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BIDIRECTIONAL POLLINATION OF ANGIOSPERM AND GYMNOSPERM OVULES

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Pollen grains of *Pinus wallichiana*, *P. mugo*, *P. ponderosa* and *Ephedra distachya* germinated at various intensities on in vitro cultured placentas of 32 angiosperm species. Pollen of the same gymnosperms did not form tubes on stigmas of pistils cultured under the same conditions as the placentas. Pollen of several angiosperms germinated on semi in vitro cultured opened ovules of *Larix decidua* and nucelli of *Taxus baccata*. Angiosperm pollen did not germinate in vivo in the pollination drop secreted by ovules of *T. baccata*. This report shows that (1) gymnosperm pollen produces fully formed tubes on ovules of angiosperms but do not germinate on their stigma, and (2) pollen representing angiosperms is able to germinate and form tubes on ovules of gymnosperms.

Key words: Semi in vitro pollination, gymnosperms, angiosperms.

INTRODUCTION

Pollen-pistil interaction in angiosperms and pollen-ovule interaction in gymnosperms play an important role in sexual reproduction. In the majority of angiosperms, the earliest arrest of incompatible pollen tubes may occur in the stigma and style, and thus fertilization does not take place. In different gymnosperms the ovules receive pollen at various stages of development, and the incompatibility mechanism that exists is later-acting and occurs within the ovule (Biswas and Johri, 1997). The barriers to foreign pollen may occur during pollen germination, pollen penetration of the nucellus, and pollen penetration of the megagametophyte. Our knowledge of in vitro pollination and fertilization of ovules of gymnosperms is poor. Recently, in the first report concerning in vitro crosses among conifers it was shown that megagametophyte cells produce a secretion of an attractive nature for pollen development and growth towards and into megagametophytes belonging to different genera (Dumont-BeBoux et al., 1998). There are only two papers (Zenkteler, 2000; Zenkteler and Bagniewska-Zadworna, 2001) concerning germination of pollen of some gymnosperm species on placentas of angiosperms. Those investigations demonstrated that pollen grains of gymnosperms were capable of germinating soon after placement on the ovules of angiosperms, and that pollen tubes occasionally were entering the micropyles.

This paper compiles more data on semi in vitro pollination of placentas and pistils of angiosperms with gymnosperm pollen grains, and ovules of *Larix decidua* and *Taxus baccata* with pollen grains of some angiosperms.

MATERIALS AND METHODS

The plants used in these experiments were grown in the Botanical Garden of Adam Mickiewicz University, Poznań. Flower buds of angiosperms shortly before anthesis were surface-sterilized with chlorine water and sterile water. Pistils and placentas were

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excised with part of the calyx. Whole pistils and open placentas of 32 species bearing ovules were used for stigma and placenta pollination. To avoid the possible harmful effect of the chlorine water on stigma function, additional pistils were excised from unsterilized flower buds. Pollen of *Pinus wallichiana*, *P. mugo*, *P. ponderosa* and *Ephedra distachya* was collected during anther dehiscence (April, early May 2001, 2002) and refrigerated at 4˚C. Ovulate cones of *Larix decidua* and ovules of *Taxus baccata* were bagged in March, that is, about 2 months before anther dehiscence. Meanwhile, the developmental stages of megagametophytes were determined by sectioning (10–12 μm) ovules and staining with safranine and fast green. In May, scales of *Larix decidua* with whole ovules or with part of the nucellus removed at the stage of archegonia were dissected from the ovuliferous cones, pollinated with angiosperm pollen grains and transferred to culture medium. Nucelli of *Taxus* at the stage of pollination drop were dissected from the ovules and pollinated with angiosperm pollen. The pollinated pistils and placentas of angiosperm species as well as pollinated ovules and nucelli of gymnosperms were cultured in glass tubes on Murashige and Skoog (1962) minerals without sucrose. At 24, 48 and 72 h after cross-pollination the ovules were removed, stained in 1% aniline blue and examined for pollen tube growth using epifluorescence microscopy. All the cultures were kept in daylight at 20–24˚C.

RESULTS

GYMNOSPERM POLLEN ON PLACENTAS OF ANGIOSPERMS

Fresh or refrigerated pollen grains of *Pinus wallichiana*, *P. mugo*, *P. ponderosa* and *Ephedra distachya* germinated on ovules of 32 species representing 6 families of Angiospermae (Tab. 1). Pollen started to germinate 24–48 h after transfer to the ovules, and on the third day the pollen tubes became much longer and grew in various directions. Pollen grains of *P. wallichiana* germinated abundantly (80–85%) on ovules of 17 species, but on 6 species germination ranged from 15 to 55%. The percentage of pollen germination of *P. mugo*, *P. ponderosa* and *Ephedra* was generally lower (40–55%) and depended on the ovule species. The highest number of germinating pollen of gymnosperms was noted on ovules representing Liliaceae species. This was probably due to the large amount of exudate, as ovules of those species were covered with sticky substances. In general the best pollen germination and the longest tubes were observed in species whose placentas were covered with "wet" ovules. On placentas covered with "dry" ovules (e.g., *Atropa*, *Dianthus*) fewer pollen grains germinated and the pollen tubes were short. None of the pollen grains of gymnosperm germinated on angiosperm stigmas. Generally on the third and fourth day after pollination only drying pollen grains were observed on the stigmas.

ANGIOSPERM POLLEN ON THE OVULES OF *LARIX DECIDUA* AND *TAXUS BACCATA*

Pollen grains of angiosperm species (Tab. 2) adhering to the ovule (Fig. 2) of *L. decidua* at the stage of fully developed archegonial cells initiated germination a few hours after pollination and 24 h later the tubes became long and grew in various directions (Fig. 3). This suggests that the factors responsible for tube formation may be present in the ovules. In some combinations of crosses, the micropyle seemed to be attracting the pollen by providing a non-specific signal. For example, there were long pollen tubes of *Spathiphyllum* concentrated around the micropyle (Figs. 4, 5). Isolated ovules of *Taxus* did not attract pollen germination. Better results were only obtained when the integuments were removed and the pollen was put directly on the nucellus (Fig. 1). In that case, pollen of 5 species from 16 applied (Tab. 3) in our experiments produced well-developed tubes growing around the nucelli. The number of pollinated pistils of each species was 10–12, placentas 15–18, and ovules/nucelli 20–25.

DISCUSSION

In our semi in vitro experiments, pollen of gymnosperm species developed tubes normally on angiosperm ovules, but the results do not clearly indicate

Fig. 1. Opened ovule with exposed nucellus of *Taxus baccata* pollinated with pollen of *Laburnum anagyroides*. × 18. **Fig. 2.** Ovuliferous scale of *Larix decidua* with ovule pollinated with pollen of *Laburnum anagyroides* (arrow). × 18. **Fig. 3.** Germinating pollen grains of *Laburnum anagyroides* on ovule of *L. decidua*. × 175. **Figs. 4–5.** Pollen grains of *Spathiphyllum wallissi* concentrating around ovule micropyle of *Larix decidua*. × 175 and × 350, respectively.

TABLE 1. In vitro pollination of placentas of angiosperms with pollen grains of gymnosperms

Female parent	Male parent	Pollen germination status
Caryophyllaceae		
Dianthus caryophyllus	*Pinus ponderosa*	++
Dianthus caryophyllus	*P. wallichiana*	+++
Dianthus gratianopolitanus	*P. mugo*	++
Lychnis coronaria	*P. wallichiana*	+++
Melandrium album	*Ephedra distachya*	++
Melandrium rubrum	*P. mugo*	++
Melandrium album	*P. wallichiana*	++
Silene caucassica	*P. ponderosa*	+++
Silene vulgaris	*P. ponderosa*	++
Silene vulgaris	*P. mugo*	++
Silene vulgaris	*P. wallichiana*	+++
Liliaceae		
Galanthus nivalis	*P. wallichiana*	+++
Allium moly	*P. wallichiana*	+++
Allium odorum	*P. wallichiana*	+++
Anthericum liliago	*P. mugo*	++
Anthericum liliago	*P. wallichiana*	+++
Convallaria majalis	*P. wallichiana*	+++
Gagea lutea	*P. wallichiana*	+++
Hemerocallis flava	*P. wallichiana*	+++
Lilium martagon	*Ephedra distachya*	++
Lilium martagon	*P. wallichiana*	+++
Ornithogalum umbelatum	*P. mugo*	+++
Polygonatum multiflorum	*P. mugo*	+++
Polygonatum multiflorum	*P. ponderosa*	+++
Polygonatum multiflorum	*P. wallichiana*	+++
Brassicaceae		
Brassica napus	*P. mugo*	+
Brassica napus	*P. ponderosa*	++
Brassica napus	*P. wallichiana*	+++
Fabaceae		
Lupinus polyphyllus	*P. wallichiana*	+
Lathyrus latifolius	*P. wallichiana*	+++
Pisum sativum	*P. wallichiana*	++
Vicia faba	*P. mugo*	++
Solanaceae		
Nicotiana tabacum	*Ephedra distachya*	+++
Nicotiana tabacum	*P. mugo*	+++
Nicotiana tabacum	*P. wallichiana*	+++
Nicotiana silvestris	*P. wallichiana*	+++
Datura suaveolens	*P. wallichiana*	+++
Petunia violacea	*P. wallichiana*	++
Atropa belladonna	*P. wallichiana*	+
Ranunculaceae		
Helleborus niger	*P. wallichiana*	+
Paeonia delawayi	*P. mugo*	+
Delphinum elatum	*P. wallichiana*	+
Delphinum elatum	*Ephedra distachya*	+
Aquilegia vulgaris	*P. wallichiana*	++

+++ – 80–85% pollen germinated and mostly formed long tubes
++ – 40–55% pollen germinated and formed long and short tubes
+ – 15–20% pollen germinated, short tubes prevailed

TABLE 2. Angiosperm pollen on unsterilized ovules of *Larix decidua*

Angiosperm pollen	Results
Allium moly	–
Anemone silvestris	–
Brassica napus	–
Cytissus sp.	+
Hemerocalis middendorfii	–
Laburnum anagyroides	+
Lychnis coronaria	+
Lychnis viscaria	+
Paeonia officinalis	+
Papaver orientalis	–
Spathiphyllum wallissi	+*
Vincetoxicum hirundinaria	+
Potentilla delphinensis	+

\+ abundant germination
– nil
+* pollen tubes concentrated at the micropyle

TABLE 3. Angiosperm pollen on unsterilized nucelli of *Taxus baccata*

Angiosperm pollen	Results
Allium moly	+
Brassica napus	+
Dictamnus albus	–
Eremurus robustus	–
Hemerocallis middendorfii	–
Laburnum anagyroides	–
Lychnis coronaria	–
Lychnis viscaria	+
Lilium martagon	+
Ornithogalum umbelatum	–
Paeonia officinalis	–
Polygonatum multiflorum	–
Rosa glauca	–
Silene vulgaris	–
Spathiphyllum walissii	+
Vincetoxicum hirudinaria	+

\+ abundant germination
– nil

the presence of a recognition mechanism because the ovules of all species tested did not discriminate the foreign pollen tubes. It may be that contact with any ovule generally attracts the pollen and suits germination and formation of fully developed tubes. It was not demonstrated that mainly the micropyle facilitated pollen germination, as pollen tubes were usually dispersed all over the ovules. In some crosses such as *Larix decidua* × *Spathiphyllum wallissi*, pollen tubes concentrated around the micropyle and even entered inside the micropylar canal, but this was an exception.

Particularly interesting is that even pollen grains of some angiosperm species were capable of producing tubes shortly after landing on the ovules (*Larix*) and nucelli (*Taxus*). Thus, barriers to angiosperm pollen might be absent on naked ovules/nucelli of gymnosperms. However, angiosperm pollen discrimination may occur in the pollination drop of the micropyle. As mentioned by Owens et al. (1998), the pollination drop, rich in sugars, amino acids, peptides and organic acids, restrains germination of foreign pollen, thus acting as one prezygotic incompatibility mechanism. Our preliminary investigations (not included in this paper) of pollinating in vivo ovules of *Taxus baccata* demonstrated that the pollination drop prevents germination of angiosperm pollen grains.

This report of wide crosses between angiosperms and gymnosperms shows that no recognition mechanism affects foreign pollen growth on ovules. It will be most interesting to obtain more information on the possibility of pollen tube growth inside female gametophytes and the fate of alien gametes.

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