study the possibility of facilitating the gene transfer and reducing the number of required backcrosses through pollen irradiation and subsequent selection of F_1M_1 plants containing a very high proportion of sterile pollen as male parent for backcrossing. Anthers of a donor line, C-3-1, were irradiated with 1,500 rad γ-rays and the pollen used for pollination of a recipient genotype W-8 which posseses a number of recessive marker genes. Five F_1M_1 plants containing more than 80% sterile pollen grains and one semi-sterile plant were selected and used for backcross to W-8. The segregation pattern of four characters expressed in the first backcross generation [W-8×(W-8×C-3-1↯)] was assessed and compared with the non-irradiated control. A changed segregation pattern was observed (in some cases even non-transfer of a paternal allele) as well as a shift towards more plants possessing the investigated maternal alleles. A scheme for backcross procedure in combination with pollen irradiation is discussed.

Key words: Pollen irradiation – Gene transfer – Backcross – *Capsicum*

Introduction

The transfer of useful genes from a cultivar or related species to the recipient genotype with conventional breeding techniques can be achieved by a cumbersome backcrossing procedure that requires many generations. Moreover, the gene transfer is quite often hampered by the lack of chromosome pairing or by gene linkages.

Molecular genetics and the development of gene engineering techniques offer possibilities for single step transfer of genes but the methods applicable to the higher plants are still at an experimental stage.

Pandey (1975, 1981) reported that in *Nicotiana* it is possible to transfer single genes by irradiation of pollen of the donor plant with very high doses (50–10,000 rad) of ionizing radiations. Virk et al. (1977); Jinks et al. (1981) and Calligari et al. (1981), applying the same techniques in *Nicotiana*, reported positive results as well. The major limitation of this technique, as pointed out by Pandey (1981), is the very low number of seeds produced and their poor germinability.

Davis (1981) stressed the importance of this new approach and urged more investigations.

Powell et al. (1983) reported similar effects in barley using pollen irradiation with much lower doses (500–2,000 rad) of γ-rays. Zamir (1983), on the contrary, observed only minor effects on enzymic gene transfer after pollen irradiation in tomato.

Studies in *Capsicum* on pollen irradiations with gamma ray doses from 1,500 to 125,000 rad were carried out at the Seibersdorf Laboratory. Doses from 3,000 to 125,000 rad yielded only a few shrivelled seeds which were not able to germinate.

The results suggested a new idea for investigating another approach for facilitation of gene transfer. It consists of using pollen irradiated with high doses which still allow fertilization and formation of viable seeds and subsequent selection of F_1M_1 plants possessing a very high proportion of sterile pollen (indicating heterozygote chromosomal aberrations) as male parent for backcrossing.

Material and methods

The experimental work was carried out with two lines of *Capsicum* at the FAO/IAEA Agricultural Biotechnology Laboratory in Seibersdorf near Vienna during the period 1981–83.

The line Wibault ms-8 (W-8) (Daskalov 1977) possessing recessive alleles for male sterility (ms-8), lack of anthocyanine (al), sulfury white immature fruits (sw) and sweet taste of the fruits (c) was used as female parent for crosses with the line C-3-1 possessing the corresponding dominant genes. The use of a male sterile female parent helped avoid selfing and contamination of the material and facilitated the crossings.

The anthers of the ms-8 plants are reduced in size, shrunken and do not contain any pollen grains. The male sterility determined by this gene is very easily identifiable and cannot be confused with sterility caused by chromosome aberrations. In the latter case, the anthers contain thousands of sterile pollen grains.

Anthers from the line C-3-1 were irradiated shortly before dehiscence (pollen at binucleate stage) with 1,500 rad γ-rays at a dose rate of 1,400 rad/min, and the pollen was immediately used for pollination of the line W-8. The female and the male parent were grown in different compartments of the greenhouse to avoid the possibility of cross-pollination.

The pollen viability of the F_1M_1 plants was assessed at anthesis with the help of acetocarmine technique.

F_1M_1 plants having more than 80% sterile pollen were selected and used as male parent for backcrossing to the line W-8. The segregation pattern expressed in the BC-1 generation [(W-8 × (W-8 × C-3-1♮)] was investigated and compared with the non-irradiated control.

The pollen viability of 121 BC-1 plants which were randomly selected was also assessed.

Results and discussion

The seed set after pollination with the pollen irradiated with 1,500 rad γ-rays was approximately 5% compared to the control.

One hundred and five F_1M_1 plants have been checked with respect to pollen sterility: 46 plants were normal fertile, 37 plants were semi-sterile (50% sterile pollen) and 22 plants had more than 80% sterile pollen.

For backcrosses with the female line, W-8, six plants were selected. Five of these plants (No. 65, 72, 91, 95 and 98) contained more than 80% sterile pollen and had anthocyanin spots on the nods and fruit and green immature fruit with pungent flavour. Some anthers of plant No. 95 were irradiated again with 1,500 rad γ-rays. The sixth plant (No. 48) was semi-sterile, lacked anthocyanin (most probably a deletion of the gene al^+) and had pungent green immature fruit.

The selection of F_1M_1 plants with a high percentage of pollen sterility was aimed at screening plants heterozygous for chromosomal aberrations (translocations, inversions, deletions). Such plants are usually characterized by a changed meiotic behaviour. The cytological investigations carried out by many research workers have revealed that the chromosomal aberrations in heterozygote conditions influence the frequency and the distributions of chiasmata and crossing over as well as the co- orientation of the centromeres (for comprehensive review see Rieger and Michaelis 1967). Intrachromosomal, as well as interchromosomal, effects have been reported (Hinton 1965; Hewitt 1967; Lucchesi and Suzuki 1968), which means that structural heterozygosity in one part of the genome increases recombination in the remainder of the genome.

The intention was to use the above described phenomenon for facilitating the gene transfer in a backcross programme using pollen irradiation of the donor plant. It was also expected that viable and competitive M_2 gametes would possess more maternal chromatin because only the chromosomes of the male parent are subject to gene and chromosome mutations

Table 1. The segregation pattern expressed in the BC-1 generation

	Phenotype	Control	[W-8 × (W-8 × C-3-1♮)] BC-1 generation derived from the selected M_1 plants							
			No. 65	No. 72	No. 91	No. 95	No. 95 ♮	No. 98	No. 48	Pooled
No. of plants	al^+	212	63	30	2	108	132	24	–	359
	al	226	75	11	42	16	37	11	166	358
Ratio (1:1)		0.94:1	0.84:1	2.72:1**	1:21***	6.75:1***	3.56:1***	2.18:1*	–	1:1
No. of plants	sw^+	346	58	35	36	78	102	31	129	469
	sw	92	80	6	8	46	67	4	37	248
Ratio (3:1)		3.76:1	0.73:1***	5.83:1	4.50:1	1.70:1**	1.52:1***	7.75:1	3.49:1	1.89:1***
No. of plants	*Ms-8*	212	1	0	26	58	86	0	87	258
	ms-8	226	137	41	18	66	83	35	79	459
Ratio (1:1)		0.94:1	1:137***	0:41***	1.44:1	0.88:1	1.04:1	0:35***	1.10:1	0.56:1***
No. of plants	al^+ sw^+ *Ms-8 C*	430	116	41	42	122	166	35	151	673
	al sw ms-8 c	8	22	1	2	1	3	0	15	44
Ratio (31:1)		53.75:1	5.27:1***	41:1	21:1	122:1	55.33:1	35:0	10.06:1***	15.30:1***

Significantly deviating from the expected segregation ratios (in brackets): * $P<0.05$; ** $P<0.01$; * $P<0.001$

and that will reduce the number of required backcrosses. This assumption has similarity with the proposed model for irradiation induced gene transfer via gametophytic selection described by Zamir (1983). In a preliminary report, Powell et al. 1983, have shown that after pollen irradiation of barley, with the increased radiation dose the M_1 as well as the M_2 plants become progressively more similar to the maternal parent.

The experimental data are summarized in Table 1 where the segregation pattern of the investigated genes expressed in the BC-1 generation [(W-8 × (W-8 × C-3-1♮)] is compared with the non-irradiated control [(W-8 × (W-8 × C-3-1)].

Presence or absence of anthocyanin spots on the nodes and fruits was the first analysed character. In five out of six progenies statistically significant deviations from the expected 1 : 1 ratio were observed. In one case the shift was towards the maternal allele (No. 91), with almost all plants having the maternal allele, and in four cases the shift was to the paternal type (No. 72, 95, 95♮ and 98).

As expected, all plants from the backcross with the F_1M_1 plant No. 48 lacked anthocyanin.

The second investigated character was sulfury white immature fruit versus light-green to dark-green fruit. In three cases significant deviations from the expected 3 : 1 ratio were observed (two pairs of alleles conditioned the sulphury white immature fruit colour; Peterson 1959). The shift was in the direction of more plants having the maternal alleles.

As regards genetic male sterility versus normal male fertility, three progenies (No. 65, 72 and 98) drastically deviated from the expected 1 : 1 ratio having exclusively plants with the maternal allele. Zamir (1983) in one case has also observed non-transfer of a parent allele.

The progenies No. 95 and 95♮ showed similar segregation patterns.

The segregation ratio of all analysed characters, including the test for pungent or sweet fruits, was also examined. Significant deviations from the expected 31 : 1 ratio were observed in two cases in the direction of more plants having all the investigated maternal alleles.

The pooled data from all BC-1 irradiated progenies showed clear evidence of a shift towards more plants possessing the maternal alleles.

The investigations on the pollen fertility of the 121 BC-1 plants showed that 83 of the analysed plants were normal fertile, 26 plants were semisterile and 12 plants had more than 70% sterile pollen. These data indicate that balanced gametes lacking chromosome aberrations preferentially take part in the fertilization.

From the data presented, the conclusion may be drawn that a procedure using pollen iradiation of the donor plant in combination with a selection of F_1M_1 plants expressing high percent pollen sterility for backcrosses may be useful for facilitating gene transfer, especially in cases of gene linkages.

Table 2. Simplified scheme of backcross procedure in combination with pollen irradiation

A × B ♮ ↓	Irradiation of pollen from the donor line for induction of chromosome and gene mutations and pollination of the recipient genotype A
A × (A × B) M_1 ↘	Selection of plants with a high percentage of sterile pollen. Backcross to A
A × (A × B) BC-1 ↓	Selection of plants that resemble A + useful genes of B. Backcross to A.
etc. ↓	(Second irradiation of pollen if necessary to delete undesirable, linked dominant genes)
A^B	Genetic background of A + desired gene or gene from B

A simplified scheme of backcross procedure in combination with pollen irradiation is shown in Table 2.

The pollen of the donor plant B must be irradiated with high but not lethal doses for induction of high frequencies of gene and chromosome mutations.

Plants expressing a high percentage of pollen sterility in the F_1M_1 generation and, if possible, lacking undesirable dominant characters, must be selected and used as the male parent for backcross to cultivar A, which is to be improved. In the BC-1 generation, fertile plants that resemble cultivar A and also contain useful characters of cultivar B have to be selected for the continuation of the backcross. In some cases pollen may be irradiated again to delete undesirable linked genes.

The procedure ends when the breeding objective is fulfilled, i.e. the desired gene or genes of the donor plant B are transferred to the genetic background of cultivar A. Due to the changed segregation pattern and the shift to the maternal parent one may expect a shortening of the backcross procedure normally required.

The proposed scheme might also be applicable with some modifications for other species as well as in connection with interspecific gene transfer.

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