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Pollen irradiation and possible gene transfer in *Nicotiana* species

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Summary. Progeny from crosses of *Nicotiana langsdorffii* with gamma irradiated pollen of *Nicotiana alata* 'Crimson Bedder' showed skewed segregation in the F_2 favoring the maternal parent. This is probably not gene transfer in a strict sense, rather just an extreme case of reduced transmission of irradiated chromosomes, leading to massive overrepresentation of maternal genes. Gene transfer or mutational loss may explain some anomalous F_1 plants. Segregation in the F_2 progeny showed the presence of several genes from the irradiated pollen. Crosses of *Nicotiana sylvestris, N. plumbaginifolia, N. paniculata,* and *Petunia parodii* with irradiated pollen from *N. alata* and *Petunia hybrida* showed no evidence of gene transfer, nor did experiments with irradiated mentor pollen. This indicates that gene transfer with irradiated pollen between non-crossing species or between species giving sterile hybrids is probably a rare phenomenon.

Key words: *Nicotiana* – Pollen irradiation – Gene transmission – Egg transformation – Mutation

Introduction

Transfer of a single gene or a few genes into a plant after pollination with X-irradiated pollen or with mixtures of irradiated nonself "mentor pollen" and incompatible self pollen has been reported in *Nicotiana* by Pandey (1975, 1980a, 1980b). The change was usually apparent in the F_1 where some plants looking exactly like the maternal plant showed paternal flower colour or selfincompatibility genes.

Several groups have reported a less dramatic phenomenon after pollination with irradiated pollen where the results are visible in the F_2 as strongly skewed Mendelian segregation ratios favoring the maternal parent. Such results have been obtained in *Nicotiana rustica* (Virk et al. 1977; Jinks et al. 1981; Caligari et al. 1981), in barley (Powell et al. 1983), in wheat (Snape et al. 1983), in tomato (Zamir 1983) in maize (Pandey 1983), and in pea (Davies 1984).

Such phenomena might have very important applications in plant breeding (Davies 1981). The skewed F_2 segregations might reduce the need for backcrossing when transferring desired genes from primitives into advanced cultivars. And Pandey's gene transfer visible in F_1 in maternal plants promises tremendous opportunities of transferring genes maybe even from distantly related species in one step.

The purpose of the present work was to see if the phenomena were repeatable and widely applicable by testing a number of *Nicotiana* combinations.

Materials and methods

Nicotiana sylvestris Spegazzini et Comes (2n = 24), *Nicotiana paniculata* L. (2n = 24) and *Nicotiana langsdorffii* Weinmann (2n = 18) were obtained from the Botanical Garden, Copenhagen, Denmark. *Nicotiana alata* Link et Otto (2n = 18) and *Nicotiana forgetiana* Hort. ex Hemsley (2n = 18) from Dr. K. K. Pandey, Palmerston North, New Zealand. *Nicotiana alata* (2n = 18) 'Idol' (white, dwarf) and *Nicotiana alata* 'Crimson Bedder' (red), from Ohlsens Enke Seed Company, Tåstrup, Denmark and *Nicotiana plumbaginifolia* Viviani (2n = 20) from Dr. Michael Freeling, University of California, Berkeley, USA. *Petunia parodii* (2n = 14) was obtained from Dr. K. C. Sink, Michigan State University, East Lansing, USA, and *Petunia hybrida* (2n = 14) 'Ratsherr' and 'Violaceae' from FDB Seed Company, Glostrup, Denmark.

Plants were grown throughout the year, in a greenhouse at more than 20 °C with continuous illumination during winter.

The plants were potted in 'K-jord', a commercial, fertilized gardener's mixture of sphagnum peat, sand and clay. Crosses usually took place from March to October.

Emasculated flowers were pollinated on the same or the following day. The flowers were not bagged to avoid abscission and to facilitate removal of growing neighbor flower buds. Progeny from accidental contamination or selfing would be classified as true hybrids or as maternals and fall outside the interesting category: maternal plants with one or a few genes from the irradiated pollen.

Abscission was prevented by spraying pollinated flower buds once or twice with 50 ppm 2,4-D or by applying 'drops' of 1% naphthoxyacetic acid in lanolin to the calyx.

Newly dehisced anthers were collected in small plastic vials and irradiated in a ^{60}Co gamma-source, calibrated with the Fricke dosimeter. Pollination took place on the day of irradiation.

Experiments with pollen mixtures were done by mixing irradiated donor pollen with selfincompatible self-pollen (1 : 1) or by pollinating the unemasculated selfincompatible flower with irradiated donor pollen and selfing the flower with a pipe cleaner immediately afterwards.

Seeds were sown on 1% agar with $^{1}/_{10}$ Murashige and Skoog salts in tap water. Seeds from *N. paniculata* and *N. plumbaginifolia* and their offspring were soaked in 0.1% gibberellic acid in water 1–2 h before sowing to induce germination.

Plants were scored in the flowering stage of F_1 for flower colour and morphological characters. In many cases also the F_2 was scored.

Chromosomes were counted in root tips after Feulgen staining and pollen viability was scored after staining in acetocarmine.

Results

Three types of progeny were obtained from fertilization of a maternal plant with irradiated pollen in many experiments: a) true hybrids, b) anomalous hybrids and c) maternal plants.

a) true hybrids could not be distinguished from the ordinary hybrids between unirradiated parents, except that they were often sterile or semifertile in normally fertile combinations. Pollen stainability was often low, e.g. 3 to 30%. The problem with sterility was a major reason for not including selfincompatibility genes in the scoring for gene transfer. Scoring for selfincompatibility would involve distinguishing selfincompatibility from pollen or egg sterility.

b) anomalous hybrids were like the true hybrids minus one or several characters (Fig. 1). In usually fertile combinations they were often sterile. Other anomalous hybrids were just monsters. Anomalous hybrids are probably mutations.

c) maternal plants were indistinguishable from the maternal parent. They were most common in crosses with *N. paniculata* as already stated in the literature (Goodspeed 1954). All maternal plants have been fully fertile with normal seed set.

Figure 2 shows the viable seed set of *N. langsdorffii* × irradiated *N. alata* pollen as a function of the irradiation dose. The 63 seeds set at 0 krad is much below the normal seed set of *N. langsdorffii*. Seed viability goes down to about 1 seed per capsule at 20 krad; but seeds are still set at 100 krad. This type of radiation inactivation curve with a tail has been reported repeatedly (Brewbaker and Emery 1962; Pandey and Phung 1982). The seed set figures have been variable in my experiments, partly because differences in pollen humidity and other environmental factors were not controlled. The other experiments showed that seed set varied with the mother plant species. It was easier to get viable seeds of *N. plumbaginifolia* × *N. alata* at 50 krad than seeds of *N. langsdorffii* × *N. alata* at 50 krad.

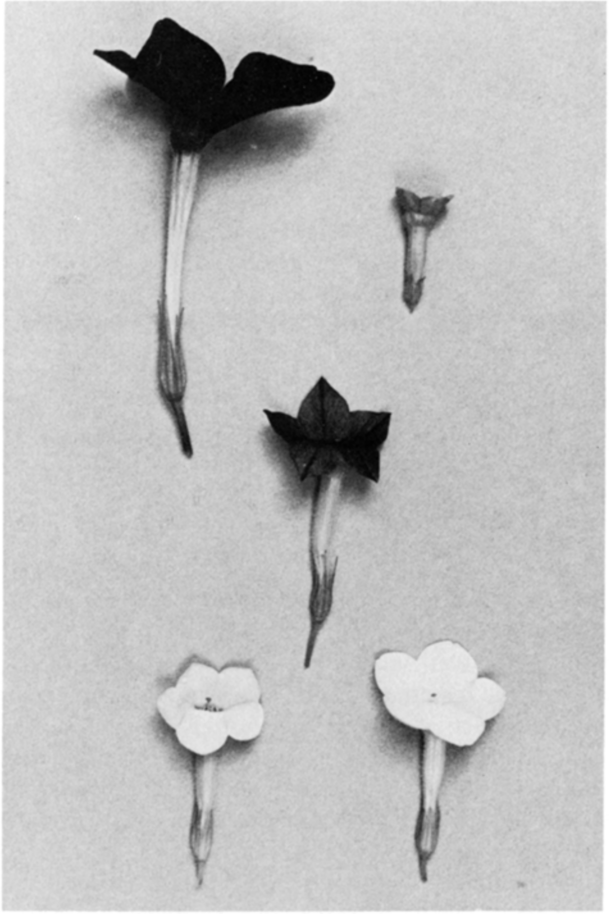

Fig. 1. Red *Nicotiana alata* 'Crimson Bedder' and green *N. langsdorffii*. The dirty red standard F_1 hybrid and below two anomalous whitish hybrids obtained after pollen irradiation

The results of a large number of small experiments are summarized in Table 1. The data have been collected over several years. Attempts to repeat Pandey's results with his own material failed. Crosses with irradiated pollen set no seeds, and no seeds were obtained for propagation.

The first experiments were mainly done with mixtures of irradiated mentor pollen and the plants own pollen. In case of selffertile species consecutive pollinations were tried first with irradiated pollen, then with

Table 1. A summary of the results of *Nicotiana* (and *Petunia*) crosses using irradiated pollen. Each entry usually represents several experiments

Maternal plant	Pollen donor	Irradiation krad	Capsules	F_1 plants	F_2 plants
N. alata Pandey	*N. forgetiana* (as mentor)	100	–	–	
N. alata 'Idol' white	*N. alata* 'Crimson Bedder'	0, 10, 20, 30, 100	33	hybrids 4 'Idol' white	
N. alata 'Idol' white	*'Crimson Bedder'* (as mentor)	0, 10, 30, 100	58	410 'Idol' white (pseudoselfcomp.)	
N. alata 'Idol' white	*P. hybrida* (as mentor)	100	63	'Idol' white (pseudoselfcomp.)	
N. langsdorffii	*N. alata* 'Crimson Bedder'	20, 40, 60, 80, 100	259	10 hybrids 2 *N. langsdorffii*	skewed segr. *N. langsdorffii*
N. langsdorffii	*N. alata* 'Crimson Bedder'	0, 10, 15, 20, 100	84	32 fertile hybrids 54 sterile hybrids	Table 2
N. paniculata	*N. alata* 'Crimson Bedder'	0, 20, 50, 100, 150	206	*N. paniculata*	*N. paniculata*
N. paniculata	F_1 of *N. langsdorffii* ×'Crimson Bedder'	0, 20, 50, 100	187	16 hybrids, sterile 106 *N. paniculata*	*N. paniculata*
N. plumbaginifolia	*N. alata* 'Crimson Bedder'	0, 20, 50, 100, 150	121	67 hybrids, sterile 32 *N. plumbaginifolia*	*N. plumbaginifolia*
N. plumbaginifolia	F_2 of *N. langsdorffii* ×'Crimson Bedder'	20, 50, 100	69	4 hybrids, sterile *N. plumbaginifolia*	*N. plumbaginifolia*
N. sylvestris	'Crimson Bedder'	100	97	–	
N. sylvestris	*P. hybrida*	100	109	–	
N. sylvestris	*P. hybrida* delayed *N. sylv.*	100	43	*N. sylvestris*	*N. sylvestris*
P. parodii	*N. alata* 'Crimson Bedder'	100	8	–	

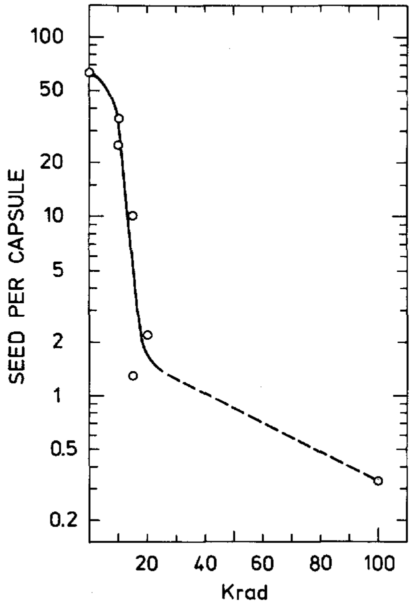

Fig. 2. Viable seed set per capsule in the cross *Nicotiana langsdorffii* × *N. alata* 'Crimson Bedder' after irradiation of the *N. alata* pollen. Note high variability and the irradiation inactivation "tail"

the plants own pollen. Sometimes the sterilizing effect of irradiated pollen was observed (Brown and Cave 1954).

Later most experiments were done with irradiated pollen alone. Anomalous hybrids were obtained in a few cases: *N. alata* white 'Idol' pollinated with *N. alata* 'Crimson Bedder' pollen irradiated with 100 krad gave a progeny of two plants ressembling white 'Idol' and two plants ressembling 'Crimson Bedder'. The standard F_1 hybrid between the two parents ressembles 'Crimson Bedder'. The four plants were classified as two maternal plants and two ordinary hybrids. Unfortunately, no progeny was tested.

Results looking like diploid parthenogenesis were obtained with *Nicotiana paniculata* and *N. plumbaginifolia* with pollen of *N. alata*. A number of plants ressembling the mother were obtained, and when scored in F_2 after selfing, they showed no genes from the irradiated pollen. Maternal plants after pollination with pollen of different species is quite common, especially in *N. paniculata* (Goodspeed 1954).

Table 2 shows the combined results of crosses between *N. langsdorffii* and pollen of *N. alata* 'Crimson

Table 2. Skewed segregation ratios of F_2 plants from the green-flowered *N. langsdorffii* × irradiated pollen of *N. alata* 'Crimson Bedder'. Combined results of two experiments. In the lower part of the table figures have been transformed relative to the number of green-flowered plants

Radiation Krad	Flowers		
	Coloured	Green	White
0	245	42	4
10	227	53	19
15	90	33	4
20	96	49	4
100	104	60	0
0	5.8	1	0.1
10	4.3	1	0.4
15	2.7	1	0.1
20	2.0	1	0.1
100	1.7	1	0

Bedder' irradiated with various doses of gamma rays from ^{60}Co. The segregation from this cross is very complicated, virtually no two F_2 plants being alike. In this work all flowers with a trace of colour were classified as coloured; flowers with a white inner corolla were classed as white, even if the back of the corolla was green. Green flowers comprised both green-whites and greens.

The Table shows skewed segregation favoring the unirradiated maternal parent in the cross *N. langsdorffii* × *N. alata* 'Crimson Bedder'. It is evident from the lower part of the Table that the dominant colour genes become less frequent in the F_2 with higher radiation dose. The Chi square values are above 15 and the skewing therefore highly significant. The over-representation of white flowers in F_2 of the 10 krad treatment is also highly significant. The white flower character comes from *N. alata* but is normally masked by the coloured flower characters.

Six anomalous hybrids between *N. langsdorffii* and *N. alata* 'Crimson Bedder' were obtained in the experiments of Table 2. They had white or white green flowers instead of the dirty red colour of the hybrid (Fig. 1). Chromosome counts showed that all plants had the $2n = 18$ chromosome number in the root tips. All the plants were sterile, while normal hybrids usually set seeds, at least after artificial selfing. Pollen staining with acetocarmine showed one plant with 3%, three plants with 10%, and two plants with about 30% viable pollen. After much coaxing with artificial selfing and repeated spraying with 50 ppm 2,4-dichlorophenoxyacetic acid, some viable seeds were set on one plant where the parent *N. alata* pollen had been irradiated with 100 krad. Twenty-eight progeny plants showed segregation for flower size, shape, and colour and for blue/white pollen, showing that the plant contained several genes from the irradiated pollen.

Discussion

On the basis of the experiments carried out by myself and others, I propose the two theses presented below.

The first is based on observations in several laboratories and is thus fairly certain; the second is more speculative and based on much less experimental evidence.

1) "Gene transfer" by pollen irradiation within species and between species giving fertile hybrids is possible. The results are visible as skewed segregation in the F_2 plants.
2) "Gene transfer" between non-crossing species or species which usually give sterile hybrids is probably a rare phenomenon.

It is certain that "something" may happen after crossing with irradiated pollen. Skewed F_2 segregation ratios, where genes from the mother predominate have been observed in *Nicotiana rustica* by Virk et al. (1977); Caligari et al. (1981); and Jinks et al. (1981), in *N. langsdorffii* × *N. alata* by myself, in barley by Powell et al. (1983), in wheat by Snape et al. (1983), in maize by Pandey (1983), and in pea by Davies (1984). A minor effect has also been observed in tomato by Zamir (1983).

However, I do not believe this to be gene transfer in any strict sense of the word. Rather it might be an extreme case of reduced transmission of chromosomes from the irradiated pollen. This has already been proposed by Snape et al. (1983) and in a more narrow sense as male gametophyte elimination by Zamir (1983). Reduced transmission after irradiation is a well-known phenomenon (Stadler and Roman 1948; Gaul 1963; Dellaert 1980), but the skewing of segregation has not been nearly as dramatic as the one observed in pollen irradiation experiments. Several mechanisms of reduced transmission would probably be at work simultaneously: gametophyte or zygote elimination caused by e.g., translocation sterility, lethal homozygous recessives, deletions, dominant mutations, chromosome elimination, etc. Zamir (1983) found that male gametophyte elimination played only a minor role in tomato pollen irradiation experiments.

Sometimes "gene transfer" is also visible in F_1 hybrids as a lack of one or several characters. This is most simply explained as mutational loss of genes from the pollen. Something like this has been seen by the maize mutation people (Stadler and Roman 1948) as well as by Caligari et al. (1981) and Virk et al. (1977) in *N. rustica,* by Powell et al. (1983) in barley, by Pandey (1983) in maize and by myself in *Nicotiana* crosses.

Gene transfer by pollen irradiation between non-crossing species or species which normally give sterile hybrids is probably rare phenomenon. It has been attempted in *Nicotiana* by Pandey (1980a); Pandey and Phung (1982); by Shizukuda et al. (1983) and by myself. Pandey observed transfer of selfincompatibility from *N. glauca* (2n = 24) to *N. bonariensis* (2n = 18) in seven cases. Pandey and Phung (1982) do not mention gene transfer in crosses of *N. tabacum* (2n = 48) with irradiated pollen of *N. alata* (2n = 18) and *N. glutinosa* (2n = 24), although they observed many maternal and anomalous hybrids. In the experiments described in this paper I have not seen any cases of gene transfer in many species crosses.

Occasionally something looking like gene transfer has been obtained without pollen irradiation, e.g. by East (1930) in strawberry species and by Katayama et al. (1969) in solanaceous species.

I may not have seen single gene transfer because too few genotypes were used as donors of irradiated pollen or because I did not score for selfincompatibility genes. As pollen donors I deliberately chose populations with many genotypes, but I have not tested a large range of donor types of different cultivars. The reason for not working with selfincompatibility is the level of expertise and the amount of extra crossing required. Radiation induced sterility is quite difficult to distinguish from selfincompatibility, and selfincompatibility in species crosses is quite complicated to handle. New selfincompatibility genes arize spontaneously, especially when there is a chance for homozygoty. This is the case when maternal plants are formed (de Nettancourt 1977). I think it is safe to conclude that one should not expect gene transfer between noncrossing species on the first try. It may be possible only under quite specific circumstances like special selfincompatibility genes (Pandey 1980a, b) or it may be a very rare phenomenon like the formation of paternal haploids after species crosses (Goodspeed 1954).

It is still too early to evaluate the possibilities of using pollen irradiation as a substitute for extensive backcrossing. One might introduce so much havoc by the irradiation that one could only get rid of it by several backcrosses.

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