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Pollen irradiation and the transfer of maternal genes in *Pisum sativum*

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Summary. Pollen of *Pisum sativum* was exposed to doses of 900 to 6,000 r of X-rays prior to pollinating a multiply marked genotype. The first generation progeny closely resembled that produced with unirradiated pollen. In the second generation, five loci were monitored, and the results showed that irradiation enhanced the proportion of maternal information transmitted to the progeny; the practical implications of the data, as well as the mechanism underlying the effect are discussed.

Key words: Pollen – Irradiation – Gene transfer – *Pisum*

Introduction

Sexual hybridisation is the only method of gene transfer that can be used in plant breeding programmes at present; while novel methods involving cell fusion or some form of transformation may be possible in the future, they do not yet offer an alternative. In many breeding programmes the objective is the transfer of one or a few characters from a given genotype into another and this is generally achieved by repeated backcrossing. Recent reports (Pandey 1980; Caligari et al. 1981; Jinks et al. 1981) have indicated that such a transfer could be attained more rapidly if pollen was exposed to high doses of ionising radiation prior to crossing. Pandey (1980) exposed pollen of *Nicotiana* species to doses of 75–100 Kr of γ-rays prior to using it for intra- or inter-specific pollinations, and obtained a few progeny which were similar to the maternal parent but had in addition on or two paternal characters. Caligari et al. (1981) and Jinks et al. (1981) similarly treated pollen of *Nicotiana rustica* with X- or γ-rays, albeit lower doses (10–20 Kr) and obtained progeny from intraspecific crosses which were more similar to the maternal parent than those derived from unirradiated pollen. In addition in these latter experiments, the frequency of particular recombinants involving both paternal and maternal markers was significantly increased over that found when using unirradiated pollen. If similar results were to be obtained in crop plants, then pollen irradiation could offer a useful method of enhancing the rate of introduction of one or a few attributes into cultivars. The present report describes our attempts to test this assumption using *Pisum sativum.*

Materials and methods

The following genotypes were used:
JI 813 and JI 717 – homozygous for *A*, af^+, tl^+, st^+, i^+, R_a
JI 1201 – homozygous for *a, af, tl, st, i,* r_a
In addition, each of these genotypes has a characteristic legumin subunit of ca. 40,000 Mr (Casey 1979).

a influences pigment formation, *af* and *tl* leaf morphology, *st* stipule morphology, *i* cotyledon colour, and r_a starch conformation (Blixt 1972). *a, af* and *i* are on chromosome 1, *af* and *i* being 8.3 units apart (Kielpinski 1982); r_a, *tl* and the structural gene for the 40 Mr legumin subunit are on chromosome 7, r_a and *tl* being 5 units apart and *tl* and the legumin locus *Lg*-1 10 units apart (Blixt 1972; Matta and Gatehouse 1982). The availability of linked markers would be useful in evaluating the size of chromosome fragments transferred, if transformation were to occur; such linked markers have not been available in previous studies.

Pollen was exposed to X-rays, and all plants were grown in a glasshouse – the parental generation in the spring and summer of 1981, and the F1, M1, F2 and M2 generations in 1982. (M1 and M2 are the progeny of irradiated pollen, as opposed to the F1 and F2 progeny of unirradiated pollen).

Results

As species differ in their radiosensitivity it is necessary to calculate the appropriate dose for each. This calculation is based on the relationship which exists between radiosensitivity (measured in terms of lethal damage) and target size, which is the average number of nucleotides per chromosome (Underbrink et al. 1968). The formula which describes this relationship is:

$\log D_0 r = 11.98 - 1 \log$ nucleotide/chromosome.

The calculated D_0 for *Nicotiana rustica* is approximately 1,400 r and for *Pisum sativum,* 340 r. On this basis it is assumed that an approximately 4 fold reduction in the doses used by Caligari et al. (1981) and Jinks et al. (1981) for *N. rustica* would be appropriate for *P. sativum.* Pollen was therefore exposed to doses of 900–6,000 r of X-rays which encompassed the calculated range. The numbers of crosses undertaken and of seed obtained are given in Table 1 and Fig. 1. Above 3,200 r no seed was set.

Over 95% of the M1 seed germinated and all M1 plants were morphologically similar to the F1. In this report the results obtained after exposing pollen to doses of 900, 1,200 or 1,800 r will be considered, as these were the doses which gave substantial numbers of M2 plants. Comparable numbers of M2 or F2 seed were produced on the M1 and F1 plants and were in the range 35 to 70; the few exceptions were semi-sterile plants in which the number of progeny could be as low as 20. In view of the large total number of M2 plants to be screened, attention was concentrated on markers which could be scored at the seedling stage; these were *af, i, st,* r_a and *tl.* Thirty-two phenotypic classes could be recognised from all the possible combinations of 32 male and 32 female gametic genotypes. Knowing the distances between the linked markers, the expected percentage of progeny in each phenotypic class was computed; the values obtained in the F2 populations agree well with these expectations (Table 2). The

Table 1. Numbers of seed obtained following pollination of JI 1201 with irradiated pollen of either JI 717 or JI 813

Dose (r)	No. flowers pollinated	No. seed obtained	No. M2 families analysed
0	36	87	13
900	130	137	20
1200	118	87	72
1800	138	45	15
2200	59	11	
3000	93	1	
3200	40	1	
4500	40	0	
6000	101	0	

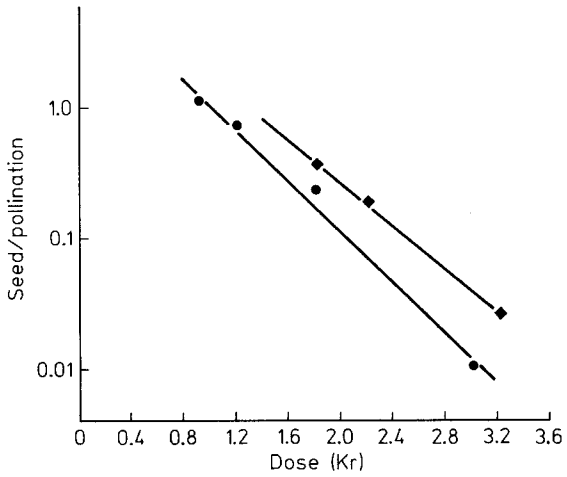

Fig. 1. Relation of seed set per pollination to dose of X-rays. ● Pollinated immediately after emasculation; ◆ pollinated 24 h after emasculation

Table 2. The distribution of phenotypes predicted in the F2 on the basis of linkage values, together with the actual frequencies obtained in the F2 and M2 generations

	Predicted	Or	900r	1200r	1800r
R_a + + + +	38.8	40.86	40.33	37.34	39.6
R_a *af* + + +	2.06	0.49	0.99	1.03	0.63
R_a *af tl* + +	0.56	0.16	–	0.26	–
R_a + *tl* + +	1.27	0.16	2.15	1.26	2.5
R_a + + *st* +	12.93	11.13	12.07	15.45	11.4
R_a *af* + *st* +	0.68	0.32	0.16	0.33	0.16
R_a *af tl st* +	0.019	–	–	0.04	0.32
R_a + *tl st* +	0.42	0.32	0.64	0.59	1.4
R_a + + + *i*	2.06	0.49	1.28	1.26	0.94
R_a *af* + + *i*	11.56	12.79	11.73	10.78	13.9
R_a *af tl* + *i*	0.38	–	0.49	0.44	0.63
R_a + *tl* + *i*	0.056	–	0.16	–	–
R_a + + *st i*	0.68	0.16	0.32	0.11	0.16
R_a *af* + *st i*	3.86	6.15	2.97	4.67	4.23
R_a *af tl st i* (b)[a]	0.13	0.16	–	0.37	0.16
R_a + *tl st i*	0.019	–	–	0.04	–
r_a + + + +	1.27	0.49	0.83	0.96	1.25
r_a *af* + + +	0.056	0.16	–	0.04	0.16
r_a *af tl* + +	0.65	0.49	0.99	1.07	0.16
r_a + *tl* + +	12.08	13.62	13.55	13.63	11.6
r_a + + *st* +	0.42	0.16	0.64	0.37	0.32
r_a *af* + *st* +	0.019	–	0.16	0.08	–
r_a *af tl st* + (b)	0.22	0.16	0.16	0.16	–
r_a + *tl st* +	4.03	4.64	3.97	4.37	4.7
r_a + + + *i*	0.056	0.16	–	0.04	–
r_a *af* + + *i*	0.38	0.32	0.83	0.67	0.48
r_a *af tl* + *i* (b)	3.59	4.81	3.47	3.22	2.5
r_a + *tl* + *i*	0.65	0.16	0.16	0.18	0.16
r_a + + *st i*	0.019	–	–	–	–
r_a *af* + *st i* (b)	0.13	–	0.16	0.11	0.32
r_a *af tl st i* (a)	1.2	1.49	1.82	1.37	2.04
r_a + *tl st i* (b)	0.22	–	–	0.11	0.16

[a] See text for definition

average values obtained for all the M2 progenies of a given pollen irradiation treatment were also calculated (Table 2); no marked difference in the distribution of phenotypic classes was observed from that seen in the control. For example the percentage of progeny which had all 5 marker alleles from the maternal parent did not increase with dose (*a* in Table 2); neither did the frequency of those classes which had 4 of the 5 maternal alleles and 1 paternal allele (b in Table 2). Furthermore there was no marked increase in the classes which showed a recombination event between both linked sets of markers. Thus when the data from all the M2 progenies of a given treatment were summed, there was no detectable effect of radiation on the distribution of phenotypic classes amongst the progeny. The next analyses deal with each F2 and M2 family separately. Within each family, the numbers were too low to consider the distribution of phenotypic classes as in the data of Table 2; however, the segregation ratio of each allele in any family could be tested to establish whether this was in agreement with expectation.

The number of progeny examined, and of families showing a statistically significant deviation at any locus, from the 3 : 1 segregation ratio expected are given in Table 3.

A similar proportion of F2 and of M2 (900 r) families, showed a deviation in the segregation ratio at any one of the five loci. After exposing the pollen to 1,200 r or 1,800 r, 11.1% and 20% of the M2 families respectively showed aberrant segregations. In two instances there was an excess of a paternal allele, and this is important in relation to possible mechanisms which underlie the effect (see Discussion). In one of these, an M2 family segregated $35 R_a$ to $3 r_a$ and in the other $24 st+ : 0 st$. In total there were 5 progenies showing an aberrant segregation at the r_a, 3 at the *i*, 3 at the *af*, 4 at the *st* and 2 at the *tl* locus. The r_a and *tl* loci (5 cross over units apart) were simultaneously affected in two progenies and the *af* and *i* loci (8.3 cross over units apart) in two progenies. In only two M2 families was there a complete absence of a particular allele in the progeny; in the one family just mentioned a segregation of $24 st+ : 0 st$ and in the other family a ratio of $0 st+ : 36 st$. In both of these as well as in all the other aberrant families mentioned, all alleles other than the one (or two) mentioned in any given instance segregated normally.

Table 3. Number of M2 or F2 families showing aberrant segregation ratios at any of the 5 loci tested

Dose (r)	Total no. of progenies	No. and % of families showing aberrant segregation, and locus (i) involved
0	602	1 (7.7%) (Excess r_a)
900	704	1 (5%) (Excess r_a and *tl*)
1200	3322	8 (11.1%) (Excess *i*; r_a; *af*; *st*+; *st* (2 families) r_a and *tl*; *i* and *af*)
1800	638	3 (20%) Excess R_a; *st*; *af* and *i*)

Discussion

The assumption which underlies experiments of the kind reported here is that exposure of pollen to heavy doses of irradiation will allow a more rapid, and thus a more economic transfer of one or a few paternal alleles into an otherwise desirable maternal genotype, than is possible by conventional backcrossing. In the present experiments, if the data from all the progenies derived from a given dose are summed, they show no increase in the frequency of plants having all 5 maternal alleles at the loci studied, or of plants having any 4 maternal alleles together with one paternal allele. Thus if single gene transfer from the paternal parent to the maternal genome does occur, the rate is not high enough, with the doses used in the present experiment, to distort the overall distribution of phenotypic classes in the M2 generation. However, if the data from each individual M2 progeny are examined separately, then many show a deviation from the expected segregation ratio of particular alleles; these deviations are not associated with the occurrence of semi-sterility in the progenies. Twenty percent of progenies derived from the 1,800 r treatment showed an aberrant segregation at at least one of the 5 loci studied. The proportion of the total linkage map (Blixt 1972) represented by these 5 loci is small; ca. 3.5% of chromosome 1 is represented by the *af* to *i* region, ca. 5% of chromosome 7 by the *r* to *tl* region, and all 5 loci probably represent no more than 1% of the total genome. It is reasonable to assume that these loci are representative of all loci, and do not represent "hot spots" of damage. Over the whole of the genome of these M2 plants, we would therefore expect a comparable probability of having an excess of maternally derived alleles.

These data substantiate the claim of Caligari et al. (1981) and of Jinks et al. (1981) that the use of heavily irradiated pollen can result in the production of progeny which show a greater resemblance to the maternal parent than do the unirradiated progeny. However, a more important comparison is between the M_2 and a backcross generation, and clearly the proportion of maternal alleles would be greater in the latter than in the present experiments. There could be one advantage in that linked markers might be separated more easily by irradiation than by recombination; there is a suggestion of this in the data for the *af* and *i* loci after 1,200 r although the frequencies are too low to be

certain of this. It remains to be seen whether the technique can be made more attractive as a practical breeding tool by the use of higher doses. While in theory an enhanced transfer of maternal genes should be observed, the seed set per pollination is also reduced and thus the amount of effort to produce each seed increased. This low seed yield after irradiation is a major problem in those plants which have few ovules per ovary – a situation typical of many temperate crop plants, and in contrast to *Nicotiana* species which have several hundred ovules per ovary. In the latter, even the very low rates of seed set following exposure to high doses of radiation can still give significant numbers of progeny.

The mechanism involved in the effect observed remains uncertain. Pandey (1980) has suggested that pollen exposed to very high doses of irradiation (75 to 100 Kr) transfers small fragments of chromatin and triggers a parthenogenetic development of the embryo. This results in the production of M1 progeny closely resembling the maternal parent. In the present experiments and those of Caligari et al. (1981) and Jinks et al. (1981), there has been a substantial transfer of paternal information from the irradiated pollen since the M1 generally resembles the F1. The lesions induced in the paternal chromosomes do not impair the expression of their information content in the M_1; they do however reduce but do not inhibit, their transfer to the next generation. Very rarely were families found in which a particular allele was completely absent – the usual situation was a change in the ratio of maternal to paternal alleles. Thus the excess of maternal alleles, and in some instances of linked alleles and the occasional excess of paternal alleles, must reflect either a disturbed meiosis in the M1 or/and a selective survival of particular M_1 gametes. The end result is an excess of maternal information in the progenies.

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