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'Hertwig Effect' in Plants: Induced Parthenogenesis Through the Use of Irradiated Pollen

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Summary. Studies of four combinations of *Nicotiana* involving four species, *N. langsdorffii* (N. l.), *N. alata* (N. a.), *N. glutinosa* (N. g.) and *N. tabacum* (N. t.), have shown that parthenogenetic haploid and diploid maternal individuals may arise with the use of male gametes (pollen) treated with high doses of ionising radiation in plants, similar to that found in animals ('Hertwig Effect'). At lower doses (10–20 Kr) rapidly diminishing numbers of seedlings were produced and many of these died soon after germination or before reaching maturity. In the intraspecific combination N. l. × N. l., viable seeds were produced only at the lower doses of 10 and 15 Kr. In the interspecific combination N. l. × N. a., at lower doses, all plants that came to bloom showed variable hybrid morphology. There were no plants resembling the female parent. In the combination N. t. × N. a., at lower doses there were rare surviving plants which were maternal dihaploid (1 plant out of 4 at 15 Kr) or tetraploid *N. tabacum* (all 5 plants at 20 Kr). All surviving plants at higher doses (50 and 100 Kr) were maternal tetraploids. In the combination N. t. × N. g., plants produced at lower than 20 Kr were almost all either aneuploid or triploid hybrids. Dihaploid, maternal *N. tabacum* plants appeared at 20 Kr and higher doses. After 50 Kr the large proportion of plants produced were maternal dihaploid or tetraploid *N. tabacum*.

Key words: Hertwig Effect – Induced-parthenogenesis – Ionising radiation – *Nicotiana* species

Introduction

In 1911, O. Hertwig found that when frog spermatozoa were exposed to various doses of ionising radiation (radium) and subsequently used to fertilise normal eggs, a paradoxical situation emerged in that, in the lower dose range, increasing dosage led to an increasing amount of embryonic death and abnormality, but that higher dosage was compatible with the production of apparently normal offspring. The phenomenon has been termed the 'Hertwig Effect', and was explained on the grounds that with lower doses the irradiated spermatozoa both penetrated the eggs and effected syngamy, but that with higher doses penetration only was effected and the egg was stimulated to gynogenetic development (parthenogenesis) giving rise to mostly haploid animals. Subsequent work confirmed both the observation and the explanation (Hertwig 1912; Rugh 1939; Rugh and Exner 1940). The present study shows that the 'Hertwig Effect' occurs in plants as well as animals.

Rate maternal haploids occur naturally in intra- and interspecific pollinations in *Nicotiana* and other plants (Goodspeed 1954; de Nettancourt and Stokes 1960; Burk 1962; Burk and Gerstel 1979; Kimber and Riley 1963; Asker 1979; Todua et al. 1973). In addition, parthenogenetic diploidy, the egg doubling its chromosome number and developing into an embryo without normal fertilisation, has been reported in *Nicotiana*, in experiments where irradiated pollen of the same or a related species was used in attempts to overcome intra- and interspecific incompatibility (Pandey 1974a, b, 1977). A major factor spotlighting the phenomenon of parthenogenetic diploidy in plants is the recent discovery of egg transformation in *Nicotiana* (Pandey 1975a, b, 1976, 1978a, b, 1979; Virk et al. 1977; Jinks et al. 1981) where the 'pseudofertilised' egg, pollinated with pollen treated with a high dose of ionising radiation, developed parthenogenetically, but incorporated certain genes from the irradiated pollen parent into its genome. A noteworthy difference between frogs, in animals, and these experiments in *Nicotiana*, in plants, regarding parthenogenetic development of the embryo after pseudofertilisation by irradiated gametes, was that in frogs such embryos were all or overwhelmingly haploids (Rugh 1939), but in *Nicotiana* these were invariably diploids (Pandey 1980a).

In this paper, we have investigated the use of irradiated pollen for inducing parthenogenetic diploidy in *Nicotiana*.

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Materials and Methods

Four species of *Nicotiana* were used, of which three were diploid, *N. alata* (N. a.) (2n = 18), *N. langsdorffii* (N. l.) (2n = 18) and *N. glutinosa* (N. g.) (2n = 24), and one was tetraploid, *N. tabacum* (N. t.) (2n = 48). They were crossed in four combinations using irradiated pollen. The interspecific cross, using normal pollen, is fully compatible in the case of ♀ N. l.×N. a. ♂ and produces fertile plants; is fairly compatible but produces sterile plants in the case of ♀ N. t.× N. g. ♂; and produces large numbers of seeds which germinate but most of which die in the case of ♀ N. t.×N. a. ♂.

The sources of seed material, growing conditions of plants and cytological techniques used have been described before (Pandey 1969). Fresh pollen was collected in glass vials and kept dry over silica gel. Vials placed in a well 30 cm from the cobalt source were irradiated with various doses ranging from 10 Kr to 100 Kr. To promote fruit and seed development after pollination with irradiated pollen, β-naphthoxy acetic acid (1% in lanolin) was applied to the calyx of pollinated flowers. Seeds were treated with commercial hypochlorite bleach (Janola), 1:60 for 5 min, rinsed in distilled water and sown on moist filter paper in petri dishes which were kept in growth chambers at 23 °C, with a light intensity of 600 lux.

Results and Discussion

Table 1 gives results of pollinations with irradiated pollen at doses 10, 15, 20, 25, 30, 50 and 100 Kr in combinations of ♀ N. l.×N. l. ♂, ♀ N. l.×N. a. ♂ and ♀ N. t.×N. a. ♂. In all three combinations at lower doses (10–20 Kr), rapidly diminishing numbers of seedlings were produced. Many of these seedlings died soon after germination and many more died before reaching maturity. In the intraspecific combination N. l.×N. l. viable seeds were produced only at 10 and 15 Kr. At higher doses only empty pods were produced. Apparently this species was more sensitive to radiation damage. In the combination N. l.×N. a., at the lower doses, all plants that came to bloom showed variable hybrid morphology for flower form, size and colour, and had various degrees of sterility including complete sterility. There were no plants resembling the female parent.

In the combination N. t.×N. a., at 10 Kr, of the two surviving plants one was maternal, fertile, *N. tabacum* (2n = 48). At 15 Kr, of the four plants which survived one was dihaploid maternal *N. tabacum* (2n = 24), two were aneuploid hybrids (2n = 24 + 7, 24 + 8) and one was full hybrid (2n = 24 + 9). All were completely sterile. At 20 Kr, however, all five plants which survived were maternal, fertile, tetraploid *N. tabacum* (2n = 48).

At higher doses some seedlings were produced in the combinations N. l.×N. a. (50 Kr) and N. t.×N. a. (50 and 100 Kr). Almost all seeds that germinated at

Table 1. Results of pollinations with irradiated pollen involving three species of *Nicotiana*

Radiation dose Kr	Species combinations					
	N. langsdorffii ×*N. langsdorffii*		*N. langsdorffii*[c] (2n = 18) ×*N. alata* (2n = 18)		*N. tabacum* (2n = 48) ×*N. alata* (2n = 18)	
	Number of pollinations	Number of germinated seeds	Number of pollinations	Number of germinated seeds	Number of pollinations	Number of germinated seeds
10	33	469[a]	20	529[d]	14	369 (2)[h]
15	32	282[b]	27	66[e]	16	280 (4)[h]
20	33	0	31	9[f]	25	46 (5)[h]
25	32	0	25	0	16	0
30	32	0	28	0	19	0
50	45	0	30	5[g]	60	24 (23)[h]
100	60	0	–	–	159	47 (39)[i]

[a] 64 seedlings died soon after germination; a number of abnormal, slow-growing plants died later; 99 random plants were grown to maturity; a few plants were completely sterile; in others, pollen fertility ranged 15 – 80%

[b] 30 seedlings died soon after germination, many others died later; 70 random plants were grown to maturity, early-flowering plants pollen fertility, 10 – 80%; many later-flowering plants were completely sterile

[c] *N. langsdorffii* has green flowers (hom. rr); *N. alata* has coloured flowers (het. Rr)

[d] 39 seedlings died soon after germination; 84 random plants were grown to maturity of which 37 had green flowers, 41 had coloured flowers; early-flowering plants had 48 – 95% pollen fertility, later-flowering plants, 0 – 46%

[e] 22 seedlings died soon after germination, many others died later or did not grow much; hybrid, abnormal, pollen fertility, 0 – 90%

[f] 6 seedlings died soon after germination, with only 3 surviving; hybrid, abnormal, pollen fertility 0, 10 and 90%

[g] From earlier published results (Pandey 1980b). These five plants were morphologically indistinguishable from normal *N. langsdorffii* and were fully fertile (2n = 18)

[h] No. of seedlings surviving

[i] 7 of the 8 seedlings which died perished as a result of accidental damage

these doses grew normally and produced maternal, fully fertile plants. The 5 plants at 50 Kr in N. l. × N. a. combination were normal, maternal diploids (2n = 18). Similarly, all 23 plants examined in N. t. × N. a. (10 plants – 50 Kr, 13 plants – 100 Kr) were normal maternal tetraploids (2n = 48). No particular significance is attached to absence of parthenogenetic plants at 25 and 30 Kr; it may be only a function of number of flowers pollinated.

The combination ♀ N. t. × N. g. ♂ was investigated in greater detail using irradiation doses which were fractionated at closer intervals (Table 2). At doses between 10 and 19 Kr the percentage of fruits that developed after pollination with irradiated pollen remained, in general, the same as with nonirradiated pollen, between approximately 40 and 50 per cent. After this dose there was a sudden jump, which remained generally constant for all higher doses (20–100 Kr), between 80 and 100 per cent. This observation is consistent with the suggestion that at high radiation doses growth promoting substances are released from the pollen coat which promote parthenocarpic fruit and seed testa development (Pandey 1974a, b, 1977). A similar influence of highly irradiated pollen was also shown in interspecific combinations in *Populus* (Stettler et al. 1980) by a higher catkin retention and a higher hollow seed production.

Another interesting phenomenon in the combination N. t. × N. g. concerned the proportion of germinated seeds per pod which survived as mature plants. Although at lower doses, owing to large number of seedlings involved, practical considerations prevented the growth of all the germinated seedlings from a pod into mature plants, the general indication was that this proportion decreased with increasing dose and was relatively low until about 50 Kr after which it rose and became stable, with almost all germinating seeds growing into mature plants. Reasons for this phenomenon become apparent from cytological observations given in Table 3. Cytological studies show that plants produced at lower than 20 Kr were almost all either aneuploid hybrids with various degrees of genetic contribution from the irradiated pollen parent, or they were triploid hybrids (2n = 24 + 12). Dihaploid, maternal, *N. tabacum* plants appeared at 20 Kr and higher doses in varying proportion (30–100%). Triploid hybrids were not found at doses higher than 30 Kr. Aneuploid hybrids appeared in substantial numbers until 50 Kr. At higher doses they became scarce, only five out of 53 plants examined from doses 60 to 100 Kr being aneuploid hybrids. Maternal tetraploid *N. tabacum* plants appeared at 50 Kr at low frequency and appeared at a higher frequency at 70 Kr and 100 Kr. Thus, after 50 Kr the overwhelming proportion of plants produced were the relatively genetically bal-

Table 2. Pollination, fruit set, seed germination and mature plants obtained in the combination ♀ *Nicotiana tabacum* × *N. glutinose* ♂, where pollen was treated with increasing doses of ionising radiation

	Dose in Kr																										
	0	10	11	12	13	14	15	16	17	18	19	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
No. of flowers pollinated	45	42	43	46	43	45	43	40	44	42	42	45	20	30	30	30	30	91	30	30	30	31	30	30	30	30	31	72
% pods formed/flowers pollinated	42	50	60	41	47	40	44	40	41	45	52	78	90	100	97	83	87	84	97	97	84	87	87	90	87	93	100	93
[a] Average no. of seeds germinated per pod	———		500	–	200	———		144	65	47	35	...	...	...	12.5	5.9	2.9	3.5	0.7	1.4	0.6	0.5	0.8	0.4	0.7	0.3	0.6	0.6
% plants formed/seeds germinated	...	...	...	...	...	...	...	...	...	...	...	...	...	...	29	48	55	49	86	93	100	80	88	100	100	100	100	100

[a] At doses higher than 50 Kr, 30 – 80% of the pods produced gave rise to one or more seedlings each

Table 3. Characteristics of plants obtained in the combination *N. tabacum* × *N. glutinosa*, where pollen was treated with different doses of ionising radiation

Combination ♀ *N. tabacum* ($2n=48$) × ♂ *N. glutinosa* ($2n=24$)	Radiation dose to *N. glutinosa* pollen Kr										
	10	15	20	30	40	50	60	70	80	90	100
Total No. of plants examined	8	9	8	10	10	25	10	13	10	8	12
[a] Number of plants in different groups											
Dihaploids ($2n=24$)	0	0	3	6	7	14	10	9	9	8	4
Aneuploids (24+1 – 24+11)	4 (24+10, 24+11 24+11, 24+11)	3 (all 24+11)	4 (24+1, 24+8, 24+8+24+9)	3 (24+1, 24+1, 24+8)	3 (24+1, 24+1 24+8)	9 (24+1, 24+1, 24+1, 24+4, 24+5, 24+7, 24+8, 24+9, 24+10)	0	0	1 (24+4)	0	4 (24+2, 24+2, 24+4, 24+4)
Triploids (36) (24+12)	3	6	1	1	0	0	0	0	0	0	0
[b] Tetraploids (48)	0	0	0	0	0	2	0	4	0	0	4
Tetraploids^{+} (48+)	1 (48+12)	0	0	0	0	0	0	0	0	0	0

[a] Dihaploids, aneuploids and triploids were all sterile (0 – 1% pollen fertility), tetraploids were all fertile (over 90% pollen fertility). Tetraploid^{+} plants had 42% pollen fertility and produced ample viable seed when selfed

[b] Plants came from a single pod at each dosage, the 70 Kr pod also included one dihaploid

anced dihaploid or tetraploid *N. tabacum.* This explains why almost all rare germinated seeds arising from doses higher than 50 Kr grew normally to maturity.

An interesting aspect of the combination N. t. × N. g. was the persistence of a relatively large number of aneuploid hybrids until dose 50 Kr, some with as many as 10 chromosomal bodies or fragments, and the rare persistence of such plants with up to four bodies or fragments even at 80 or 100 Kr. The diploid nature of the egg in the tetraploid species *N. tabacum* may allow not only a considerable, viable development of dihaploid embryos but also a greater survival of aneuploids in terms of number as well as chromosomal range (Tables 1 and 3). Even so, the situation in the combination N. t. × N. g. appears unusual. An alternative explanation may be that the plants obtained at higher doses had received specific genetic segments from the irradiated pollen that were conducive to parthenogenetic development. These segments may have been incorporated into the genome of the plants which lacked additional fragments. This hypothesis is consistent with data from egg transformation studies in *N. alata, N. forgetiana* and *N. langsdorffii,* where S gene transformed parthenogenetic diploids which contained no additional fragments were obtained (Pandey 1975a, 1980a). In fact all 18 parthenogenetic diploid plants obtained in *N. alata* and *N. langsdorffii* were transformed for an S allele, suggesting that a gene or genes closely linked to the S locus were responsible for the promotion of parthenogenetic seed development (Pandey 1980a, b). The fact that there are species and strains of plants (Asker 1979; Gustafsson 1947; Hermsen and Ramanna 1981) and animals (Cuellar 1977; Mittwoch 1978) where parthenogenesis without normal fertilisation is a regular occurrence supports the suggestion that there are specific genes which promote parthenogenetic seed development.

A further feature of the data was the observation that in *N. tabacum,* at higher doses, irradiated pollen from *N. glutinosa* promoted predominantly dihaploidy (Table 3), while that of *N. alata* promoted exclusively maternal tetraploidy (in the text discussion of Table 1), as it also does in its own species and in *N. forgetiana* and *N. langsdorffii* (Pandey 1980a). It therefore seems likely that *N. alata* has specific genes which when transferred to the egg in segmented fashion promote parthenogenetic diploidy, while *N. glutinosa* has, in addition, genes which promote parthenogenetic haploidy. On the other hand, *N. langsdorffii* and *Pisum sativum* used in this and earlier studies (Pandey 1980b), gave no parthenogenetic plants with their own self, irradiated pollen, and therefore appear to lack such genes. Evidently, the genetic constitution of both maternal and paternal parents, particularly of the latter, donor parents, is a significant factor in the successful induction of parthenogenetic embryos through the use of irradiated pollen.

The occurrence of five normal, maternal, tetraploid *N. tabacum* plants at 20 Kr in the combination N. t. × N. a. (in the text discussion of Table 1) shows that parthenogenetic diploidy may occasionally occur at lower doses. The recent results of Jinks et al. (1981) who confirmed an earlier observation of Virk et al. (1977) concerning egg transformation in *N. rustica* ($2n=48$), also suggested that parthenogenetic diploidy may occur at low doses (20 Kr). Indeed, in the present experiments, in the combination N. t. × N. a. one maternal tetraploid ($2n=48$) was obtained at an even lower dose of 10 Kr (in the text discussion of Table 1). Parthenogenetic, maternal tetraploid plants have not been reported in normal crosses with unirradiated pollen in *N. tabacum.* Furthermore, in the combination N. t. × N. g. (Table 3), one pentaploid hybrid plant, tetraploid$^+$ ($2n=48+12$), occurred at the dose of 10 Kr. This shows that parthenogenetic diploidy may, occasionally, not only occur at much lower doses but that it may occur, as in the latter case, in combination with 'normal' fertilisation, resulting in the maintenance of as many as 12 chromosomal bodies or fragments from the irradiated parent. It is also possible that in tetraploid species *N. rustica* gene transfer may be associated with tetraploid-aneuploid plants, having certain genetic segments from the irradiated donor parent, which are partially fertile, producing viable seed. For egg transformation combined with parthenogenetic diploidy in *Nicotiana,* it appears that the results may be sharper and the efficiency improved at higher doses.

The present study shows that parthenogenetic haploid and diploid maternal individuals may arise with the use of male gametes treated with higher doses of ionising radiation in plants as well as in animals. In animals, egg transformation by microinjection of foreign DNA into fertilised eggs has been reported in *Drosophila* (Fox et al. 1975) and in mouse (Gordon et al. 1980; Harbers et al. 1981). Thus the Hertwig Effect combined with egg transformation may have wide implications in the genetic manipulation of higher organisms. In plants, a better understanding of the process of parthenogenesis induced by irradiated pollen would not only greatly facilitate plant improvement through the refinement of the egg transformation technique but would also expedite plant breeding techniques generally.

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