GENE TRANSFER IN *NICOTIANA RUSTICA* BY MEANS OF IRRADIATED POLLEN. I. UNSELECTED PROGENIES

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SUMMARY

The effects of varying high doses of X- and γ -ray radiation (10, 15 and 20 krad) on the pollen of the V_{12} variety of *Nicotiana rustica* are examined when used in crosses with variety V_{27} . It is shown that the M_2 families derived from such crosses show a closer resemblance to the maternal V_{27} phenotype for characters showing continuous variation as well as those controlled by major genes than does the equivalent unirradiated cross, F_2 . The degree of similarity to the mother is related to the dose of radiation used, with the resemblance being greatest after the highest dose was applied. The results suggest that only a portion of the paternal genome is expressed in such crosses but give no indication of this being a specific portion. It is concluded that the most plausible explanation is not that just a portion of the paternal DNA is being expressed in the progeny but that only fragments of it were effectively transferred.

1. INTRODUCTION

IN 1968 experiments were initiated in the Genetics Department at Birmingham to investigate the possibility of inducing matromorphy in *Nicotiana rustica*, that is, the production of offspring derived solely from the maternal genome (Dhahi 1974, Brumpton 1973). Despite reports of its widespread occurrence in this and other genera (*e.g.*, Goodspeed, 1954; Eenink, 1974*a, b*) the attempts met with only limited success. When heavily irradiated pollen was used, many of the offspring, although not strictly matromorphic (since families derived from them segregated for one or more of the parental differences), did show a general resemblance to their mothers (Virk *et al.*, 1977). Subjecting the pollen of *N. rustica* to near-lethal doses of radiation therefore appeared not to produce true matromorphy but to offer the possibility of transferring parts of the DNA from one genotype to another (Mather, 1981). The experiments reported here were thus initiated to investigate this possibility both in terms of characters controlled by major genes and those displaying continuous variation.

2. IRRADIATED CROSSES

The parents chosen for this study were two highly inbred varieties of *Nicotiana rustica*, V_{27} and V_{12} . These varieties differ at a number of major gene loci, with V_{27} displaying the recessive characters of yellow ovary and flower, mophead inflorescence and yellow stem and leaf, and V_{12} which was used as the pollen parent having the dominant characters of black

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ovaries, non-mophead inflorescence and green stem and leaf. Although these characters are controlled by major genes their inheritance is not simple; for example two loci, with three alleles displaying duplicate interactions control ovary colour and at least three loci control inflorescence morphology (Pooni and Jinks, 1981; Jinks and Coombs, 1979). These two varieties were also thought, from earlier work, to show large differences for some of the quantitative characters that could readily be scored.

Pollen was collected from V_{12} and divided into seven groups: one group was not irradiated while the other six were subject to 10, 15 or 20 krad of either γ -rays or X-rays. The pollen was then used to pollinate previously emasculated V_{27} flowers. At the same time, some of the emasculated V_{27} flowers were left unpollinated to test for spontaneous parthenogenesis: however, no seeds formed in any of these. As would be expected the crosses using unirradiated pollen produced a large number (>500) of viable seeds while in the crosses with irradiated pollen the number of viable seeds fell with increasing dose.

3. M_1 GENERATION

The plants from the seeds produced from the above crosses were raised in the glasshouse and designated M_1 for the irradiated crosses, the F_1 being the unirradiated cross.

An upper limit of 96 plants/treatment was imposed for practical reasons, but, this was not reached for the two highest radiation doses (table 1). Samples of the two parents and the F_1 were raised under similar conditions. All the plants were scored for the expression of the major gene characters as well as their general appearance being noted. They were then selfed, but a number failed to produce any seed (table 1).

TABLE 1
Irradiated pollen crosses. $V_{27}\text{♀} \times V_{12}\text{♂}$

Treatment (symbol)	Number of M_1 plants grown	Percent of M_1 plants producing no seed	Percent of M_1 plants showing maternal characters	Number of M_1 plants contributing to M_2
γ -rays				
10 krad (E)	96	23%	13%	28
15 krad (F)	96	29%	38%	28
20 krad (G)	44	66%	36%	5
X-rays				
10 krad (H)	96	19%	29%	21
15 krad (I)	96	48%	58%	10
20 krad (J)	3	100%	—	0

All the F_1 progeny were uniform in appearance and showed the dominant characters of the pollen parent including its general morphology. In contrast, the M_1 progeny were extremely variable in their phenotypes, showing varying degrees of resemblance in their general appearance to either parent as well different combinations of the recessive and dominant major gene controlled characters. The only plants which were phenotypically identical to the maternal V_{27} parent were 29 in treatment H (10 krad

X-rays). These plants could be V_{27} selfs, or matromorphic in origin. These plants were selfed, but no segregation was detected for any of the major genes in their progeny or for the continuously varying characters, and are not considered further. Of the remaining plants in H as well as in the other treatments, the number of plants showing any of the recessive maternal characteristics have been grouped together within treatments and expressed as percentages in table 1.

From the plants in this generation which produced a reasonable amount of seed, representatives were chosen within each treatment, of every phenotypic class present, in addition to a random sample of the remaining plants. In treatment J (20 krad X-rays) none of the three plants gave any seed and in treatment G (20 krad γ -rays) only five plants produced sufficient seed, so all were taken. Thus in none of the treatments has there been any selection for the more maternal types: the only strong selection pressure was, for obvious practical reasons, for fertility. The numbers of M_1 plants contributing M_2 families, chosen on this basis for further investigation, are shown in the final column of table 1. These chosen M_2 families were grown on the experimental field at Birmingham in an experiment composed of two parts. The first, which contained 20 individuals per treatment family together with 25 plants of each of the parents, 18 F_1 individuals and 100 F_2 was based on complete single plant randomisation. The second part of the experiment contained 40 individuals per family grown together in blocks in a non-randomised manner. For some of the major gene characters plants from both the randomised and non-randomised design were scored while only plants in the randomised design were used for assessing the quantitatively varying characters.

4. M_2 RESULTS

The yellow to green differences in stem colour, ovary colour and flower colour were invariably linked in their appearance so suggesting, in this cross at least, that they were really a single character, which it was decided to call plant colour. Also the black pigmentation in both ovaries and flowers proved difficult to score unambiguously. Therefore only the segregation of yellow to green plant colour and "mop" to "non-mop" inflorescence structure will be presented here. Eight continuously varying characters were measured, including height assessed at various stages of growth, the day of first flower appearance, and some leaf dimensions, but the only suitable ones, in which there was not only a clear difference between the means of the two parents but also a difference between the F_2 mean and the maternal parent, were height at flowering time (H.Ft.), height at 10 weeks after planting (H_{10}) and the final height reached at the end of the growing season (F.H.). These three characters are, obviously, measurements of height taken towards the second half of the growing season and because they are likely to have genes in common will be expected to show a certain degree of correlation. In fact, amongst F_2 individuals the estimates of the correlation coefficients are; between H.Ft and H_{10} $r = 0.68$, H.Ft and F.H. $r = 0.73$ and H_{10} and F.H. $r = 0.93$. The three characters cannot, therefore, be regarded as completely independent in the information they provide; but H.Ft is not particularly closely correlated with the other two, the relationship accounting for only 46 and 53 per cent of the variation in

the two cases, especially since no allowance has been made for the likely inflationary effects on the correlations of any linkage present between the relevant loci.

The segregation ratios for the two characters controlled by major gene differences and the means and standard errors for the three continuously varying characters are presented in table 2. For yellow to green plant colour, only 5 out of the 140 F_2 plants scored were yellow (*i.e.*, a ratio of 1 yellow to 27 greens). If the treatment of the pollen with radiation had no effect the treated families would be expected to show a segregation in agreement with that of the F_2 . However, all five treatments produce segregation ratios which, when tested by χ^2 , are significantly different from the F_2 and in every case the deviation is in the direction of an excess of the recessive maternal phenotype. Furthermore, an effect of X-ray dose can be seen, with the excess of maternal type increasing with dose, resulting in treatment G giving a ratio as high as 1 yellow to every 4 green plants. The ratios in the two X-ray treatments give no evidence of any similar heterogeneity. A comparable picture emerges when the segregation of mophead inflorescence is examined.

The results for the three quantitative characters all show a very similar pattern which may, of course, reflect the pleiotropic effects of the underlying genes particularly between H_{10} and FH. The most important comparison to be made for each of these characters is again between the M_2 and the F_2 . When the mean over all the treatment groups is compared with the F_2 mean there is a significant difference for all three characters and the mean of M_2 always deviates in the direction of the mean of the maternal parent, V_{27} . Also, the means for the different doses of radiation within both X-rays and γ -rays vary significantly, the resemblance to V_{27} increasing with increasing dose as was apparent for the major genes. From these results it is clear that as the primary objective was to produce genotypes which, while generally resembling the mother, contain some of the paternal characteristics, the highest dose of radiation used, 20 krad γ -rays (G), is the treatment of most direct interest on which attention will now be focused.

5. TREATMENT G (20 krad γ -rays)

The results for the continuously varying characters from treatment G and from the two parents and F_2 can be represented as frequency distributions and plotted in the form of histograms. The picture obtained is very similar for all three characters and so only the results of H.Ft. are presented (fig. 1). The change in the mean of the treated group towards the maternal mean, compared with the F_2 distribution, is again clearly seen. For this character only approximately 12 per cent of F_2 individuals fall exactly within the phenotypic range displayed by the maternal parent, V_{27} , while in treatment G it is as high as 40 per cent. However, it can be seen that although there is a distinct increase in the frequency of maternal types among the treated group the range of phenotypes exhibited is if anything greater than that displayed by the unirradiated cross and the distribution has a long upward tail. One obvious explanation for such an increase is that the 5 M_2 families are themselves heterogeneous and thus the overall distribution consists of 5 separate and different distributions.

TABLE 2
Results for M_2 generation

Major gene characters, no. of plants			Quantitative characters			
	N ₁ *	N ₂ *	plant colour yellow: Green	inflorescence mop: non-mop	mean (cms)± s.e.	
					H.Ft	
					H ₁₀	
					F.H.	
γ-rays						
	10 krad (E)	1130	549	142:1537 (1:10.8)	35:514 (1:14.7)	110.96±1.02
	15 krad (F)	1100	551	153:1498 (1:7.1)	68:483 (1:9.79)	126.46±2.64
	20 krad (G)	154	97	43:208 (1:4.8)	23:74 (1:3.3)	135.24±3.72
X-rays						
	10 krad (H)	842	414	104:1152 (1:11.1)	42:372 (1:8.9)	121.39±2.08
15 krad (I)	294	185	36:443 (1:12.3)	30:165 (1:5.5)	173.68±1.94	
Scored on N ₁ + N ₂ plants					171.21±2.79	
Scored on N ₂ plants only					147.31±2.65	

* N_1 = number of plants raised in the non-randomised experiment.

N_2 = number of plants raised in the randomised experiment.

FIG. 1.—Frequency distributions of the character height at flowering time (H.Ft.), grouped into 5 cm. classes, for the maternal parent V₂₇, the pollen parent V₁₂, the F₂ generation from the normal unirradiated cross and the equivalent M₂ generation from treatment G(20 krad γ -ray irradiated pollen cross). ▼ signifies the mean of the distribution. The shaded area of G denotes the distribution for the family derived from a single M₁ plant, G₁₁.

The results for the 5 G families are shown separately in table 3. For all characters there is significant heterogeneity between families. The mean of each family for each of the quantitative characters can be compared with its respective F₂ mean by a t-test, although it should be noted that these tests will not be statistically independent of one another. All show

TABLE 3
Results from M_2 generation for families from 20 krad γ -ray treatment (G)

M_2 family	N_1	N_2	Number of plants		Means (cms) $\pm$ s.e.		
			Plant colour yellow: Green	inflorescence mop: non-mop	H.Ft	H ₁₀	F.H.
G ₄	38	18	18:38***	2:16*	112.83 $\pm$ 8.24	157.77 $\pm$ 6.61	176.59 $\pm$ 6.20
G ₅	40	20	4:56	5:15***	94.24 $\pm$ 6.79	135.20 $\pm$ 7.54	149.79 $\pm$ 6.55
G ₁₀	0	20	1:19	2:18	79.80 $\pm$ 4.45	139.65 $\pm$ 6.51	143.80 $\pm$ 6.67
G ₁₁	36	20	12:44***	9:11***	81.53 $\pm$ 4.39	114.10 $\pm$ 7.00	124.53 $\pm$ 8.10
G ₁₉	39	20	8:51***	5:15***	102.40 $\pm$ 6.17	134.60 $\pm$ 6.07	150.95 $\pm$ 6.18
			Scored on $N_1 + N_2$ plants		Scored on N_2 plants only		

(Departure of segregation ratios from those of the F_2 results; *** $P < 0.001$, * $P < 0.05$.)

a significant difference except family G_4 where the means displayed for H.Ft and F.H. show no significant differences and for H_{10} only a marginally significant difference from the F_2 . Without exception all the deviations, including those for G_4 , are towards the V_{27} mean. Conversely, however, although deviating towards V_{27} , four of the families differ significantly from it for all three characters with the single exception of H.Ft in family G_{10} . The fifth family, G_{11} , shows no significant differences from V_{27} for any of the three characters, and the distribution of one of these, H.Ft, is shown as the shaded region of the overall distribution of G families in fig. 1.

The different G families all clearly showed greater resemblance to V_{27} than would be expected if irradiating the pollen had no effect, but they display the influence of the paternal parent to varying degrees. Whether this heterogeneity is a sufficient explanation of the apparent increase in the range and skewness of the overall distribution relative to the F_2 is difficult to assess with certainty since each of the families comprises, at most, only 20 individuals. Judging by the standard errors, or the distribution of H.Ft for G_{11} given in fig. 1, there appears to be more variation within a family than that exhibited within the maternal parental family. This, however, might be expected since if any of the paternal genes are present and active in such families they would be expected to lead to a phenotypic segregation in the M_2 .

6. DISCUSSION AND CONCLUSIONS

Exposing the pollen of variety V_{12} of *Nicotiana rustica* to 10 to 20 kr doses of X or γ radiation before using it to pollinate variety V_{27} , an M_1 generation was produced in which phenotypic variation was apparent. In particular, plants were observed which displayed to varying degrees the recessive maternal characteristics. On selfing these plants an M_2 generation was raised in which, for obvious reasons, only those M_1 plants which set a reasonable number of seeds were represented. The phenotypic distributions for both the characters controlled by major genes and those showing quantitative variation deviated from those of the equivalent unirradiated cross and did so consistently in the direction of being more like those of the maternal parent, the similarity increasing with dose. The observations on the M_2 generation thus confirmed those on the M_1 that the radiation was tending to decrease the expression of paternal DNA. When the families derived from the highest dose of radiation were examined it was found that while they all deviated, for at least some of the characters, away from the F_2 and towards the maternal parent there were significant differences between them. It would, therefore, appear that not only was it just a part of the paternal DNA which was being expressed in all of them, but that it was a different part in families derived from different M_1 individuals. Thus there was no evidence of specific regions of the genome being preferentially affected.

There are at least three possible explanations for the results reported here. First, the effects that have been observed could be attributed to mutations, defined in a broad sense, caused either directly or indirectly by the radiation treatment. However, if this was the case it would be necessary to assume, for all the characters reported here, that such "mutations" were in general directional, leading to a closer resemblance to the maternal

parent, which would be difficult to reconcile with most mutation studies. The second possibility is that the high doses of radiation cause a large part of the genome present in the pollen to be inactivated and therefore although present in the M_1 plants and passed on to the M_2 families only restricted parts of it would be expressed. Such a possibility cannot be definitely excluded by the present results.

The third, and perhaps the most likely, explanation is that only a part of the paternal DNA is present. In other words only fragments (or possibly in the extreme a single fragment) of paternal DNA are effectively present in the resulting M_1 plants from irradiated pollen crosses. The details of the processes involved can, at present, only be speculated upon but the general appearance of the majority of M_1 plants, their fertility and resulting M_2 families suggest that they are unlikely to be haploid or grossly aneuploid in constitution. Indeed, earlier preliminary cytological observations of progeny from irradiated pollen crosses of other *Nicotiana* species onto *N. rustica* have shown them to have the normal diploid chromosome number (Dhahi, 1974). Similarly Pandey (1975, 1978) working with irradiated pollen crosses between different *Nicotiana* species has presented results of a similar kind to those reported here for the characters controlled by major genes and again found no evidence of cytologically observable abnormalities. Such observations would suggest that if only fragments of paternal DNA are present, the maternal genome must double some time after "fertilisation" with irradiated pollen. These are, however, no more than tentative speculations at this stage. Firmer conclusions must await further cytological observations and examination of the segregation patterns in later generations of our material. One claim we can make, however, is that the results of the irradiated pollen crosses, which have now been obtained on a number of occasions in this Department, are repeatable and whatever the underlying mechanisms they are consistent with the transfer of fragments of DNA from one genotype of *Nicotiana rustica* to another. Indeed, Jinks, Caligari and Ingram (1981) have shown how by a combination of selection and this technique, specific paternal characteristics can be transferred into a pure breeding maternal genotype.

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