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Sexual reproduction in *Populus* II. Information molecules of the pollen grain

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Introduction

Sexual interactions in flowering plants depend upon pollen–pistil biocommunications, involving biochemical signals exchanged between the mating partners. Such components involved in self-incompatibility are known to be the primary products of the *S* (self-incompatibility) gene. They are of protein and glycoprotein nature (Nasrallah and Nasrallah, 1986; Gaude and Dumas, 1987). On the other hand, no data are available on the pollinic signals implicated in interspecific incompatibility of self-compatible species.

Cellular localization and biochemical identification of pollinic molecules, likely to be implicated in interspecific incompatibility in Poplars, are presented in this paper. Our model involves 2 species of section *Aigeiros (Populus nigra, P. deltoides)* and one species of section *Leuce (P. alba)*. Crosses between species of these 2 botanical sections are strictly incompatible.

Materials and Methods

P. nigra, P. deltoides and *P. alba* branches were obtained from the INRA Forestry Station in Orléans (France). Pollen was collected and stored in closed vials at –18°C.

For cytochemistry, ultrathin pollen sections were prepared according to Gaget (1988). They were stained with uranyl acetate/lead citrate (Reynolds, 1963), and observed at 80 kV with the Hitachi HU 12A microscope at CMEABG, University of Lyon, France.

Freeze–fracture was performed according to Kerhoas *et al.* (1987) and replicas were observed by transmission electron microscopy (TEM).

Pollen prints were obtained by placing pollen grains onto a formvar-coated grid for 30 min in humid Petri dishes. Grids were then stained (uranyl acetate/lead citrate; Reynolds, 1963) and observed by TEM.

Biochemical analysis: pollen diffusates were obtained by putting pollen grains in Tris buffer (pH 7.5, 25 mM) for 10 min at 4°C. After centrifugation, the supernatant was filtered and concentrated by acetone precipitation. The resuspended extracts were assayed for total proteins, according to Bradford (1976), with bovine serum albumin as a standard. Samples

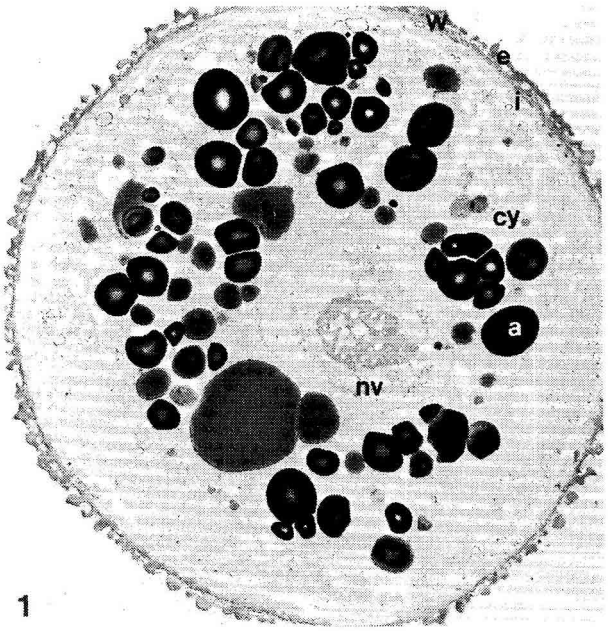

Fig. 1. TEM observation of the pollen grain of *P. alba*. At maturity, the bicellular pollen grain contains a vegetative nucleus (**nv**) and a generative nucleus (not visible here). The generative cell lies within the cytoplasm (**cy**) of the vegetative cell. The pollen grain is surrounded by a complex wall (**w**), with 2 distinct layers, intine (**i**) and exine (**e**). Numerous amyloplasts (**a**) are observed in the cytoplasm of the pollen grain (x 5000, PATAg test).

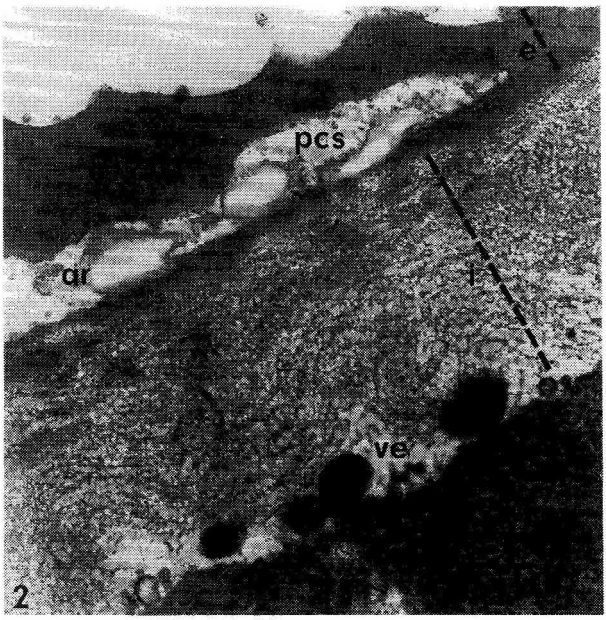

Fig. 2. Pollen wall structure of *P. alba*. Exine (**e**): the arcades of the exine (**ar**) contain pollen coat substances (**pcs**), likely to be involved in pollen–pistil interactions. Intine (**i**): osmiophilic vesicles (**ve**) are observed within the intine. (TEM, x 48 000, uranyl acetate/lead citrate stain).

Fig. 3. Freeze–fracture replica of *P. alba* pollen. This replica shows the presence of microchannels (**c**) through the intine (**i**) allowing recognition material to diffuse from the cytoplasm (**cy**) of the pollen grain. (TEM, x 48 000).

containing 10 µg of protein were loaded on an SDS–gel, according to Laemmli's procedure (Laemmli, 1970). After running, proteins were stained with silver nitrate (Morrissey, 1981). Proteins were electrophoretically transferred onto a nitrocellulose sheet (Towbin *et al.*, 1979) and the glycoproteins revealed by the Con A–peroxidase method (Hawkes, 1982).

Results and Discussion

Ultrastructural observations (TEM) show a bicellular pollen grain containing a vegetative nucleus, a generative nucleus, the cytoplasm of the vegetative cell with numerous amyloplasts and a bilayered pollen wall with a thin semi-tected exine (Figs. 1 and 2). The presence of a great amount of osmiophilic components in the pollen coat is observed within the arcades of the exine (Fig. 2). These components were also reactive in the polysaccharide and glycoconjugate detection test (Gaget, 1988). These components are able to diffuse rapidly from the pollen grain, as shown by pollen prints (not illustrated). Such diffusible materials are of polysaccharidic nature and contain glycoconjugates (Gaget, 1988). The freeze–fracture replicas show the presence of microchannels through the fibrillar intine (Fig. 3). These structures might be the pathway of diffusible pollinic components from the vegetative cell to the external surface. Such microchannels have been observed in the papillar wall of the female partner

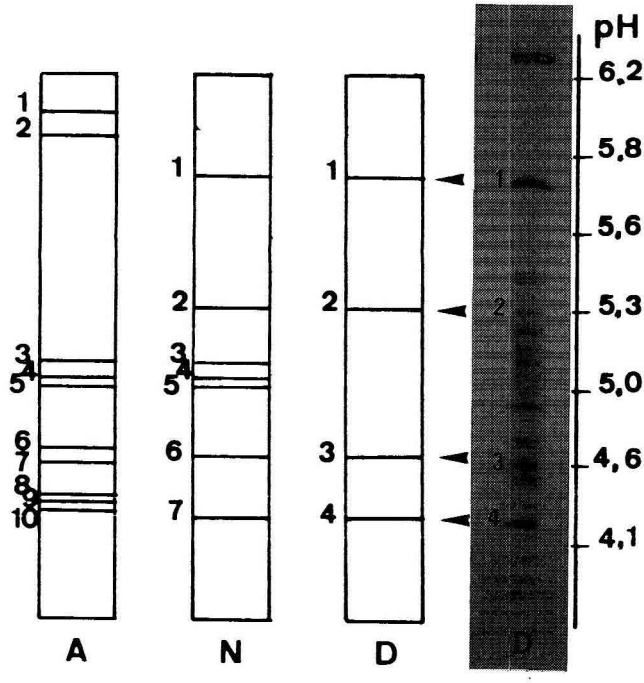

Fig. 4. Diffusable pollinic glycoproteins (Con A-binding proteins). Arrowheads: similar glycoprotein bands of species of section *Aigeiros (P. nigra and P. deltoides).* N: *P. nigra;* D: *P. deltoides;* A: *P. alba.*

and were implicated in the transport of recognition proteins to the female surface (Clarke *et al.*, 1980).

Globular proteins with molecular mass of 100 000 Da may be able to diffuse across the pollen wall by means of canaliculi whose diameter was estimated to be 30 nm. Protein assay showed that, depending upon the species used, 5–20% of the pollinic proteins diffuse in 10 min in Tris buffer, without affecting pollen viability. This protein fraction contains numerous protein bands whose molecular masses ranged between 10 000 and 100 000 Da. Among them, we found some peculiar bands associated with the group of incompatibility (Fig. 4). Some are specific to *Aigeiros* species (present in *P. nigra* and *P. deltoides*). Others are specific to *Leuce* species (present in *P. alba*). Moreover, these bands were also revealed by the Con A–peroxidase procedure and could be considered as glycoproteins.

Conclusion

Our work clearly demonstrates the existence of specific glycoproteins, whose presence in pollen is related to sexual incompatibility. Such a study should be extended to other species of the 5 sections of the genus *Populus.* Moreover, extraction and use of these specific glycoproteins implicated in pollen–pistil recognition could be an attractive tool for overcoming interspecific incompatibility in poplars.

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