Self-Incompatibility System of *Oenothera organensis* for the Detection of Genetic Effects at Low Radiation Doses

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The self-incompatibility system of *Oenothera organensis* is used to analyze the frequency of S locus mutations induced by low doses (2.5–20 rad) of fast neutrons and x-rays. The principles and methods of the screening system for detection of low dose effects have been presented.

The results show that low doses induced significantly higher frequencies of seeds and seedlings than those produced spontaneously. The analyses of part of the progenies derived from the control and treated series suggested that they were due to revertible mutations. A modified *in vitro* method of culturing pollinated cut styles has been developed for rapid screening of compatible pollen tubes.

**Introduction**

Genetic effects of low doses of radiation are attracting more attention, because of public concern in the increasing use of nuclear energy. In higher organisms, analyses of frequencies of mutation induced by low doses of radiations are difficult, because they require extremely large populations for the detection of a statistically significant increase in the mutation frequency above the spontaneous level. The establishment of the dose-effect relationships and RBE values at low dose ranges requires the development of special test-systems.

Certain test systems in higher plants have already been used for radiation studies at low doses, namely somatic mutations for flower color in *Tradescantia* (1–4) and the chlorotic spots in *Nicotiana tabacum* (5, 6), and gametic mutations for the waxy character in pollen grains of barley and maize (7–11).

The self-incompatibility system in monofactorial gametophytic species of higher plants, which is based on the rejection of incompatible pollen tubes, bearing the same S alleles as those present in the pistil, by styal barrier can be a unique test-system for detecting and analyzing the frequencies of mutation induced at low radiation doses. The S locus has a tripartite structure (12, 13): two activity parts control the reaction in the pollen and in the style respectively, and one specificity part, common to both pollen and style, which determines the specificity. It is uniquely suitable for analysis of different types of mutations:

1. **self-compatibility mutations**, namely styal-part or pollen-part mutation resulting from negative changes (deletions or inactivation) in pollen-or styal-part of the S locus, and
2. **new self-compatibility alleles** resulting from a constructive (positive) modification in the specificity part of the S locus (14). These modifications are, when they lead to a breakdown of the incompatibility relationship, automatically screened by the styal barrier, and selected out of millions of individual cells for transmission to the next generation. Thus, extremely large number of individuals can be analyzed.

With respect to the mapping of S locus in higher plants, the genetic segregation of S alleles and the involvement of chromosomal aberrations in the manifestation of self-compatibility mutations clear-
ly suggest a chromosomal location of self-compatibility loci, but little information is available to date on the site of the S gene within the genome of a self-incompatible plant. The attempts made to identify S-bearing chromosome in Lycopersicon and Nicotiana have been recently reviewed by de Nettancourt (14).

The S system of Oenothera organensis exhibits rigorous incompatibility, i.e., complete absence of pseudo-fertility (15, 16). Lewis (16–18) demonstrated a close agreement between the pollen tube growth method (compatible pollen tubes in the style) and seed-set method for determining mutation rates at the S-locus of O. organensis.

Furthermore, because of its long style and due to pollen tube growth inhibition at or near the stigma, O. organensis can also be a suitable material to develop biological screening methods for pollen mutated at the S-locus by in vitro culture of pollinated style segments (19, 20).

Research with radiations on the one locus gametophytic system has been so far limited to the study, at high doses, of physiological and genetic effects of acute and chronic irradiations (14, 21).

The present article reports on the principles and methods used for the detection and analysis of genetic effects of low doses (2.5, 5, 10, 20 rad) of fast neutrons and x-rays, the results obtained so far on the frequencies of S mutations as determined from the number of seeds and seedlings, and some attempts made to develop a semi in vitro screening method for pollen mutated at the S locus.

**Material and Methods**

**Genotype**

To deal with a genetically homogeneous population, of Oenothera organensis a self-incompatible diploid clone III-4/55 was established for a single S$_3$S$_4$ plant by stem-cutting technique (22). The plant (S$_3$S$_4$) was obtained from seeds kindly provided by Prof. A. Hecht.

The clone, III-4/55 has an extremely good capacity for vegetative propagation. The propagation and planting were carried out under controlled conditions (16 hr day, 12,000 lux, 23°C; 8 hr night, 17°C; relative humidity 70–80%). Under these conditions, the plants flower almost throughout the year.

**Irradiation**

The source of fast neutrons was the BARN-reactor at ITAL, Wageningen, The Netherlands. The reactor was set at 800 W and the irradiation treatments were given at a dose rate of 0.17 rad/min the γ-contamination was approximately 3% on GY basis. The x-irradiation was applied with a Philips deep-therapy apparatus operating at 160 kVp and 9 mA with an additional 0.25 Cu 1.0 Al 0.4 Sn filter. The dose rate was 0.25 rad/min.

**Treatment of Pollen Mother Cells (PMC)**

Since S gene mutations induced after the tetrad stage are too late to express themselves in the pollen (18, 23), the irradiation treatments were applied to meiotic cells. The flowering shoots of Oenothera organensis have a graded developmental series of buds, the youngest being at the tip and the oldest at the base of the shoot. Under our conditions of growing, about 9 mm flower bud size coincides with prophase and 14 mm bud size with tetrad stage. Buds that were 9–13 mm long at the time of irradiation were marked and the pollen from these flowers was used. For plants in the control series, the same procedure was followed. The marked buds opened into flowers after 19–22 days in the control and irradiated series.

**Pollination Procedures and Detection of Irradiation Effects**

Totals of 585 and 417 flowering plants were treated with fast neutrons and x-rays, respectively. The control series consisted of 600 plants. Five to fifteen plants of the same age at each dose were irradiated each time (replication), the interval between one replication and the other being two or more weeks. In all, 34 replications in fast neutron series and 23 replications in x-ray series have been carried out.

To transmit modifications in the self-compatibility character, mature pollen from flowers which had been in meiosis at the time of irradiation, was applied to the stigmas of flowers of untreated plants from the same S$_3$S$_4$ clone. All the pollinations have been made by the method of Lewis (17): pollen from two anthers was applied on the receptive surface of each stigma in a thin layer.

Attempts were made to score the effects of irradiation on the pollen component of the S-gene because large-scale scoring is possible.

To prevent abscission of flowers and to induce swelling of the ovaries, 1% naphthalene acetamide in lanolin was applied around the calyx of the flowers immediately after pollination (17, 24). Every capsule, whether big or small, which developed after pollination with the pollen from treated or untreated plants has been harvested and checked for seed-set.
The seeds were sown in petri dishes on a Perlite medium (soaked with water) and germinated under controlled conditions as described above. The seedlings at the 2–4 leaf stage were transplanted into soil in small pots. At anthesis the plants were self-pollinated and test-crossed with the mother clone.

**Estimation of Pollen Grains on Pollinated Stigmas**

To determine the mutation rate it is necessary to know the number of pollen grains that are being tested. To do this, the mean number of pollen grains per pollinated stigma must be estimated. The dilution method described by Lewis (17) for *O. organensis* was used with some modifications. A mixture of 1:3.7 parts (by volume) of absolute alcohol and chloroform has a density in which the pollen grains remain suspended. Two pollinated stigmas were placed in a flask containing 10 ml of the mixture and two samples of 1 ml were withdrawn by pipet from each flask and placed on a calibrated slide for counting. The pollen from two anthers was applied on the surface of each stigma in a thin layer (approximate monolayer). Estimation of pollen grains was made from samples of about 20 stigmas collected periodically. The pollinations were made by one person. The number of aborted pollen grains was subtracted while estimating the number of tested pollen grains for the treated and control series. The mean number of pollen grains per stigma was 4483 ± 121.

**Estimation of Mutation Rates**

Several factors can influence the estimation of mutation rates, the important ones being the number of pollen grains deposited on a stigma, time of *S* gene action and the type of self-incompatibility system of the species.

The number of pollen grains accommodated on a stigma can be influenced by several variables (25) which may be overcome to a certain extent by adopting a standard pollination technique, by growing the plants under controlled conditions and by performing experiments each time (replication) with a few selected plants; the replications were well spaced in interval.

The time of *S* gene mutation during microsporogenesis is another important variable. If a mutation has been induced in a late stage of pollen development, there will be only one mutated pollen grain and hence one pollen tube in the style or one seed in the capsule. The earlier in development the mutation is induced the higher will be the number of mutated pollen grains, hence the larger number of seeds in the capsule. For comparative analyses of mutation frequencies treatments can be applied to buds in a particular stage, e.g., post pachytene (but before tetrad), being the most sensitive stage: a mutation at this stage results in a single seed per capsule.

The species chosen must have a rigorous incompatibility system, so that interference by pseudo-fertility during scoring procedures can be avoided.

In the present study all these factors have been considered for analyses of frequencies of mutations. Yet, the number of seeds or seedlings determined per total number of pollen grains tested may be considered as approximate estimates.

**Pollen Fertility**

Pollen stainability was assessed by tests with Alexander's stain (26). The counts were based on 200 pollen grains per flower and 1–2 random flowers which were in meiosis at the time of irradiation per plants. In all, 40 plants in the control and 30 plants per dose in the treated series were analyzed.

**Method of Culturing Pollinated Cut Styles for Scoring Compatible Pollen Tubes**

The modified *in vitro* method is briefly summarized below. It involves incubation at 32°C in dark for 30 min, of detached flowers (without any self-pollen on the stigmas) from plants grown under controlled conditions; pollination of flowers followed by incubation at 32°C in dark for 4 hr; collection of the styles from flowers; cutting with a sharp razor the first 60 mm of style (with stigma) in the culture medium; planting the style segment on the medium, the cut-end (½–1 cm) being in the medium in the Petri dish; incubation of petri dishes at 32°C for about 20 hr; counting of pollen tubes grown from the cut-ends into the medium with the aid of a stereomicroscope.

The culture medium adopted by Brewbaker and Kwack (27) for pollen germination in several plant species was slightly modified and tested for pollen germination and tube growth in the clone III–4/55 of *O. organensis* (28) and also used in the present study for culturing the pollinated cut-styles. It has the following composition: 10% sucrose, 300 ppm Ca(NO₃)₂·4H₂O, 200 ppm MgSO₄·7H₂O, 100 ppm H₃BO₃, 100 ppm KCl, 0.5% agar.

**Calculations**

The frequency of seeded capsules was calculated per 100 pollinations and the frequency of seeds per
pollination. The statistical analysis is based on the assumption, that the variable (the number of seeded capsules and seeds) follow a Poisson distribution with parameter \( N\lambda \), where \( N \) is the number of flowers pollinated and \( \lambda \) is the expectation of the variable (capsules or seeds) per pollinated flower.

### Results

#### Pollen Abortion

The treatments with low doses of fast neutrons and x-rays significantly (\( p < 0.01 \)) increased pollen abortion (Fig. 1). Percentage abortion increased with increasing dose, being significantly higher following treatment with 20 rad fast neutrons than after 2.5–10 rad fast neutrons or 2.5–20 rad x-rays.

#### Frequencies of Capsules and Seeds

The frequencies of seeded capsules and seeds after pollination of unirradiated \( S_2S_4 \) plants with the pollen from the irradiated or unirradiated \( S_2S_4 \) plants are given in Table 1. In the control the mean frequency of seeds per pollination was 0.027. The low doses of fast neutrons and x-rays induced significantly higher frequencies of seeded capsules, and seeds than those produced spontaneously.

Within the dose range of 2.5–20 rad x-rays, no significant differences in the frequencies of seeded capsules or seeds were observed. In the case of fast neutron treatment (2.5–10 rad), the frequencies of

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**Table 1. Frequencies of capsules and seeds after crosses between unirradiated \( S_2S_4 \) plants, used as female parents and irradiated and control \( S_2S_4 \) plants in *Oenothera organensis*.

| Irradiation treatments, rad | Flowers pollinated | Capsules | Seed per 100 pollinations | Seeds | Viable seeds |
|-----------------------------|--------------------|----------|--------------------------|-------|-------------|
|                             | No.                | ± S.E.   | No.                      | ± S.E. | No.                      |
| Control Fast neutrons       | 5380               | 54       | 1.00 ± 0.14              | 23    | 0.43 ± 0.09              |
| 2.5                         | 1081               | 51       | 4.72 ± 0.66             | 17    | 1.57 ± 0.38             |
| 5                           | 1056               | 36       | 3.41 ± 0.57             | 15    | 1.42 ± 0.37             |
| 10                          | 889                | 26       | 2.92 ± 0.57             | 11    | 1.24 ± 0.37             |
| X-Rays                      |                    |          |                          |       |                          |
| 2.5                         | 455                | 15       | 3.30 ± 0.85             | 24    | 0.053 ± 0.01           |
| 5                           | 479                | 20       | 4.17 ± 0.96             | 28    | 0.058 ± 0.02           |
| 10                          | 480                | 17       | 3.54 ± 0.88             | 25    | 0.052 ± 0.01           |
| 20                          | 430                | 15       | 3.49 ± 0.90             | 26    | 0.060 ± 0.02           |

\( a \ 0.01 < p < 0.025 \quad b \ 0.025 < p < 0.05 \quad c \ 0.05 < p < 0.10 \)

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Table 2. Frequencies of capsules with varying number of seeds after crosses between unirradiated S₃S₄ plants, used as female parents and irradiated and control S₃S₄ plants in Oenothera organensis.

| Seeds per capsule | Control | Fast neutrons | X-Rays |
|-------------------|---------|---------------|--------|
|                   |         | 2.5 rad | 5 rad | 10 rad | 2.5 rad | 5 rad | 10 rad | 20 rad |
| 0                 | 5326    | 1039    | 1020     | 863     | 440     | 458    | 463    | 415    |
| 1                 | 32      | 43      | 35       | 22      | 10      | 14     | 15     | 12     |
| 2                 | 8       | 7       | 1        | 2       | 2       | 4      | 1      | 1      |
| 3-4               | 9       | 1       | 0        | 2       | 3       | 2      | 0      | 1      |
| >4                | 5       | 0       | 0        | 0       | 0       | 0      | 1      | 1      |

capsules, a high percentage (60%) also had one seed, 15% had 2 seeds, and 25% had 3 or more seeds.

Capsules with Seedlings

Data on germination are not yet available for the x-ray series. In the fast neutron series, 90% of the capsules, which gave rise to seedlings were single-seeded type (Table 3). Among the spontaneously generated capsules that produced seedlings, 70% were of the type with one or two seeds; the other had three or more seeds.

Progeny Testing for S Mutations

Of the 277 seeds, 87 germinated. These eventually gave rise to 61 mature plants, the others died at different stages of the growth and development (Table 4). Xantha seedlings occurred often in the progenies, because the clone III-4/55 (S₃S₄) is heterozygous for Xantha. Some morphological variants were also found which were characterized by having grasslike leaves, leaves with anthocyanin pigmentation, and by poor growth and development.

From the 61 surviving plants, 43 have been so far tested for S mutations, 20 being from the fast neutron series and 23 from the control. All were highly fertile and normal in growth and morphology. The results of self-pollinations and reciprocal crosses with the original mother clone (S₃S₄) showed that all the 43 progeny plants were both self- and cross-incompatible. Thirty-six plants were S heterozygotes, i.e., S₃S₄, and seven S homozygotes. The data of the crosses among the homozygotes indicated that all contained the same S allele, S₃ or S₄, in homozygous condition; it is yet to be determined if all are of S₃S₄ or S₄S₄ constitution.

Figure 2. Style, capsules, and seeds in S₃S₄ of Oenothera organensis: (a) style with ovary and stigma; (b) capsule formed after a cross-compatible pollination; (c) capsule as in (b), showing seeds after opening; (d) single-seeded capsule development after pollination with the pollen of irradiated plants; (e) capsule as in (d), showing a seed after opening.
Table 3. Frequencies of capsules with varying number of viable seeds after crosses between unirradiated $S_3S_4$ plants, used as female parents and irradiated and control $S_3S_4$ plants in Oenothera organensis.

| Seeds per capsule | No. of capsules with viable seeds |
|------------------|----------------------------------|
|                  | Control | 2.5 rad | 5 rad | 10 rad |
| 1                | 11      | 16      | 14    | 9      |
| 2                | 5       | 1       | 1     | 1      |
| 3–4              | 3       | 0       | 0     | 1      |
| >4               | 4       | 0       | 0     | 0      |

* Viable and nonviable seeds.

Modified in vitro Method of Culturing Pollinated Cut Styles

The basic requirements of the method are to allow the compatible (mutated) pollen tubes to grow out of the basal ends of the cut styles into culture medium and to inhibit all the incompatible pollen tubes near the stigma. The effects of some factors have been investigated, namely temperature (25, 32, 37°C), duration of incubation (1, 2, 4 hr) and the length of cut styles (20, 40, 60 mm). Three or four pollinated cut styles were placed in each petri dish and the number of pollen tubes which grew from the cut ends were scored (Fig. 3). The optimum conditions for the screening method are: an incubation temperature of 32°C, a 4-hr period of post-pollination incubation and 60 mm long cut styles.

In all, 755 flower after self-pollination of $S_3S_4$ have been scored by a modified in vitro method in several replications. As can be seen from the results given in Table 5, the mean number of pollen tubes per pollination was 0.21 ± 0.02. Among the styles that carried pollen tubes, a high percentage (60%) contained a single pollen tube (Fig. 4), the others having 2 or more pollen tubes.

Under the conditions of the screening method, cross-compatible pollinations ($S_3S_4 \times S_27S_{28}$) gave an average of about 60 pollen tubes per pollination.

This method can be of use for a rapid scoring of genetic and physiological effects of chemical mutagens and radiations.

Discussion

Effects of Low Radiation Doses on Pollen Abortion

Pollen stainability although not necessarily identical to pollen fertility, is from a practical point of view the easiest method for large-scale scoring of radiation damage induced in pollen grains (pollen abortion ≈ nonstainable pollen). The results of the present study show that the low doses of fast neutrons and x-rays significantly increased pollen abortion when compared to that in control series. Fast neutron treatments at 2.5, 5, and 10 rad induced similar levels of pollen abortion to those

Table 4. Germination, type and number of plants after crosses between unirradiated $S_3S_4$ plants, used as female parents and irradiated and control $S_3S_4$ plants in Oenothera organensis.

| Irradiation treatment, rad | Seeds sown | Seeds germinated | Type and no. of seedlings* |
|---------------------------|------------|-----------------|---------------------------|
|                           | Control    | 147             | 44                        | Normal | Xantha | Morphological variants |
|                           | Fast neutrons | 60             | 17                        | 39 (11) | 3 (3) | 2 (1) |
| 2.5                       | 15 (3)     | 0               | 2                         |
| 5                         | 11 (1)     | 2 (2)           | 2 (2)                     |
| 10                        | 9 (1)      | 2 (2)           | 0                         |

* Number of lethals are given in parentheses.
induced by 2.5–10 rad x-rays, but were highly effective.

Spontaneous and Induced Mutations at the S locus of *O. organensis*

Because of rigorous incompatibility in *O. organensis*, a close agreement exists between the pollen tube growth method and seed-set method for determining mutations rates at the S locus (16–18). Therefore, the frequencies of S locus mutations can be analyzed at three different levels of viability, as has been done by Lewis (16–18) in different S genotypes of *O. organensis*: (1) the number of compatible pollen tubes, which gives a maximum measure of the proportion of pollen grains with a changed incompatibility reaction, including those with an inviable sperm nucleus, (2) the number of seeds, which gives an estimate of the number of pollen grains with a mutated S gene and at the same time a sperm nucleus which is able at least to stimulate the initiation of a zygote and (3) the number of seedlings, which gives an estimate of the S mutations which are fully viable.

When compared to the spontaneously produced frequencies of seeds per million pollen grains reported by Lewis (17) for three clones of *O. organensis*, the present clone which differs in origin and S genotype showed a higher frequency of seeds. In regard to the frequencies of seedlings, although the frequencies of capsules with viable seeds were similar, the number of seedlings per million pollen grains differed, being higher than that found by Lewis (16) in a clone which was similar in S genotype, i.e. S3S4. Differences in the spontaneous mutation rates between different clones on S genotypes have been previously demonstrated by several studies in various species (16, 17, 24, 29).

Concerning the effects of low radiation doses on the S locus, the results obtained show that both fast neutrons and x-rays produced significantly higher frequencies of seeds and seedlings than those obtained spontaneously. The frequencies doubled after treatment with 2.5 rad. However, the present data, which are incomplete concerning the screening of populations for mutations and determination of the frequency and type of mutations, do not permit a conclusion on dose-effect relationships. The studies by Lewis (16–18) on spontaneous and induced S gene mutations in five different S genotypes of *O. organensis* revealed mutations of two types: permanent self-compatible mutations, namely pollen-part mutation resulting from negative changes (deletions or inactivation) in pollen-part of the S locus; and revertible events. The arguments to demonstrate the genetic nature of revertible events and the four possible explanations given by Lewis (16) for supporting the hypothesis that revertible mutations are summarized in Figure 5 (21). Lewis (16) and Pandey (30, 31) considered that such mutations result from labile premutations which subsequently revert, after a certain number of cell divisions to the original allelomorph.

Further, Lewis (16) demonstrated the novelty of revertible mutations in a clone, which was similar in S genotype (S3S4) to that used in the present study. The S3 allele did not give even a single case of permanent or revertible mutation either spontaneously or after x-irradiation in spite of screening

**Table 5. Frequency of pollen tubes after incompatible pollinations.**

| Flowers pollinated | Styles with pollen tubes | Pollen tubes |
|--------------------|--------------------------|--------------|
| Total no. 162      | No. per pollination (mean ± S.E.) 0.21 ± 0.02 |

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approx. 62 million pollen grains. The $S_4$ has given spontaneously revertible mutations only, being 0.9 per million pollen grains. But, after x-ray treatments, it gave both revertible and permanent mutations in higher frequencies. The results obtained with $S_3S_4$ in the present study after self-pollinations and testcrosses of 43 progeny plants showed that they are due to revertible events. Because the data obtained fit Lewis’ arguments and explanations (Fig. 5), it can be concluded that these revertible events correspond to revertible mutations. All the seven homozygotes so far tested contained the same allele. However, the present sample is still too small to permit a conclusion on the allele mutability differences. The analysis is being completed.

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REFERENCES

1. Underbrink, A. G., Sparrow, R. C., Sparrow, A. H., and Rossi, H. H. Relative biological effectiveness of 0.43 MeV-and lower energy neutrons on somatic aberrations hair-length in Tradescantia stamen hairs. Radiat. Biol. 19: 215 (1970).
2. Underbrink, A. G., Sparrow, R. C., and Sparrow, A. H. Relations between phenotypic aberrations and loss of reproductive integrity in Tradescantia stamen hairs. Radiat. Bot. 11: 473 (1971).
3. Sparrow, A. H., Underbrink, A. G., and Rossi, H. H. Mutation induced in Tradescantia by small doses of x-rays and neutrons. Analysis of dose-response curves. Science 176: 916 (1972).
4. Ichikawa, S., and Takahashi, C. S. Somatic mutation frequencies in the stamen hairs of stable and mutable clones of Tradescantia after acute gamma-ray treatments with small doses. Mutat. Res. 45: 195 (1977).
5. Dulieu, H. L., and Dalebroux, M. A. Spontaneous and induced reversion rates in a double heterozygous mutant of Nicotiana tabacum var. Xanthi NC: Dose response relationship. Mutat. Res. 30: 63 (1975).
6. Fabries, M., and Delpoux, M. Genetic effects of low and very low chronic doses of gamma-irradiation and the $a_1 +/a_1$, $a_2 +/a_2$ system of tobacco. Mutat. Res. 49: 377 (1978).
7. Ehrenberg, L., and Eriksson, G. Mutation in the waxy character in barley pollen grains following $^{88}$Sr-incorporation at low activities. Mutat. Res. 1: 139 (1964).
8. Ehrenberg, L. and Eriksson, G. The dose dependence of mutation rates in the rad range in the light of experiments with higher plants. Acta Radiol. Suppl. 254: 73 (1966).
9. Eriksson, G. Variation in radiosensitivity and the dose effect relationship in the low dose region. Hereditas 68: 101 (1970).
10. Lindgren, D., Eriksson, G., and Ehrenberg, L. The mutagenic effect of $^{137}$Cs in barley. Mutat. Res. 10: 335 (1970).
11. de Nettancourt, D., Eriksson, G., Lindgren, D., and Puite, K. Effects of low doses by different types of radiation on the waxy locus in barley and maize. Hereditas 85: 89 (1977).
12. Lewis, D. Genetic control of specificity and activity of the S antigen in plants. Proc. Roy. Soc. (London) B151: 408 (1960).
13. de Nettancourt, D. Self-incompatibility in basic and applied researches with higher plants. Genet. Agr. 26: 163 (1972).
14. de Nettancourt, D. Incompatibility in Angiosperms. Springer-Verlag, Berlin-Heidelberg-New York, 1977.
15. Emerson, S. Growth of incompatible pollen-tubes in Oenothera organensis. Bot. Gaz., 101: 890 (1940).
16. Lewis, D. Structure of the incompatibility gene. III. Types of spontaneous and induced mutation. Heredity 5: 389 (1951).
17. Lewis, D. Structure of the incompatibility gene. I. Spontaneous mutation rate. Heredity 2: 219 (1948).
18. Lewis, D. Structure of the incompatibility gene. II. Induced mutation rate. Heredity 3: 339 (1949).
19. Devreux, M., Laneri, U., Magnien, E., and Celestre, M. R. Biological screening method for mutated pollen at the S-locus by in vitro culture of pollinated pistil. Incomp. Newslett. 5: 17 (1975).
20. de Nettancourt, D. and Devreux, M. Incompatibility and in vitro cultures. In: Plant Cell, Tissue, and Organ Culture. J. Reinert Y. P. S Bajaj, Eds., Springer-Verlag, Berlin-Heidelberg-New York, 1977, pp. 426-442.
21. de Nettancourt, D. Radiation effects on the one-locus-gametophytic system of self-incompatibility in higher plants. Theoret. Appl. Genet. 39: 187 (1969).
22. Sree Ramulu, K., Bredemeijer, G., Dijkhuis, P., de Nettancourt, D., and Schibilla, H. Mentor pollen effects on gametophytic incompatibility in Nicotiana, Oenothera and Lycopersicum. Theoret. Appl. Genet. 54: 215 (1979).
23. Pandey, K. K. Elements of the S-gene complex. VI. Mutations of the self-incompatibility gene, pseudo-compatibility and origin of new incompatibility alleles. Genetica 41: 477 (1970).
24. Pandey, K. K. Elements of the S-gene complex. II. Mutation and complementation at the SI locus in Nicotiana alata. Heredity 22: 255 (1967).
25. Sree Ramulu, K. On some factors influencing estimation of S-gene mutation rates. Incomp. Newslett. 10: 94 (1978).
26. Alexander, M. P. Differential staining of aborted and non-aborted pollen. Stain Technol. 44: 117 (1969).
27. Brewbaker, J. L., and Kwack, B. H. The essential role of calcium ions in pollen germination and pollen tube growth. Am. J. Bot. 50: 859 (1963).
28. Sree Ramulu, K., and Dijkhuis, P. In vitro pollen germination and growth in self-incompatible Oenothera organensis. Incomp. Newslett. 10: 98 (1978).
29. Gastel, A. J. G., and de Nettancourt, D. The effects of different mutagens on self-incompatibility in Nicotiana alata Link and Otto. II. Acute irradiations with x-rays and fast neutrons. Heredity 34: 381 (1975).
30. Pandey, K. K. Mutations of self-incompatibility alleles in Trifolium pratense and T. repens. Genetics 41: 327 (1956).
31. Pandey, K. K. Mutations of the self-incompatibility gene (S) and pseudo-compatibility in angiosperms. Lloydia 22: 222 (1959).