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Sexual reproduction in *Populus* I. Some physiological and biochemical events of the progamic phase

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Introduction

Hybridization in *Populus* breeding programs is limited by sexual incompatibility barriers, whose cellular and molecular mechanisms are not yet known. In an attempt to understand the nature of interspecific incompatibility in *Populus*, we have explored the interactions between male and female partners (pollen–pistil) in compatible and incompatible crosses *P. nigra* (female) x *P. nigra* (male) *and P. nigra* (female) x *P. alba* (male) (Villar, 1987).

Materials and Methods

P. nigra 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. Kinetics of pollen tube growth *in vivo* were studied in growth chambers (20°C). Pollen tubes in the stylar tissues were visualized using the aniline blue fluorescence method (ABF method, Dumas and Knox, 1983).

Isoelectric focusing (IEF) of pollinic and stigmatic proteins, gel preparation, focusing conditions, silver nitrate staining and homogenates of pollinated stigma were performed according to the procedures of Villar *et al.* (1988). Each of the 2 crosses was represented by 3 series of stigmatic extracts, in accordance with the growth kinetics (0.6 and 20 h after pollination). Protein patterns of these extracts were compared on a single gel. Glycoprotein revelation (concanavalin A-binding proteins) on IEF polyacrylamide gel was adapted from Hawkes (1982). β-Galactosidase visualization was optimized from the protocol of Singh and Knox (1985).

Results and Discussion

Kinetics of pollen tube growth (visualization using the ABF method) have demonstrated distinct behaviors of *P. nigra* and *P. alba* pollen tubes inside *P. nigra* pistils (Fig. 1). *P. alba* pollen tubes exhibit a unique S-shaped growth curve and an arrested growth near the stylodium. On the other hand, *P. nigra* pollen tubes exhibit 2 growth phases, respectively, in the stigmatic tissues and in the ovarian cavity. *P. nigra* and *P. alba* curves diverge 5 h after pollination (20°C), corresponding precisely to the deposition of the first

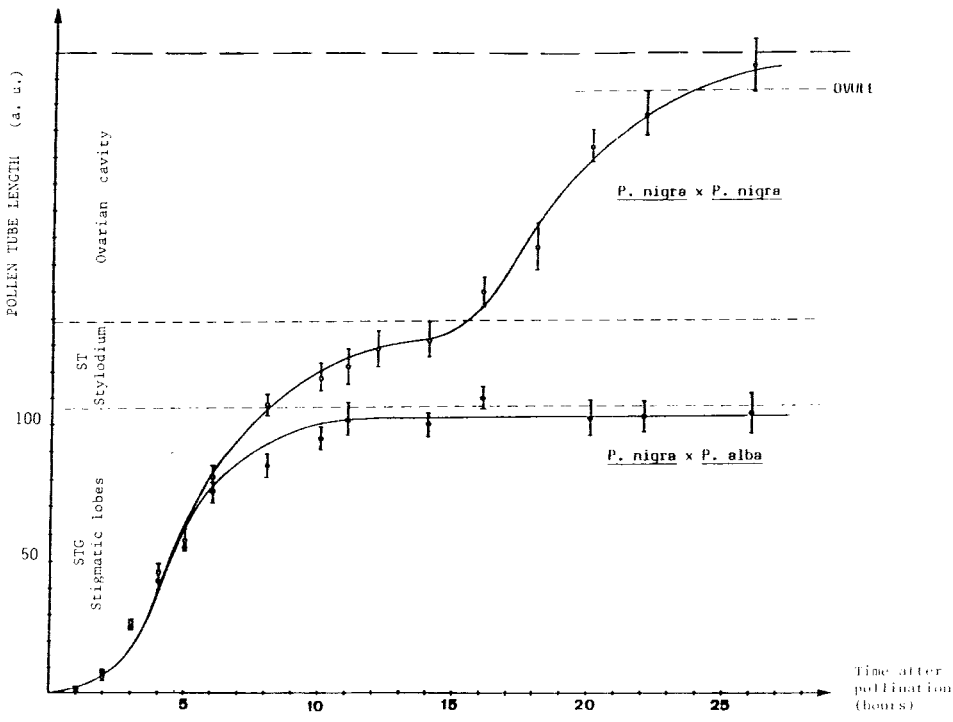

Fig. 1. Kinetics of pollen tube growth *in vivo*. (From Villar *et al.*, manuscript in preparation)

callose plug inside pollen tubes. This divergence could likely be related to a change in the physiology of the compatible pollen tube, shifting from an autotrophic to a heterotropic type of nutrition.

Biochemical studies focused on pollinic and stigmatic proteins, known to be involved in male–female interactions (Gaude and Dumas, 1987). After silver nitrate staining, qualitative and quantitative differences could be observed, related to the presence of *P. nigra* and *P. alba* pollen tubes inside stigmatic tissues. However, increasing protein bands were detectable, 0–20 h after pollination, only in compatible pollinated stigmas. The concanavalin A–peroxidase reaction allowed the visualization of 15 stigmatic and pollinic glycoproteins. Differences were observed, according to the type of cross: one distinct glycoprotein increases only in the compatible cross. Moreover, β-Galactosidase activities were revealed with a similar electrophoretic technique in pollinated stigma. This pollinic enzyme could play a role in heterotropic pollen tube nutrition (Singh and Knox, 1985). An increase of its activity (one isoenzyme of isoelectric point about pH 4.2) from 6–20 h after pollination was detected only in the compatible cross.

Conclusion

The compatible progamic phase in *P. nigra*, *i.e.*, pollen tube growth up to the

embryo sac, could be related to pollinic enzymes involved in pollen tube metabolism, such as β-galactosidase. Its activity could be the final result of a series of interactions started by initial pollen–stigma communications. This dialogue probably implicates protein compounds, detected in pollen and pollen tube diffusates *in vitro* (Villar, 1987; Gaget, 1988).

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