Theor Appl Genet (1985) 70:433–439

THEORETICAL AND APPLIED GENETICS

"Egg transformation" induced by irradiated pollen in *Nicotiana:* a re-examination

Y. S. Chyi and J. C. Sanford

Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, NY 14456, USA

Received December 24, 1984; Accepted January 17, 1985
Communicated by H. F. Linskens

Summary. We have attempted to confirm previous reports of "egg transformation" in seven *Nicotiana* species, including genetic stocks obtained from the original experimenter. The methods employed in the original experiments were duplicated as closely as possible. In total, 1,622 pollinations were made involving irradiated pollen and mixtures of irradiated and self pollen. Consequently, 995 seedlings from 9,052 seeds were screened for 1,594 potentially detectable transformation events. A very low frequency of unexpected progeny resulted, but these results were not repeatable and appear to have arisen by mechanisms other than transformation. These results are strongly at odds with previous claims, when 50% of offspring were found to be transformed. We conclude that the previous observations of high frequency egg transformation are not reproducible. However, due to the ambiguous nature of the markers employed, it is very difficult to prove that such transformation events do not occur as very rare events.

Key words: *Nicotiana* – Pollen – Irradiation – Egg – Transformation

Introduction

Since 1975, Pandey has published extensively on his observations of an unusual genetic phenomenon he has called "egg transformation" (Pandey 1975a, b, 1976, 1978, 1979, 1980a, b, 1981a, b, 1983; Pandey and Patchell 1982). His observations could be summarized by the following four points: 1) Some phenotypic characters of donor male stocks were transferred to non-hybrid (maternal) progenies after using heavily irradiated donor pollen, either alone or as mentor pollen mixed with self-incompatible maternal pollen. 2) The rate of gene transfer was found to be above 50% for the traits he tested, which were self-incompatible specificities, flower color, and pollen color. 3) The proposed mechanism underlying these observations was that the egg cell was transformed by the fragmented chromatin of radiation-treated pollen, and that the transformed egg either developed parthenogenetically into a diploid embryo or was additionally fertilized by self-pollen. 4) Based on the proposed mechanism, it was suggested that this novel technique of gene transfer could be applied to most other plant species by providing a second fertilization with normal pollen after "pseudofertilization" with heavily irradiated donor pollen.

These reports have been of great interest to many plant breeders, for the following reasons: 1) This method might achieve gene transfer in a single generation based simply on selection of a whole-plant phenotype, so it could be developed as an alternative to backcrossing. 2) The only pre-requisite for successful gene transfer would be the ability of donor pollen to traverse the style and discharge its genetic contents into the embryo sac of the recipient species, circumventing the problems of hybrid sterility or inviability inherent in wide crosses. 3) This technique would be simple and easy, not requiring gene identification and cloning at the molecular level, thereby offering an alternative to more sophisticated molecular approaches to gene transfer.

Unfortunately, experiments involving several plant species having reproductive mechanisms somewhat different from *Nicotiana* have failed to extend Pandey's findings outside of the *Nicotiana* genus (Sanford et al. 1984a; Sanford et al. 1984b; Chyi et al. 1984). Therefore,

this research attempted to confirm Pandey's observations of "egg transformation" in *Nicotiana* species, employing, among others, genetic stocks supplied by his lab.

Materials and methods

The same *Nicotiana* taxa and genetic characters used by Pandey were chosen for this study. The specific stocks used in these experiments were from five different sources: 1) Dr. K.K. Pandey, Genetic Unit, Grasslands Division, DSIR, Palmerston North, New Zealand. 2) Drs. L.G. Burk and V.A. Sisson, USDA Tobacco Research Laboratory, Oxford, North Carolina. 3) Dr. D.U. Gerstel, Crop Science Department, North Carolina State University, Raleigh. 4) Dr. H. Takahashi, Otani Women's College, Tondabayashi, Osaka, Japan. 5) Interspecific hybrids produced by the authors. The genetic stocks used, their compatibility type, and their phenotypic descriptions are listed in Table 1.

Experimental procedures, including radiation dosage, followed the methods of Pandey (Pandey 1975a, 1978, 1980a). Many donor/recipient combinations were tested. The donor usually had two dominant (flower and pollen color) or co-dominant (S allele) marker traits, while the recipient was generally multiple recessive. Cross compatibility between the donor and the recipient was confirmed in each case, unless stated otherwise.

Pollen was collected from flowers on the day of blooming or on the next day. Dehiscing anthers were separated from their filaments and stored in gelatin capsules at 4 °C. Generally, donor pollen was collected on the same day as irradiation, to be used within the following 4 days. Radiation dosages were either 80 or 100 kr, and were controlled by adjusting the distance between samples and the Co^{60} source, for a fixed 1 h treatment. Pollen was kept at 4 °C at all times except during irradiation.

In 1982 experiments, no specieal measures were taken to prevent possible cross pollen contamination. In 1983 and 1984, flowers of recipients were protected from possible pollen contamination by cutting off the limbs of the corolla and placing one end of a gelatin capsule over the corolla tube one day before blooming. A pair of forceps were used to apply pollen to the stigma of the flowers, with time of pollinations ranging from a day before blooming to 2 days after. Two methods were adopted to achieve mixed pollination: 1) the two types of pollen were mixed at 1:1 by volume in a gelatin capsule before application, using an electric buzzer; and 2) the stigma was pollinated first with a light covering of irradiated pollen and then with a heavy covering of self pollen. After pollination, a lanolin paste with 1% NAA was applied to the calyx for better fruit and seed set.

Seed pods were harvested at maturity, before splitting. Seeds were extracted, counted, and stored at room temperature for one month before planting. A sample of seeds resulting from mixed pollination was dissected and inspected. The great majority of such seeds were found to be hollow. In a few cases, seeds were sown aseptically in agar medium as described by Pandey (1980a). Flower and pollen colors of resulting progeny were visually screened and S genotypes were identified using tester plants which were derived from the parental stocks by bud-pollination (Pandey 1963). At least two flowers were pollinated for each S tester. Only rejection reactions of special interest were examined under a fluorescence microscope for in vivo pollen tube growth (Kho and Baer 1968).

Results

The results of crosses intended to induce "egg transformation" are summarized in Table 2, 4, and 5, by the year of study. Over the three years, 1,622 pollinations were made, and 14,795 "seeds" (most of which were

Table 1. *Nicotiana* stocks and genetic characters used in egg transformation studies

Species	Code	Compatibility[a]	Flower[b]	Pollen[b]	Source
N. langsdorffii	L	S.C. (S_fS_f)	green	blue	Burk
N. alata	A0	S.I. ($S_{F4}S_{F6}$)[c]	white	white	Sisson
	A1	S.I. ($S_{F11}S_{F11}$)	white	white	Pandey
	A2	S.I. ($S_{F10}S_{F11}$)	white	white	Pandey
	A3	S.I. (S_3S_3)	pink	white	Pandey
	A4	S.I. (S_3F_{F10})	pink	white	Pandey
N. forgetiana	F	S.I. (S_9S_8)	red	blue	Takahashi
N. sanderae	S	S.C.	red	white	Sisson
N. bonariensis	B	S.I.	brown	white	Sisson
N. glauca	G	S.C.	orange	white	Gerstel
N. rustica	R	S.C.	green	white	Sisson
N. tabacum	T	S.C.	pink	white	Cornell
Hybrids:					
L×A3	LA	S.C. (S_fS_3)	pink	blue	Cornell
B×L	BL	S.I.	green	blue	Cornell

[a] SC: self-compatible; SI: self-incompatible; S_{FI}: self-incompatible alleles that are also incompatible to *N. langsdorffii* pollen (Pandey 1964)

[b] See Brieger (1935) for inheritance of flower and pollen colors

[c] Several seedlings with different S genotypes, only one shown here

Table 2. Results of transformation-inducing crosses made in 1982

Treatment[a]	Genes[b]	No. pollinations	No. fruit	No. seeds	No. sown	No. sdlg	No. unexpected
L × *i*A3	R	384	66	357	318	34	0
*i*A3+	R	14	14	2,193	2,193	113	2[c]
*i*LA	R	12	1	1	1	0	–
*i*S	R	20	0	–	–	–	–
*i*F	R	17	4	25	25	5	0
A0 × *i*A4	R, S	9	8	55	55	0	
*i*A4+	R, S	40	33	807	415	54	11[d]
*i*LA+	R, B	5	3	177	177	18	0
*i*S+	R, S	6	5	633	633	38	0
A1 × *i*A3	R	24	6	49	42	16	0
*i*A3+	R	48	12	321	149	27	0
*i*S+	R, S	2	2	46	46	1	0
*i*F+	R	4	2	115	36	0	0
A2 × *i*A3	R, S	17	8	15	15	0	–
*i*A3+	R, S	42	21	118	116	7	0
*i*S+	R, S	3	3	5	5	0	–
B × *i*A3+	R	39	19	69	69	1	0
*i*S+	R, S	19	3	24	24	0	0
*i*LA+	R, B	4	1	43	43	0	–
*i*L+	B	8	6	72	72	0	–
*i*G+	R, S	16	9	120	120	3	0
BL × *i*G+	R, S	40	10	128	128	13	3[e]
*i*A3+	R, S	60	0	–	–	–	–
R × *i*A4	R	38	0	–	–	–	–
*i*A4+	R	17	2	988	988	3	0
*i*S+	R	6	3	410	410	9	0
*i*T+	R	8	2	60	60	21	0
Others[f]	R, S, B	55	13	1,383	1,383	110	0

[a] See Table 1 for stock codes; *i*: irradiated; +: mixed with self pollen
[b] Indicates genes differing between the donor and the recipient, which were screened (R = flower color, S = S allele, B = pollen color)
[c, d, e] See text for descriptions of unexpected seedlings. [f] Included A3, AA, LA, and S as recipients, and L, LA, S, and A3 as donors, in 8 combinations

Table 3. Some unexpected seedling populations from 1982 crosses and their S genotypes

Population	Recipient	Donor	Progeny plants
82-1	$S_{F4}S_{F6}$	S_3S_{F10}	3 S_3S_{F6}, 2 S_3S_{F4}, 1 $S_{F10}S_{F6}$, 3 $S_{F4}S_{F6}$, 1 unknown
82-2	$S_{F4}S_{F6}$	S_3S_{F10}	1 S_3S_{F6}, 1 $S_{F4}S_{F4}$
82-3	$S_{F5}S_{F7}$	S_3S_{F10}	1 S_3S_{F7}, 1 $S_{F10}S_{F5}$
82-4	$S_{F8}S_{F9}$	S_3S_{F10}	1 S_3S_{F8}, 1 $S_{F10}S_{F9}$

apparently empty seed coats) were produced, of which 9,052 were planted. Some 995 seedlings were obtained and screened for multiple markers, which represented 1,594 potential transformation events. The vast majority of the seedlings screened did not show any signs of transformation. However, a few unexpected families were observed, which superficially appeared to be transformed. These require further discussion.

Case 1

Several families from the 1982 experiments involved white *N. alata* pollinated with irradiated red *N. alata* pollen mixed with self pollen. In four unusual families, 11 of 16 total progeny had an S allele of the irradiated red-flowered donor parent, but were themselves white flowered (Table 2, footnote d; Table 3). Several of these potential transformants were bud-selfed and test crossed. S alleles segregated normally, no "triallelic" plants were observed, and no pink-flowered progeny resulted. Since most families showed no trace of transformation while these families appeared to be mostly "transformed", we asked ourselves if pollen contamina-

Table 4. Results of transformation-inducing cross made in 1983

Treatment[a]	Genes[b]	No. pollinations	No. fruit	No. seed	No. sown	No. sdlg	No. unexpected
L × *i*A3	R, S	40	8	70	70	0	–
A0 × *i*A4	R, S	15	5	6	6	0	–
*i*A4+	R, S	34	28	587	262	95	0
*i*A0+		3	1	14	14	4	0
*i*F	R, S	5	0	–	–	–	–
*i*F+	R, S	21	7	86	84	59	0
A1 × *i*A3	R, S	8	5	22	22	3	1[c]
*i*A3+	R, S	14	3	57	57	11	0
A2 × *i*A3	R, S	36	14	91	77	37	0
*i*A3+	R, S	149	77	2,340	362	127	0
*i*A2+		11	1	47	47	29	0
*i*F+	R, S	58	24	2,514	94	18	0
A3 × *i*F	R, S	11	8	41	41	0	–
*i*F+	R, S	45	28	290	110	23	0
*i*A3+		7	4	24	14	5	0
*i*S+	R, S	13	5	113	30	10	0

[a] See Table 1 for stock codes; *i*: irradiated; +: mixed with self pollen
[b] Indicates genes differing between donor and recipient stocks, which were screened (R = flower color, S = S allele)
[c] See text for descriptions of unexpected seedlings

Table 5. Results of transformation-inducing crosses made in 1984

Treatment[a]	Genes[b]	No. pollinations	No. fruit	No. seed	No. sdlg	No. unexpected
L × *i*A3+	R, S	16	7	60	28	0
L × *i*L+		8	2	30	19	0
A0 × *i*A4+	R, S	20	8	45	17	0
A0 × *i*A0+		21	0			
BL × *i*G+	R, S	80	15	66	0	0
A1 × *i*A3	R, S	25	23	37	2	0
A1 × *i*A1		25	0			

[a] See Table 1 for stock codes; *i*: irradiated; +: mixed with self pollen
[b] Indicates genes differing between donor and recipient stocks, which were screened (R = flower color, S = S alleles)

tion had occurred. This was possible since in this type of intra-species cross, contamination with non-irradiated pollen can only be detected on the basis of flower color. After testing possible sources of contamination, we found one white flowered *N. alata* plant which had been obtained from Dr. Pandey and should have been $S_{F11}S_{F10}$ but was actually S_3S_{F10}. Non-irradiated pollen contamination from this plant would have given us the observed results. Eliminating this possible source of pollen contamination, and using more careful pollen control measures, we repeated these crosses twice. No further evidence of transformation was observed.

Case 2

A second case involved a rare female recipient which was an interspecific hybrid of *N. bonariensis* by *N. langsdorffii* (footnote e in Table 4), which was fertile but strictly self-incompatible. Bud-pollination even by the most efficient methods (Pandey 1963) failed to produce any self seeds. Irradiated mentor pollen of various species also failed to lead to seed set, except in one case, where *N. glauca* was employed as mentor. Out of the 13 plants so produced, three were pink in flower color, and one of these was a D.S. mutant, by Pandey's definition (Pandey 1980a).

Since the hybrid female parent in this cross was white flowered and the mentor *N. glauca* was orange flowered, it might appear that the pink flowers in the progeny derived their color from *N. glauca.* However, it must be kept in mind that when a large number of crosses are made using incompatible or infertile pollen, there is tremendous selection favoring seed set from stray pollen. The same cross was later repeated twice using *N. glauca* as mentor, with no viable seeds produced (Table 5). In addition, the hybrid white flowered female had *N. bonariensis* as a parent – which has anthocyanin genes expressed as reddish brown on the outside of the corolla. Therefore, the white female should be heterozygous for this gene, which must be recessive or must be repressed by other genes. These recessive genes should segregate normally in the F_2. In a new genetic background, such genes might be expressed as pink. In a similar manner, deleterious mutants such as the D.S. mutant might be expected to arise in the F_2 segregation from a wide cross. Ironically, because we were entirely unable to overcome the strict incompatibility of the hybrid female, we could not generate a proper control F_2 population. Therefore, we could not determine if the D.S. mutant and pink flowers reflected "transformations", pollen contamination, or if these traits were natural components of the F_2 segregation from this wide interspecific hybrid.

Case 3

A third possible transformant arose when a white flowered *N. alata* plant was pollinated with irradiated pollen from a red-flowered *N. alata,* with no self pollen included. Three seedlings resulted (Table 4), and one of these was unusual in having atypical leaf morphology, stunting, and small flowers. This plant had pink flower color and an S-allele different from that in the female. However, this new allele was also different from that of the donor, yet was the same as another S-allele in our genetic stocks. This plant was extremely weak and died before a chromosome count could be made, although pollen size and chloroplast counts indicated that it was not haploid. Limited crosses were made in 1984, attempting to repeat these results, but seed set was low and no unusual progeny were recovered (Table 5).

Case 4

A fourth case of possible transformation involved *N. langsdorffii* as a female recipient, pollinated with irradiated pollen from a pink-flowered *N. alata* plant (homozygous for S_3 allele) mixed with self pollen (Table 2). This resulted in 113 non-hybrid, maternal-type progeny. Two of these were unusual in being strictly self-incompatible. *N. langsdorffii* is normally a self-compatible species and all siblings of these two plants were self-compatible. Therefore, the two plants might appear to be transformants. However, self-incompatible *N. langsdorffii* plants do arise naturally, presumably by mutation (Kostoff 1943; Dr. Sisson, personal communication). The self-incompatibility alleles in these two *N. langsdorffii* seedlings appear to be fully dominant, while in typical *langsdorffii/alata* hybrids the self-incompatible phenotype is recessive (Brieger 1935). The two SI plants are mutually and reciprocally compatible, proving that they are not homozygous for the donor S_3 allele. They are also reciprocally compatible with normal *N. langsdorffii* and *N. alata.* When crossed to *N. alata* (a homozygous S-allele tester), progeny are divisible into two separate compatibility groups (17 self-compatible : 14 self-incompatible). Both compatibility groups in the *N. langsdorffii* × *N. alata* hybrid family are compatible to the original *N. alata* donor, as were the two SI *N. langsdorffii* plants. Progeny in the hybrid family have intermediate length styles, which should be long enough to allow the normal incompatibility reaction to occur (Pandey 1979). Taken together this all provides relatively good evidence that the SI alleles in the two *N. langsdorffii* are different from the S_3 allele in the original donor plant.

Discussion

Pandey (1975 a) has been the first one to report on high frequency transformation in higher plants, using heavily irradiated pollen as both the donor and the vector. Based on our present knowledge on plant reproduction, it is not unreasonable that such an event as "egg transformation" would actually occur.

A variety of plant reproductive mechanisms do exist, which in combination would yield the results observed by Pandey. First, the level of irradiation employed by Pandey will "pulverize" pollen chromosomes into fragments of chromatin, thereby precluding any possibility of true hybrids, yet providing the genetic debris appropriate for transformation. Secondly, such irradiated pollen retains the remarkable ability to physically traverse the style and "microinject" its fragmented genetic contents into the embryo sac. Therefore, such radiation-damaged pollen chromosomes are delivered into the egg sac and can "pseudo-fertilize" the egg and endosperm (Nishiyama and Uematsu 1967; Vassileva-Dryanovska 1966). Thirdly, plant eggs often have the capability to develop parthenogenically (Nygren 1967), or, a second pollen tube can enter the same egg sac (Sprague 1929, 1932; Vigfusson 1970), providing normal fertilization. Additionally, pollen tube fusions in the style might account for the mentor effect while offering a mechanism selecting exclusively for polyspermous viable fertilizations. Moreover, the reproductive properties involved in the proposed mechanism for "egg transformation" are specifically known to exist in *Nicotiana.* Self-incompatibility has been long known in *Nicotiana* (East and Mangelsdorf

1925; East and Yarnell 1929), and the mentor effect of overcoming self-incompatibility by irradiated pollen in *Nicotiana* is well established (Pandey 1975a, b, 1977, 1979; Ramulu et al. 1979). Apomixis is believed to occur in *Nicotiana,* especially after interspecific hybridization (East 1930; Goodspeed 1954). The parthenogenetic form of apomixis was also demonstrated in a recent study (Pandey and Phung 1982). At least one case of polyspermy was reported in *Nicotiana* (Goodspeed 1954, Fig. 39–17). All these properties would make *Nicotiana* one of the most promising genera to look for "egg transformation", which presumably would occur as a very rare event.

Pandey, however, has reported an extremely high frequency (> 50%) of "egg transformation" (Pandey 1980a). This could only be explained if intense cell selection for transformants was occurring in style and/or ovary. The pollen/style incompatibility system could be the basis of such cell selection, or as Pandey proposed, there might be and "embryo growth promoting gene" linked to the S gene complex (Pandey 1980b, 1983). In the present study, we have used the same plant species that Pandey used, and in many cases used direct descendants from his stocks. Our results clearly indicate that high rates of egg transformation do not occur. A few rare events appeared as possible cases of transformation, and bear further discussion.

In case 1 the occurrence of high rates of transformation within four families appeared to be too good to be true (relative to all other families). Subsequently, we identified a misclassified plant which could have served as a source of pollen contamination and would better explain our "transformants". When this possible pollen contamination was precluded, the high rates of "transformation" could not be reproduced in later experiments. Therefore, case 1 appears to be an example of genetic contamination. While such genetic contamination should theoretically be avoidable, in reality rare cases are inevitable wherever extensive work is proceeding using a wide array of genotypes. It should be kept in mind that when crosses are largely infertile, there arises a strong selective pressure favoring seed set from stray pollen grains.

In case 2, a very unusual female happened to be employed as recipient. This female was expected to produce unusual segregating progeny regardless of transformation, yet ironically its strict self-incompatibility precluded the formation of a proper control F_2 population. Therefore, the "D.S." mutant and pink flowers resulting from mixed pollinations are very interesting but inconclusive. Furthermore, these results could not be reproduced, and may again represent genetic contamination.

Case 3 does not seem to be fully explained by any hypothesis. The foreign S-allele specificity would suggest pollen contamination, but this would not explain the stunting or other plant abnormalities. Co-transformation for flower color and S-alleles is also very unlikely, since our data indicates that these genes are not linked, and the wrong S-allele occurs in the progeny. Perhaps this plant may represent a partial hybrid resulting from a pollen grain which received something less than a full dose of irradiation. Again, this abnormality was not reproducible.

In case 4 it would seem likely that the self-incompatibility in *N. langsdorffii* arose by mutation. If an S-allele was actually transferred to *N. langsdorffii,* its expression was dramatically altered in its new genetic background, since the self-incompatibility became dominant instead of recessive and had a specificity different from the original allele.

In conclusion, we feel it is doubtful that any of these cases represent examples of egg transformation. Certainly none of them constitute a clear-cut demonstration of the phenomenon. We have employed the same markers and often the same stocks as Dr. Pandey. Dr. Pandey had no difficulty in obtaining egg transformants, in fact he generated 170 of them from only 180 crosses and 331 seedlings, with an overall transformation rate of greater than 50% (Pandey 1980a). We have made over 1,622 crosses and have screened over 995 seedlings, but have not found a single clear-cut example of egg transformation.

One reason for the difficulty in obtaining clear-cut results in this research is the nature of the markers employed. A basic premise underlying Pandey's work is that flower color and self-incompatibility are simple single gene traits in *Nicotiana,* and are closely linked. Our own data show that this is not the case. Flower color frequently displayed distorted segregation ratios, and tended to show continuous rather than discrete segregation (in the closely allied genus *Petunia,* over 23 genes have been described affecting flower color, Wiering 1974). Our experience with S-alleles indicates that this also is a very poor genetic marker, being subject to numerous complicating factors. Mulcahy and Mulcahy (1983) have recently reviewed evidence that the historic characterization of self-incompatibility as a single gene is a serious over-simplification of a complex genetic trait. We tend to agree with this assertion.

Central to Pandey's hypothesis is the postulate that the flower color gene and the self-incompatibility locus are closely linked. From our test crosses we have clear evidence that these traits segregate independently, dispelling a major postulate used to rationalize previous egg transformation work. If two random unlinked genes were being transformed at very high rates, it would suggest that many other genes should also be transferred at high rates. This would mean that progeny would have large numbers of genes from the donor, and would be pseudohybrid in phenotype. This was clearly not observed by Pandey or ourselves, and raises serious doubts about the credibility of Pandey's proposed mechanisms of transformation.

Given that major research efforts have failed to extend Pandey's findings to other genera (Sanford et al. 1984a; Sanford et al. 1984b; Chyi et al. 1984), or to confirm his findings in *Nicotiana* (Engvild 1981, this study), and given that his central premise of linkage is incorrect, it would appear that Dr. Pandey's entire thesis may be mistaken. Some form of systematic error might explain this. Although numerous publications have resulted, the actual number of families Pandey studied was quite low. Since we found at least two cases of genetic contamination in stocks supplied by Dr. Pandey, this would seem to be a likely source of error. Although we are puzzled by a few rare events we have observed, as described above, we regret to say that in our view this direction of research does not seem sufficiently promising to warrant further investigation in our lab.

Acknowledgements. The authors would like to respectfully thank Dr. Pandey for supplying his genetic stocks of *Nicotiana.* We would also like to thank Dr. Burk, Dr. Sisson, Dr. Gerstel, and Dr. Takahashi for their genetic stocks. We thank Dr. H. C. Aderhold for aiding us in the use of the Gamma Cell irradiation facilities.

References

Brieger FG (1935) Genetic analysis of the cross between the self-fertile *Nicotiana langsdorffii* and the self-sterile *N. sanderae.* J Genet 30:79–100

Chyi YS, Sanford JC, Reisch BI (1984) Further attempts to induce "egg transformation" using irradiated pollen. Theor Appl Genet 68:277–283

East EM (1930) The origin of the plants of maternal type which occur in connection with interspecific hybridizations. Proc Natl Acad Sci USA 16:377–380

East EM, Mangelsdorf AJ (1925) A new interpretation of the hereditary behavior of self-sterile plants. Proc Natl Acad Sci USA 11:166–171

East EM, Yarnell SH (1929) Studies on self-sterility. 8. Self-sterility allelomorphs. Genetics 14:455–487

Engvild KC (1981) On gene transfer from radiation inactivated pollen of *Nicotiana.* Incompatibility Newslett 13: 111–113

Goodspeed TH (1954) The genus *Nicotiana.* Chron Bot 16:1–536

Kho YO, Baer J (1968) Observing pollen tubes by means of fluorescence. Euphytica 17:198–302

Kostoff (1943) Cytogenetics of the genus *Nicotiana.* Sofia State's Printing House, Bulgaria

Mulcahy DL, Mulcahy GB (1983) Gametophytic self-incompatibility reexamined. Science 220:1247–1251

Nihiyama I, Uematsu S (1967) Radiobiological studies in plants. 8. Embryogenesis following X-irradiation of pollen in *Lycopersicum pimpinelifolium.* Radiat Bot 7:481–489

Nygren A (1967) Apomixis in the angiosperms. Handb Pflanzenphysiol, vol 18, Springer, Berlin Heidelberg New York, pp 551–596

Pandey KK (1963) Stigmatic secretion and bud-pollination in self- and cross-incompatible plants. Naturwissenschaften 50:408–409

Pandey KK (1964) *S* gene polymorphism in *Nicotiana.* Genet Res 10:251–259

Pandey KK (1975a) Sexual transfer of specific gene without gametic fusion. Nature 256:310–313

Pandey KK (1975b) Transformation, incompatibility and plant improvement. Incompatibility Newslett 6:91–121

Pandey KK (1976) Genetic transformation and 'graft-hybridization' in flowering plants. Theor Appl Genet 47:299–302

Pandey KK (1977) Mentor pollen: possible role of wall-held pollen growth promoting substances in overcoming intra- and interspecific incompatibility. Genetica 49:219–229

Pandey KK (1978) Gametic gene transfer in *Nicotina* by means of irradiated pollen. Genetica 49:53–69

Pandey KK (1979) Overcoming incompatibility and promoting genetic recombination in flowering plants. New Zealand J Bot 17:645–663

Pandey KK (1980a) Further evidence for egg transformation in *Nicotiana.* Heredity 45:15–29

Pandey KK (1980b) Parthenogenetic diploidy and egg transformation induced by irradiated pollen in *Nicotiana.* NZ J Bot 18:203–207

Pandey KK (1981a) Induction of parthenogenetic diploidy and egg transformation in *Nicotiana.* Incompatibility Newslett 12:5–8

Pandey KK (1981b) Egg transformation through the use of irradiated pollen: poor man's genetic engineering. Incompatibility Newslett 13:119–123

Pandey KK (1983) Irradiated pollen-induced egg-transformation in plants: prospects for rapid plant improvement. In: Mulcahy DL, Ottaviano E (eds) Pollen: Biology and implications for plant breeding. Elsevier Biomedical, New York, pp 117–123

Pandey KK, Patchell MR (1982) Genetic transformation in chicken by the use of irradiated male gametes. Mol Gen Genet 186:305–308

Pandey KK, Phung M (1982) 'Hertwig effect' in plants: induced parthenogenesis through the use of irradiated pollen. Theor Appl Genet 62:295–300

Ramulu KS, Bredemeijer GMM, Dijkhuis P (1979) Mentor pollen effects on gametophytic incompatibility in *Nicotiana, Oenothera,* and *Lycopersicon.* Theor Appl Genet 54:215–218

Sanford JC, Chyi YS, Reisch BI (1984a) An attempt to induce "egg transformation" in *Lycopersicon esculentum* Mill. using irradiated pollen. Theor Appl Genet 67:553–558

Sanford JC, Chyi YS, Reisch BI (1984b) Attempted "egg transformation" in *Zea mays,* using irradiated pollen. Theor Appl Genet 68:269–275

Sprague GF (1929) Hetero-fertilization in maize. Science 69: 526–527

Sprague GF (1932) The nature and extent of hetero-fertilization in maize. Genetics 17:358–368

Vassileva-Dryanovska OA (1966) Fertilization with irradiated pollen in *Tradescantia.* Radiat Bot 6:469–479

Vigfusson E (1970) On polyspermy in the sunflower. Hereditas 64:1–52

Wiering H (1974) Genetics of flower colour in *Petunia hybrida* Hort. Genen Phaenen 17:117–134