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Mentor Pollen Effects on Gametophytic Incompatibility in *Nicotiana, Oenothera* and *Lycopersicum**

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Summary. Attempts were made, through mentor pollen techniques, to overcome self-incompatibility in species belonging to the genera *Nicotiana* and *Oenothera* and in a hybrid of *Lycopersicum*, which are characterized by a gametophytic system of incompatibility. While radiation-killed incompatible pollen did not generate mentor effects in any of the material tested, radiation-killed compatible pollen was found to promote a high level of illegitimate fertilizations by incompatible pollen in *N. alata*. No evidence was obtained that radiation-killed compatible pollen could induce mentor effects in strictly self-incompatible clones of *O. organensis* and of the interspecific hybrid *L. esculentum* x *L. peruvianum*.

Key words: Gametophytic self-incompatibility – Compatible and incompatible mentor pollen – *Nicotiana-Oenothera-Lycopersicum*

Introduction

The so-called compatible mentor pollen effect, the capacity of compatible pollen, in a mixture of compatible and incompatible grains applied on stigmas, to stimulate the growth of the incompatible pollen present in the mixture, was first observed by Michurin (1950) and subsequently reported by Glendinning (1960), Tsitsin (1962), Grant et al. (1962), de Nettancourt and Grant (1963), Miri and Bubar (1966) and Karpov (1966). Stettler (1968) amplified this effect and obtained hybrids of poplars between cross-incompatible species by submitting the pollen used as mentor to lethal dosages of radiations. This possibly enhanced the transmission of signals from compatible to incompatible pollen and certainly prevented ovule mobilization by the compatible mentor pollen. Knox et al. (1972) successfully repeated the experiments of Stettler and showed that compatible pollen killed with methanol or by repeated freezing and thawing was as effective as radiation-killed compatible pollen for inducing the manifestation of mentor phenomena in poplar. Going one step further, Knox et al. (1972) extracted the rapidly leachable proteins from the compatible poplar pollen and demonstrated that these extracts, presumably exine-held and of the same origin and nature as the specific recognition substances participating in the reaction of sporophytic incompatibility in *Raphanus* (see Dickinson and Lewis 1973 a, b; Heslop-Harrison et al. 1974) and in *Cosmos* (see Howlett et al. 1975), could, in mixture with incompatible pollen, induce the mentor effect. Considering that the incompatibility proteins in species with gametophytic incompatibility were in all probability located in the pollen intine and that massive interaction between different pollen grains which are each specifically determined for their incompatibility phenotype was unlikely, Knox et al. (1972) did not suggest the application of the mentor technique for overcoming gametophytic incompatibility. Pandey (1977), on the other hand, drew attention to the fact that the effects of mentor pollen upon the growth of incompatible pollen had been reported in *Lotus, Malus, Lolium* and *Nicotiana*, that is to say in genera known to be characterized by a gametophytic system of self-incompatibility. Pandey (1977) also expressed the view, from his results with *Nicotiana*, that the mentor effect involved the release by the mentor pollen of a non-specific wall-held pollen growth substance which is, as such, different from any specific recognition component. He found, in *Nicotiana*, that the killed incompatible pollen of *N. langsdorffii* stimulates the growth of unkilled incompatible *langsdorffii* pollen in styles of *N. forgetiana* and allows the production of interspecific hybrids between the two species. Pandey concluded from such results and from other related

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data that the mentor effect is essentially promoted by the killing agent itself which initiates the diffusion from the mentor pollen of a non-specific wall-held substance able to assist the growth of untreated weakly incompatible pollen. Whether the pollen used is compatible or incompatible is immaterial and the essential prerequisite is the emission of the diffusable non-specific agent by the killed pollen and the use, as receptor, of pollen which is only weakly incompatible and thus responsive to mentor treatment.

It is our objective to report, in this article, on the results of experiments carried out with radiation-killed incompatible and compatible mentor pollen in species of *Nicotiana* and *Oenothera* and in a hybrid of *Lycopersicum* characterized by a monofactorial gametophytic system of self-incompatibility.

Material and Methods

Experiments with Nicotiana alata Link et Otto

Genotypes. The two self-incompatible clones (OWL – S_2S_3 and OB2 – S_6S_7) described previously by Carluccio et al. (1974) were used as test material in the present study.

Irradiation source and treatment conditions. Pollen in small glass tubes of about 1 mm thickness was exposed to the ^{60} Co source of 1.33 MeV of the Association EURATOM-ITAL in Wageningen. The dose was measured inside the tube. The temperature during irradiation approximated 19°C and the relative humidity 60 per cent. The lethal dose of 250 krad gamma rays (determined from a preliminary experiment) was applied in 11 minutes.

Pollinations. Fresh pollen collected from plants grown under controlled conditions (16 h day, 13000 lux, 23°C; 8 h night, 17°C; relative humidity 60 to 70 per cent) was used in all cases. The pollinations were performed on emasculated flowers placed under strict conditions of isolation. For pollinations with compatible (S_6S_7) or incompatible (S_2S_3) mentor pollen, pollen samples were irradiated and subdivided into two equal portions for direct deposition on S_2S_3 stigmas and for mixtures with viable incompatible S_2S_3 pollen which were thereafter deposited on the stigmas of S_2S_3 plants. In all, more than 800 flowers[1] were submitted to pollinations with self-pollen and pollen mixtures. They are strictly self-incompatible.

Oenothera organensis Munz.

Genotypes. Five self-incompatible clones one of which (clone III – 4) was S_3S_4 in genotype and the other four (clones III – 6, III – 7, III – 8 and III – 9) S_3S_{35}, were used. These clones have been produced through material originating from seeds kindly provided by Prof. A. Hecht.

Irradiation source and treatment conditions. Irradiation treatment was applied with the Cs source of 1200 Ci at the University of Louvain. Fresh pollen collected from plants grown in the greenhouse was exposed to the lethal doses of 240 krad gamma rays at a dose-rate of 92 krad per hour.

Pollinations. Immediately after irradiation, the treated compatible (S_3S_{35}) or incompatible (S_3S_4) mentor pollen was divided into approximately two halves; one half was deposited on S_3S_4 stigmas whereas the other half was thoroughly mixed with about an equal quantity of incompatible pollen and crossed to the female parent. In all, more than 70 flowers were submitted to pollinations with self-pollen and pollen mixtures.

Lycopersicum hybrid (L. esculentum Mill. × L. peruvianum Mill.)

The self-incompatible hybrid between *L. esculentum* × *L. peruvianum* (de Nettancourt et al. 1974) was used as female parent. The plant species utilized as incompatible mentor pollen sources were *L. esculentum* varieties 'Lotina' and 'Money maker', *L. peruvianum* and some species of *Solanum.*[2]

For irradiation treatment of the incompatible mentor pollen, the same Cs source as that used for *O. organensis* was utilized. The doses, 120 krad and 240 krad, were given respectively in 80 min and 160 min.

For the compatible mentor pollen source, the tester-stocks of *L. peruvianum*, which are known to be compatible to the hybrid, were used. The pollen was irradiated with 360 krad delivered in 240 min. In all more than 200 flowers were submitted to pollinations with self-pollen or pollen mixtures.

Results

Effects of Radiation Killed Incompatible Mentor Pollen

Not a single case of seed-set or fructification was observed in any of the three genera analysed after pollinations with mixtures of self (incompatible) pollen and irradiated incompatible pollen.

Effects of Radiation Killed Compatible Pollen

The plants of *Oenothera* used as receptors and the interspecific self-incompatible tomato hybrid did not produce a single fruit after pollination with mixtures of live incompatible pollen and radiation killed compatible pollen. In the case of *Nicotiana alata*, however, high numbers of seeded fruits containing more than 60 seeds on average (Table 1) were collected after pollinations with mixtures of radiation-killed compatible pollen and living (self) incompatible pollen. In view of the fact that not a single fruit was obtained in the series which involved either the use of unirradiated incompatible pollen alone or radiation-killed compatible pollen alone, it is obvious that killed compatible pollen exerted a mentor effect, in pollen mixtures, upon untreated incompatible pollen. Under the conditions of our experiments and for the material used, this effect can be defined as leading to approximately 25% of the normal fruit yield recorded after fully compatible matings and around 7% of the normal seed yield.

1 in several replications during a 2-year period

2 *Solanum* species cv. Etna [botanical gardens, Meise, Brussels] and *S. capsicum frutescens* L. [*Solanaceae*] and *Brachiaria ruziziensis* Germain & Evrard [*Gramineae*] were used as sources of incompatible mentor pollen

Table 1. Fruit and seed yields obtained from intraspecific crosses with normal and/or radiation (250 krad)-killed compatible or incompatible pollen in *Nicotiana alata* Link et Otto

Cross	Flowers pollinated	Fruits with viable seeds	Fruits with non viable seeds	Non seeded fruits	Average no. of viable seeds		
					per pollination	per fruit	per seeded fruit
S_2S_3 ♀ X Living incompatible S_2S_3 pollen	118	0	0	0	0	0	0
" X Living compatible S_6S_7 pollen	118	118	0	0	700	700	700
" X Radiation-killed compatible S_6S_7 pollen	120	0	40	24	0	0	0
" X Mixture of radiation-killed compatible S_6S_7 pollen and living incompatible S_2S_3 pollen	233	65	66	11	16	27	60[a]
" X Radiation-killed incompatible S_2S_3 pollen	100	0	0	0	0	0	0
" X Radiation-killed incompatible S_2S_3 pollen and living incompatible S_2S_3 pollen	125	0	0	0	0	0	0

[a] Test-crosses showed that the progeny plants were S_2S_2, S_2S_3 and S_3S_3 in genotype

Discussion and Conclusions

The results obtained in the present study corroborate Pandey's report on the induction in *Nicotiana alata* of mentor effects by radiation-killed pollen which enable otherwise incompatible pollen tubes to accomplish illegitimate fertilization. Since only compatible pollen was found to induce such mentor effects, the data do not, however, confirm the claim (Pandey 1977) that mentor effects can also be promoted by radiation killed incompatible pollen.

The complete absence of mentor effects in the *Oenothera* and *Lycopersicum* experiments indicate that the manifestation of mentor phenomena, in spite of their occurrence in *Lotus*, *Malus*, *Lolium* and *Nicotiana*, is perhaps a relatively rare event in species characterized by gametophytic systems of incompatibility. Yet, the possibility remains that mentor effects would have been detected in the *Oenothera* experiments if plants with a weaker self-incompatibility phenotype had been available as pollen receptors. The same argument holds for the experiments carried out with the interspecific tomato hybrid *L. esculentum* x *L. peruvianum* which is also very strictly self-incompatible. In this latter case, however, the situation was much complicated by the fact that the hybrid combines two different incompatibility components, self-incompatibility and unilateral interspecific incompatibility, which possibly interacted to prevent induction of mentor effects by radiation-killed pollen grains. Since no attempts were made to observe the behaviour of pollen tubes in styles after pollinations with pollen mixtures, it is also possible, in addition, that the absence of fructification resulted at least in part from the zygotic and post-zygotic barriers which are known to complement reproductive isolation between *L. esculentum* and *L. peruvianum*. (McGuire and Rick 1954; Hogenboom 1972; de Nettancourt et al. 1974)

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