Theor. Appl. Genet. 49 (1977) 297-303

THEORETICAL AND APPLIED GENETICS

Ultrastructural Aspects of Pollen Tube Growth Inhibition after Gamma Irradiation in *Lycopersicum peruvianum*

M. Cresti, F. Ciampolini and E. Pacini
Istituto di Botanica, Università di Siena, Siena (Italy)

K. Sree Ramulu, M. Devreux and U. Laneri
Laboratorio Valorizzazione Colture Industriali del C.N.E.N., Casaccia (Italy)

Summary. Gamma ray treatments at various doses were applied to flowers after cross-compatible pollinations ($S_1S_4 \times S_{12}S_{13}$) and self-incompatible pollinations ($S_1S_4 \times S_1S_4$). After 200 kR treatment (highest dose) a high percentage of pollen became non-viable, and tube growth of all the germinated pollen was inhibited at the first third, or just before half the length of the style. Observations on the ultrastructural aspects revealed that the pollen tubes were destroyed by a precise degradation process which led to the disappearance of the inner wall, lysis of the tube, accumulation of several bipartite particles and alteration of endoplasmic reticulum into a whorl of concentric circles. These results indicated that the high dose of gamma rays probably interferes with protein synthesis. The ultrastructural aspects of compatible pollen tubes after gamma irradiation were similar to those of self-incompatible tubes and therefore it is suggested that cessation of protein synthesis might also be a result of incompatibility.

Electron microscopy observations after treatment with 200 kR gamma rays on flowers after self-incompatible pollination ($S_1S_4 \times S_1S_4$) showed that gamma irradiation affects the self-incompatibility reaction; but the results obtained so far are difficult to explain.

Introduction

The wild tomato species *Lycopersicum peruvianum* Mill. is characterised by homomorphic gametophytic monofactorial self-incompatibility. Although many of the genetical and biochemical features characterising the self-incompatibility phenomenon are known (see Lewis 1965; Linskens 1965; Lundquist 1965; Lewis et al. 1966; Pandey 1967, 1970; Nasrallah et al. 1969; de Nettancourt et al. 1971; de Nettancourt 1972; van der Donk 1974a, b), only a little information is available on the rejection process and on the ultrastructural changes which accompany the inhibition of pollen tube growth (de Nettancourt et al. 1973a). In the course of observations with the electron microscope on the mechanism of pollen tube rejection after incompatible pollinations in *L. peruvianum*, de Nettancourt and coworkers (1973a, b, 1974, 1975) interpreted the occurrence of endoplasmic reticulum in whorls of concentric circles, and its subsequent degradation, as an indication that incompatibility might lead to a general cessation of protein synthesis. In an attempt to gain further information on the mechanism of pollen tube rejection, experiments were carried out on *L. peruvianum:* 1) to determine the radiation dose that can inhibit pollen tube growth in the style after cross-compatible pollination, 2) to observe the ultrastructural features of pollen tube inhibition, and 3) to find out if these aspects are similar to or different from those observed after self-incompatible pollination, given that ionizing radiations also decrease the rates of both nucleic acid and protein synthesis (see Luse 1970).

Materials and Methods

1. Test-material and growing conditions

Lycopersicum peruvianum clones F13($S_{12}S_{13}$) and 10(S_1S_4) were used. The details on the origin of these clones have been given in previous publications (Laneri and de Nettancourt 1973; Sree Ramulu et al. 1976).

The plants were grown under greenhouse conditions and the experiments were conducted in the months of April to early June, when the plants flower profusely.

2. Irradiation source and treatments

Gamma irradiation was carried out with the 67000 Ci ^{60}Co source at the nuclear centre of Casaccia near Rome. The doses applied to pollinated flow-

ers were 50 kR and 100 kR at the rate of 38.7 kR/hour, and 200 kR at the rate of 60 kR/hour. The temperature during irradiation was 22° ± 1°C.

3. Self-incompatible and cross-compatible pollinations and method of treatment

Plants of genotype S_1S_4 were used as female parents and pollinated with pollen from both S_1S_4 plants ($S_1S_4 \times S_1S_4$; self-incompatible pollination) and $S_{12}S_{13}$ plants ($S_1S_4 \times S_{12}S_{13}$; cross-compatible pollination). The pollinations were performed at 9 a.m. on emasculated flowers. After 5 hours, the pollinated flowers were removed from the plants, planted in petridishes containing agar minerals, and immediately treated with gamma rays. Irradiation was applied to the flowers in petridishes in such a way that the stigmas were vertically facing the ^{60}Co source. Immediately after irradiation, the styles were fixed for observations with fluorescence and electron microscopes, or the irradiated flowers were transplanted to non-irradiated petridishes (with agar) and placed in the climate room over night (climate room conditions: about 3000 lux, TL 33 tubes, 22° ± 1°C), and the styles were fixed on the next morning (9 a.m.).

4. Fluorescence and electron microscopy

The fluorescence technique described by Martin (1958) was followed in these experiments.

For electron microscopic observations, each style was cut into three parts and prefixed in 3% glutaraldehyde buffered with 0.066 M cacodylate (pH 7.2) for 60 minutes at room temperature. The styles were then rinsed in buffer and fixed in 1% osmium tetroxide buffered with 0.066 M cacodylate (pH 7.2) for 60 minutes. The material was dehydrated and embedded in an Epon-Araldite mixture. Sections were cut on an LKB Ultratome III, stained with uranyl acetate and lead citrate and observed with a JEOL JEM 100 B electron microscope at 80 kV.

In each experiment 20 flowers for self-incompatible pollinations and 20 flowers for cross-compatible pollinations were used. Ten flowers for each type of pollination were treated with gamma rays, and 10 were used as controls.

The first and second experiments were carried out with 50 kR and 100 kR gamma rays (at the rate of 38.7 kR/hour) respectively, and observations were made on pollen germination and pollen tube growth, using the fluorescence technique.

The third experiment was performed with 200 kR gamma rays (60 kR/hour), and observations were made by the fluorescence technique. With this treatment after cross-compatible pollination ($S_1S_4 \times S_{12}S_{13}$), pollen tube growth was inhibited in the style, while in the control pistils the pollen tubes reached the ovary. In order to observe by electron microscopy the ultrastructural aspects of pollen tube growth inhibition after 200 kR gamma rays, the same experiment was repeated twice; the data were uniform throughout all these experiments.

Results

1. Effect of gamma irradiation on pollen tube growth after cross-compatible pollination ($S_1S_4 \times S_{12}S_{13}$)

With doses of 50 and 100 kR pollen germination was inhibited to some extent, but the tubes of the germinated pollen could grow through the style into the ovary (Fig. 1). However, after the 200 kR treatment not only a high percentage of pollen became non-viable, but even the growth of the surviving pollen tubes was inhibited in the first third or just before the middle of the style length. Bursting pollen tubes could also be observed under fluorescence microscopy (Fig. 2). These observations on the effects of 200 kR gamma rays were confirmed in repeated experiments, and the ultrastructural features were studied both in the controls and treated materials.

It was observed that immediately after irradiation, i.e. 8 hours after pollination, the pollen tubes could grow only up to the base of the stigma where the stylar transmitting tissue begins to differentiate. In the tube portion very near the apex (Fig. 3), the cytoplasm appeared very dense with several dictyosomes producing many vesicles which migrated towards the callosic wall (Fig. 3). The mitochondria were swollen, and had some small vesicles inside containing fibrillar material (Fig. 3). An unusual aspect of these pollen tubes was the presence of cisternae of rough endoplasmic reticulum (RER) appearing as whorls of concentric circles (CER) (Fig. 4). In the cytoplasm the free cisternae of RER seemed to be rare or absent.

Twenty-four hours after pollination the pollen tubes had grown only as far as the end of the first third of the style length. In the stylar intercellular spaces, degenerated pollen tube material was present due to the bursting of the tubes. Inside the pollen tubes the cytoplasm was similar to that observed 8 hours after pollination, i.e. with CER, mitochondria containing vesicles, dictyosomes etc. Near the apex the pollen tubes showed a very thick external wall as well as the callosic inner wall. In the space between the callose wall and the plasmalemma numerous very fine granules were present (Fig. 5). The formation of a callosic plug and the occurrence of well recognizable CER could be seen (Fig. 6). The

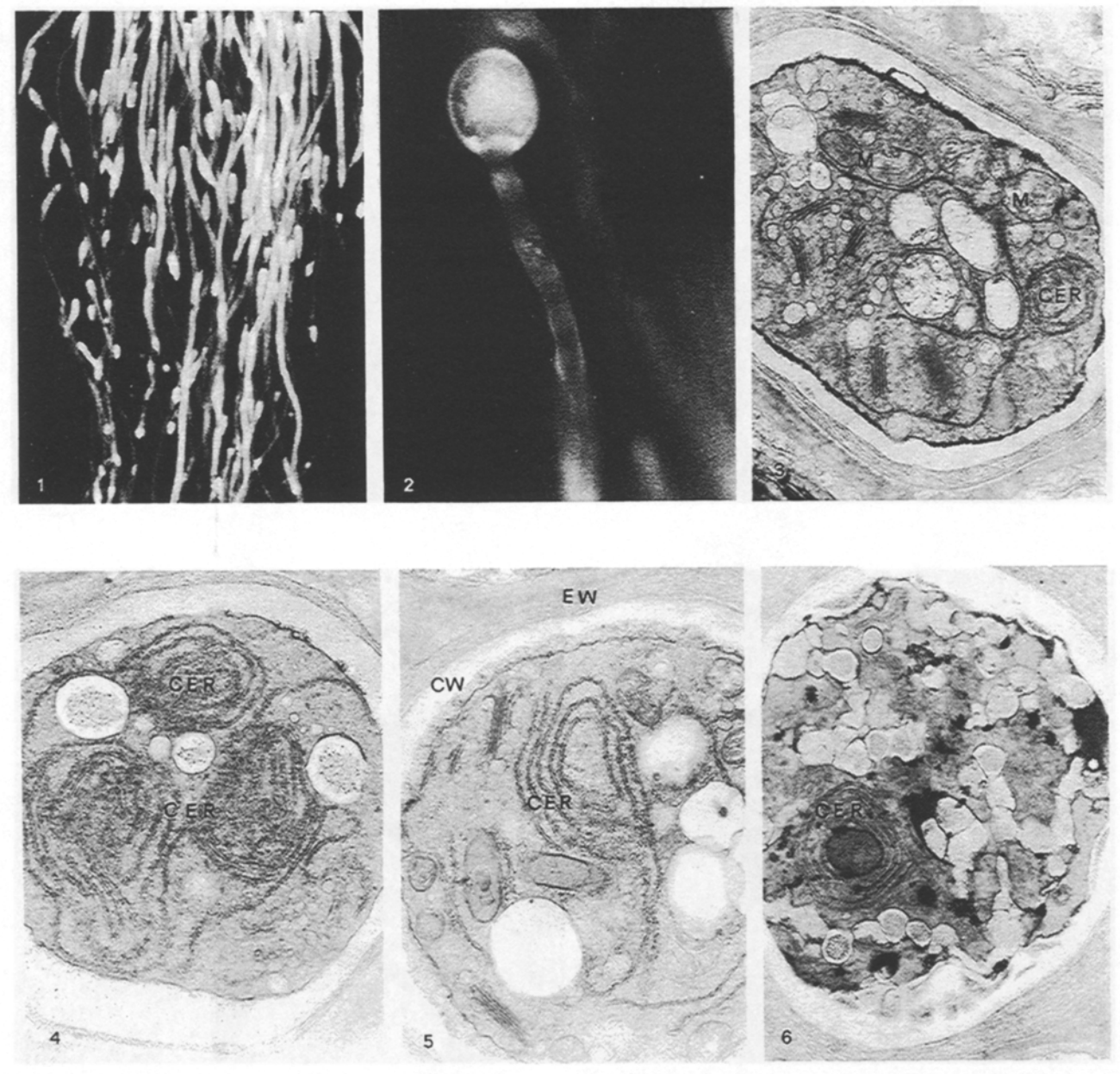


Fig.1. Pollen tubes growing normally in the style of flower irradiated with 100 kR gamma rays after cross-compatible pollination ($S_1S_4 \times S_{12}S_{13}$); × 125

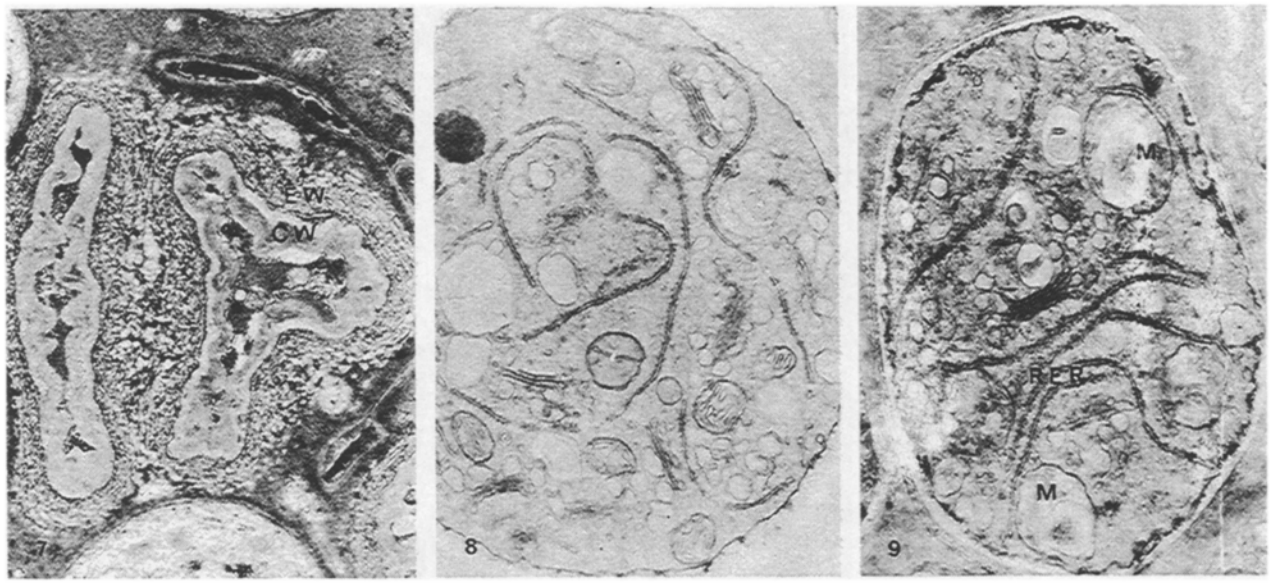
Fig.2. Bursting of pollen tube, as observed under fluorescence microscopy, in the style of flower treated with 200 kR gamma rays after cross-compatible pollination ($S_1S_4 \times S_{12}S_{13}$); × 670

Figs.3-4. Pollen tubes in irradiated (200 kR) styles 8 hours after cross-pollination ($S_1S_4 \times S_{12}S_{13}$). The tubes are sectioned near the apex: whorls of concentric endoplasmic reticulum (CER) are visible; the mitochondria (M) contain relatively small vesicles; Fig.3 (× 27.000), Fig.4 (× 30.300)

Figs.5-6. Pollen tubes as in preceding figures but 24 hours after pollination
Fig.5. Between the inner callosic wall (CW) and the plasmalemma a layer is present with finely granular material (EW = external pectocellulosic wall); × 26.200
Fig.6. Callosic plug formation zone; degenerating CER is present; × 18.200

outer wall, especially its outer layers, of the collapsed empty portion of the pollen tubes was formed of very loose fibrils (Fig.7).

In the controls, the pollen tubes had reached the last third of the style 8 hours after pollination. It was observed that the RER had long, single pro-



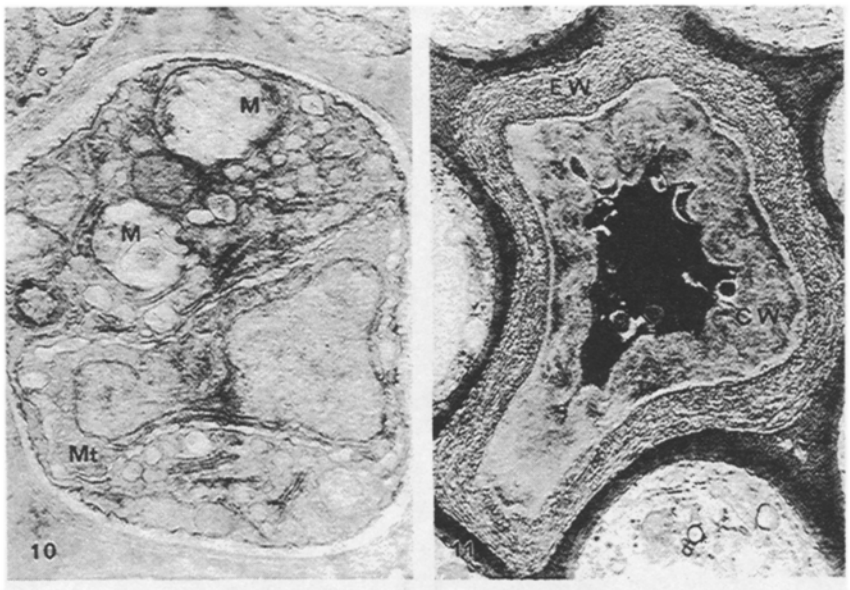


Fig.7. Pollen tubes as in preceding figures but 24 hours after pollination. Inactive and collapsed portion of pollen tube; × 10.100

Fig.8. Cross-compatible pollination ($S_1S_4 \times S_{12}S_{13}$) control. Pollen tube near the apex with cisternae and numerous organelles; × 33.000

Figs.9-11. Apical (Fig.9), nuclear (Fig.10), and collapsed (Fig.11) portions of irradiated (200 kR) pollen tubes, 8 (Figs.9-10) and 24 (Fig.11) hours after self-incompatible pollination ($S_1S_4 \times S_1S_4$). Free RER cisternae and mitochondria with small vesicles are present (Figs.9 and 10); the generative cell contains a large number of microtubules (Mt) (Fig.10); (EW = external pectocellulose wall; CW = callosic wall); Fig.9 (× 10.500); Fig.10 (× 11.100); Fig.11 (× 13.000)

files; the structure of the mitochondria appeared normal with evident membranes and cristae (Fig.8). Twenty-four hours after pollination, only collapsed pollen tubes were observed in the style; probably by that time the spermatic nuclei had reached the ovary.

2. Effect of gamma irradiation on pollen tube growth after self-incompatible pollination ($S_1S_4 \times S_1S_4$)

It was observed that immediately after irradiation with 200 kR gamma rays, i.e. 8 hours after pollina-

tion, the pollen tubes reached the intercellular substance of the style beyond the stigma. The pollen tube portion near the apex, which can be regarded as representative of other portions, showed several organelles: dictyosomes with vesicles, free RER cisternae, lipid globules and, in particular, very swollen mitochondria containing vesicles with fibrillar substances (Fig.9). This abnormal condition of the mitochondria was still more evident in the nuclear area (Fig.10).

Twenty-four hours after pollination the pollen tubes reached the second third of the style length. Only rarely could any further growth of the tubes be observed. There were several traces of degenerated cytoplasm in the intercellular substance of the style. The active portion of the pollen tubes showed a pecto-cellulosic outer wall and a well-distinguished inner callosic wall. With regard to cytoplasm organelles, the pollen tubes showed the same features as irradiated pollen tubes 8 hours after cross-pollination, except that in selfed pollen tubes no CER was observed. The inactive part of the tube showed a very regular pecto-cellulosic outer wall with very compact fibrils (Fig.11).

In the controls, 8 hours after self-pollination, the pollen tubes showed no noticeable ultrastructural difference as compared with treated pollen tubes. After 24 hours the pollen tubes could grow only up to about half the style length and thereafter the pollen tubes were inhibited in their growth and were destroyed by a degradation process which led to the disappearance of the inner wall and lysis of the tube; several whorls of CER were found which subsequently degraded in the cytoplasm.

Discussion

In many plant species pollen germination and tube growth are generally inhibited only after very high doses of radiation, e.g. LD.50 for germination was reported to vary from 35 kR *(Oenothera organensis)* to as high as 550 kR *(Saintpaulia ionantha)* (Brewbaker and Emery 1962). Nishiyama and Tsukuda (1959) treated the dehisced pollen of *Lycopersicum esculentum* with filtered (1 mm Al) 230 kvP X-rays under room conditions, and found that 250 kR treatment caused 50 % reduction in germination; a sporadic germination was encountered at doses exceeding 300 kR. In the present study with the wild tomato species *L. peruvianum*, inhibition of pollen germination was observed in the styles after cross-compatible pollination in increasing order following treatments with 50, 100 and 200 kR gamma rays. However, it was found that after 50 and 100 kR treatments, many pollen tubes grew through the style into the ovary. After treatment with high exposure 200 kR the pollen tubes were not only inhibited in their growth, but also were destroyed by a precise degradation process which led to the disappearance of the inner wall, lysis of the tube, accumulation of numerous bipartite particles and the arrangement of endoplasmic reticulum into whorls of concentric profiles. These features were quite similar to those observed in the pollen tubes after self-incompatible pollination. The occurrence of CER in the incompatible pollen tubes of *L. peruvianum* was interpreted by de Nettancourt et al. (1973a, b) as an indication that incompatibility might lead to a general cessation of protein synthesis. Similarly concentric configurations of the endoplasmic reticulum, observed in temporarily inactive cells such as those of resting potato tubers (Shih and Rappaport 1971) and *Betula* buds (Dereuddre 1971), seem to indicate the inactivation of some important biosynthetic mechanism: Shih and Rappaport (1971) demonstrated in fact that the application of gibberellin GA3 to potato tubers simultaneously induced metabolic activity and the disappearance of CER. The formation of CER seems, therefore, to indicate that one effect of gamma radiation is the cessation of protein synthesis. The presence of CER and other ultrastructural features both in irradiated cross-pollinated and untreated self-pollinated pollen tubes is further evidence that cessation of protein synthesis is also one of the main events in the self-incompatibility reaction.

A final comment may be made concerning the effects of 200 kR gamma rays on pollen tube growth in the stylar tissues after self-incompatible pollination ($S_1S_4 \times S_1S_4$). Twenty-four hours after self-pollination in the non irradiated materials, all the pollen tubes stopped growing in the first third or half of the style length showing all the usual aspects of degener-

ating incompatible tubes (de Nettancourt et al. 1973a). On the other hand, treated pollen tubes grew farther down the style than the non-irradiated ones, their active portion showing a pecto-cellulosic outer wall and an easily distinguishable inner callosic wall; no concentric layers of endoplasmic reticulum were observed. These results indicate that ionizing radiation has an influence on the self-incompatible mechanism. These data need to be confirmed but what is especially necessary is to follow the development of the pollen tubes for a longer time and farther down the stylar length: only then will it be possible to attempt an explanation of the effects of irradiation on the self-incompatibility reaction.

Acknowledgement

The authors are indebted to Prof. G. Sarfatti for critical reading of the manuscript and to Prof. de Nettancourt for advice during the course of this investigation. They appreciate the efficient technical assistance of Mrs. I. Liberti in greenhouse work.

This article is contribution n° 1384 from the Biology Division of the European Communities and contribution n° 488 from the Divisione Applicazioni delle Radiazioni, C.N.E.N. Research performed under CNR program "Biology of reproduction" and supported partially by a sub contract between the association EURATOM-ITAL and the C.N.E.N.

Literature

Brewbaker, J.L.; Emery, G.C.: Pollen radiobotany. Radiation Bot. 1, 101-154 (1962)

Dereuddre, J.: Sur la présence de groupes de saccules appartenants au réticulum endoplasmique dans les cellules des ébauches foliares en vie ralentie de *Betula verrucosa* Ehrh. C.R. Acad. Sci., Paris 273 (D), 2239-2242 (1971)

Donk, J.A.W.M. van der: Gene activity and the incompatibility reaction in *Petunia*. In: Fertilization in higher plants, pp. 279-283 (ed.: Linskens, H.F.), Amsterdam-New York: North Holland American Elsevier 1974a

Donk, J.A.W.M. van der: Synthesis of RNA and protein as a function of time and type of pollen-tube - style interaction in *Petunia hybrida* L. Molec. Gen. Genet. 134, 93-98 (1974b)

Laneri, U.; Nettancourt, D. de: Distinction between complete compatibility and semi-compatibility by means of fluorescence technique. Incompatibility Newsletter (Wageningen) 2, 56-59 (1973)

Lewis, D.: A protein dimer hypothesis on incompatibility. Proc. XIth Int. Congr. Genet., The Hague. In: Genetics Today (ed. S.J. Geerts) 3, 657-663 (1965)

Lewis, D.; Burrage, S.; Walls, D.: Immunological reactions of single pollen grains, electrophoresis and enzymology of pollen protein exudates. J. Expt. Bot. 18, 371-378 (1966)

Linskens, H.F.: Biochemistry of incompatibility. Proc. XIth Int. Congr. Genet., The Hague. In: Genetics Today (ed. S.J. Geerts) 3, 629-636 (1965)

Lundquist, A.: The genetics of incompatibility. Proc. XIth Int. Congr. Genet., The Hague. In: Genetics Today (ed. S.J. Geerts) 3, 637-647 (1965)

Luse, R.A.: Radiobiology. In: Manual on mutation breeding, IAEA Technical report series n° 119, 21-31 (1970)

Martin, F.W.: Staining and observing pollen tubes in the style by means of fluorescence. Stain Techn. 34, 125-128 (1958)

Nasrallah, M.E.; Barber, J.T.; Wallace, D.H.: Self-incompatibility proteins in plants: detection, genetics and possible mode of action. Heredity 24, 23-27 (1969)

Nettancourt, D. de; Ecochard, R.; Peroquin, M.D. G.; Drift, T. van der; Westerhof, M.: The generation of new S-alleles at the incompatibility locus of *L. peruvianum* Mill. Theor. Appl. Genet. 41, 120-129 (1971)

Nettancourt, D. de: Self-incompatibility in basic and applied researches with higher plants. Genet. Agr. 26, 163-216 (1972)

Nettancourt, D. de; Devreux, M.; Bozzini, A.; Cresti, M.; Pacini, E.; Sarfatti, G.: Ultrastructural aspects of self-incompatibility mechanism in *Lycopersicum peruvianum* Mill. J. Cell Sci. 12, 403-419 (1973a)

Nettancourt, D. de; Devreux, M.; Laneri, U.; Pacini, E.; Cresti, M.; Sarfatti, G.: Ultrastructural aspects of unilateral interspecific incompatibility between *L. peruvianum* and *L. esculentum*. Proc. Conf.: From ovule to seed. Siena (Italy) October 1972. Caryologia 25 (suppl.) 207-217 (1973b)

Nettancourt, D. de; Devreux, M.; Laneri, U.; Cresti, M.; Pacini, E.; Sarfatti, G.: Genetical and ultrastructural aspects of self- and cross-incompatibility in interspecific hybrids between self-compatible *L. esculentum* and self-incompatible *L. peruvianum*. Theor. Appl. Genet. 44, 278-288 (1974)

Nettancourt, D. de; Devreux, M.; Carluccio, F.; Laneri, U.; Cresti, M.; Pacini, E.; Sarfatti, G.; Gastel, A.J.G. van: Facts and hypotheses on the origin of S mutations and on the function of the S gene in *Nicotiana alata* and *Lycopersicum peruvianum*. Proc. Roy. Soc. Lond. B 188, 345-360 (1975)

Nishiyama, I.; Tsukuda, S.: Radiobiological studies in plants. I. Effects of X-rays upon pollen germination and fertility. Jap. J. Genet. 34, 363-370 (1959)

Pandey, K.K.: Origin of genetic variability: combinations of peroxidase isoenzymes determine multiple allelism of the S-gene. Nature (Lond.) 213, 669-672 (1967)

Pandey, K.K.: Elements of the S-gene complex. VI. Mutations of the self-incompatibility gene, pseudo-compatibility and origin of new incompatibility alleles. Genetics. Princeton 41, 477-516 (1970)

Sree Ramulu, K.; Devreux, M.; Ancora, G.; Laneri, U.: Chimerism in *L. peruvianum* plants regenerated from in vitro cultures of anthers and stem internodes. Z. Pflanzenzüchtg. 76, 299-319 (1976)

Shih, C.Y.; Rappaport, L.: Regulation of bud rest in tubers of potato *Solanum tuberosum* L. VIII. Early effects of gibberellin A_3 and abscisic acid on ultrastructures. Plant Physiol. 18, 31-35 (1971)

Received November 8, 1976
Communicated by H.F. Linskens

M. Cresti, F. Ciampolini, E. Pacini
Laboratorio di Microscopia Elettronica
Istituto di Botanica
Università di Siena
Siena (Italy)

K. Sree Ramulu
Association EURATOM-ITAL
P.O. Box 48
Wageningen (the Netherlands)

M. Devreux, member of the
European Communities
Biology Group
C.C.R. Ispra
21020 Ispra (Italy)

U. Laneri
Laboratorio Valorizzazione Colture
Industriali del C.N.E.N.
C.S.N. Casaccia
Roma (Italy)