POLLEN TUBE BRANCHING IN THE OVARY OF FIVE SPECIES OF *OENOTHERA*

RENATA ŚNIEŻKO

Department of Cell Biology, Institute of Biology,
Maria Curie-Skłodowska University,
Akademicka 19, 20-033 Lublin

ABSTRACT

Flowers of *Oenothera hookeri* Torr. et Gray, *Oe. brevistylis* and *Oe. lamarckiana* de Vries were pollinated after anthesis by insects. *Oe. biennis* L., *Oe. suaveolens* Desf and *sulfurea* were selfpollinated in the buds. Pollen morphology was slightly different: *Oe. hookeri* have regular, triporated pollen, often germinating through two pores; in *Oe. suaveolens* many pollen grains had callose patches on the intine; in *Oe. brevistylis* tetraporated pollen were more often than in other species; in *Oe. lamarckiana* many pollen grains were empty; in *Oe. biennis* and *Oe. suaveolens* pollen grain size and viability varied. The pollen tube growth and fertilization were similar in 5 species and can be considered as typical for *Oenothera*. In the ovary pollen tubes branched and changed their growth direction. Near micropyle they formed short branches to the inner integument. In the nucellus the pollen tube became swollen.

KEY WORDS: *Oenothera*, pollination, fertilization, pollen tube growth.

INTRODUCTION

Oenotheras are well known as very fertile plants, having some peculiar features in their developmental cycle. Their flowering and seed-setting were described for many species. *Oe. hookeri*, *Oe. lamarckiana* and *Oe. brevistylis* are cross-pollinating plants, while *Oe. biennis* and *Oe. suaveolens* are cleistogamic and self-pollinating. *Oe. hookeri* is an homozygote, other species are heterozygotic. Crossings between *Oenothera* species were applied for numerous breeding programs in genetical studies (Harte 1948; Renner 1946; Stubbe 1953). In those studies the fertilization process was not observed directly, but determined from morphological and genetical characteristics of the progeny (Schwemmler, 1968). Microscopic features of fertilization and embryo development was described by Ishikawa (1918), but he considered only the processes inside the ovule. Pollen tubes and stigma incompatibility was described by Dickinson and Lawson (1975). In the present paper detailed observations of the natural pollination and the successive stages of the progamic phase till fertilization were carried out in 5 *Oenothera* species.

MATERIAL AND METHODS

Plants of *Oenothera hookeri* Torr. and Gray, *Oe. lamarckiana* de Vries, *Oe. brevistylis*, *Oe. biennis* L., *Oe. suaveolens* *sulfurea* and *suaveolens* Desf. were grown in 1994 and 1995 in the experimental garden of the Biology Institute, Maria Curie-Skłodowska University in Lublin. Genetically stable forms were obtained every year by selfing.

During July and August 30-45 buds and flowers of each species were taken for microscopic observations. Pistils at dif-

ferent developmental stages were macerated in 1N NaOH, 1/2 h, 60°C, rinsed in water and stained with aniline blue. Squashed preparations were observed in Nikon fluorescence microscope in UV 430 nm. Samples of pollen grains were tested *in vitro* for germination ability.

RESULTS

The inflorescence of *Oenothera* is shown in Fig. 1. The regular arrangement of the buds and flowers along the axis allowed us to ascertain the developmental stage, which was important to study self-pollination. One or sometimes two flowers were opening every evening. Anthesis of *Oe. biennis* took place at 6 pm, *Oe. suaveolens* 1/2 hour later, and others opened about 8 pm. Each flower remained open during the night and till next day noon, then withered. The time of withering depended on the temperature – on hot days the flowers withered already at 10 am.

Morphology and germination of pollen grains were compared on the stigma and *in vitro*. *Oenothera* pollen grains were connected by viscine threads in big clusters. The triporate *Oe. hookeri* pollen grains were of the most regular shape and size. The number of empty grains was relatively low in this species. Two pollen tubes very often germinated from one pollen grain (Fig. 2). In *Oe. brevistylis* the tetraporate pollen grains were more numerous than in the other species. Such grains germinated successfully (Fig. 3). In pollen of *Oe. suaveolens* Desf. many grains had callose spots on the intine and in the pores (Figs 4, 6). Such grains did not germinate. In *Oe. lamarckiana*, *Oe. biennis* and *Oe. suaveolens* pollen grains differed in size and many of them were empty; at least 3-4 classes of pollen grains could be distinguished (Figs 5, 6). Me-

Fig. 1. Scheme of the *Oenothera* inflorescence. Explanations in text.

dium-sized pollen grains germinated well, whereas the biggest did not germinate.

On the stigma, the self and cross-pollen grains germinated 1/2-2 hours after pollination depending on the temperature and humidity. Pollen tubes grew well on the upper and lower side of the stigmatic lobes. The pollen tubes grew from the stigma to the style after crosspollination (Fig. 7) and selfpollination (Fig. 8).

In *Oe. hookeri*, *Oe. brevistylis* and *Oe. lamarckiana* flowers opened late in the evening. The stigma lobes spread, anthers opened in less than 1/2 an hour after anthesis (depending on weather conditions, temperature and humidity). One hour later some pollen were found on the stigmas. During the night a large amount of pollen grains was deposited on the stigma. Three hours after anthesis many pollen grains started to germinate. During the night they grew through the style to the ovary. At 8 am. in *Oe. hookeri* pollen tubes were found inside the placenta, one hour later on its surface. On the placental surface, numerous growing pollen tubes were branching and making many turnings (Fig. 9). There were various: long and smooth, short and swollen as bubbles. Some pollen tubes split several times and the branches grew a long distance in different directions (Fig. 14).

In each species the pollen tubes always entered the ovules through the micropyle. On the next day after pollination most ovules were fertilized (Figs 9-13, 15, 16). Near the micropyle of the ovule the pollen tube formed short branches which grew into the inner integument (Figs 11, 13, 15). The main tube penetrated into the nucellus and grew directly to the embryo sac (Figs 15, 16). It became much wider in the subterminal part and often had a bright callose spot at the tip (Figs 14, 16).

The self-pollinating and cross-pollinating *Oenothera* differed in the time of their pollination. In *Oe. biennis* and both forms of *Oe. suaveolens* the pollination and fertilization was observed in the buds at the 2nd or 3rd level above the open flower (see Fig. 1). In the "-2" bud the stigma was of the same length as anthers. They surrounded the stigmatic lobes and distributed pollen grains directly on the outer surface of the stigma. This did not disturb the pollen grain germination and pollen tube growth (Fig. 8). At first they turned to the middle part of the stigma and then inside the tissue of the style.

In *Oe. biennis* and *Oe. suaveolens* pollen tubes were found in the style in "-2" level buds and inside the ovary in the "-1" buds (Fig. 1). In the just opened flowers nearly all ovules were fertilized (Figs 10, 11).

DISCUSSION

Many specific characteristics were found and described in *Oenothera*. Plants developed their own meiosis and spores formation depending on the genetic constitution (Renner 1917; Catcheside 1932).

In recent studies of pollen germination and pollen tube growth some features characteristic for *Oenothera* were found. Two pollen tubes can germinate from more than one porus. Such pollen tube germination was seen in every species, it occurred very often in *Oe. hookeri*. Both pollen tube branches grew for a long time. Pollen tube branching inside the style is very difficult to observe in a preparation because a large number of pollen tubes forming a bundle blurred the picture. In the ovary, especially on the placental surface, the pollen tubes are branching and changing their growth direction. The pollen tube growth and fertilization were observed in female sterile form of *Oe. brevistylis* (Śnieżko and Winiarczyk 1995). In this plant branching and winding of the pollen tube in the ovary was connected with a lack of fertilization. In the investigated species branchings and turnings are frequent in very fertile *Oe. hookeri* and in other species. This phenomenon can be considered as the usual pollen tube growth in *Oenothera*. Branching of pollen tubes was described for *Spinacia* inside the nucellar tissue (Wilms 1974), but is not a very common behaviour of the pollen tubes examined. Most in them are described as direct tubes e.g. in *Liliaceae*, *Rosaceae*, *Cruciferae* (Dickinson and Lewis 1973; Dickinson et al. 1982; Heslop-Harrison and Heslop-Harrison 1986; Janson 1992; Oekendon 1972; Śnieżko and Winiarczyk 1995; Willemse and Franssen-Verheijen 1986).

The last stage of pollen tube growth in the studied of *Oenothera* species is very specific. In the vicinity of the micropyle the pollen tube formed some small branches which were directed to the inner integument. The main branch of the pollen tube entered the nucellus, where it became much wider than before. This pollen tube behaviour could be considered characteristic for all *Oenothera*.

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Fig. 2. Two pollen tubes growing from *Oe. hookeri* pollen grain. x 400.

Fig. 3. *Oe. brevistylis* tetraporate pollen grain. x 400.

Fig. 4. *Oe. suaveolens* pollen grains with callose spots on the intine and in the pores. x 400.

Fig. 5. *Oe. biennis* pollen grains of different size; many small grains are empty. x 150.

Fig. 6. Pollen grains of *Oe. suaveolens*; small, yellow are empty. x 150.

Fig. 7. Pollen tubes of *Oe. hookeri* below the stigma. x 150.

Fig. 8. Pollen grains of *Oe. biennis* with the pollen tubes from bud at 2nd level above open flower. x 150.

Fig. 9. *Oe. hookeri* pollen tubes winding on placental surface; ovules already fertilized. x 100.

Fig. 10. Fertilized ovule of *Oe. biennis*. x 100.

Fig. 11. Micropylar part of fertilized ovule of *Oe. suaveolens*; many short branches direct to the integument. x 400.

Fig. 12. *Oe. brevistylis* fertilized ovule. x 100.

Fig. 13. Micropylar part of the *Oe. lamarckiana* ovule. x 400

Fig. 14. *Oe. hookeri* pollen tube turning and branching on the placenta; wider subterminal part; a bright callose spot at the tip. x 400.

Fig. 15. Fertilization of *Oe. hookeri* ovule. x 100.

Fig. 16. Micropylar part of *Oe. hookeri* ovule; short branches of the pollen tube connected with integument. x 400

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ROZGAŁĘZIANIE SIĘ ŁAGIEWKI PYŁKOWEJ W ZAŁĄŻNI U 5 GATUNKÓW *OENOTHERA*

STRESZCZENIE

Zapylenie, faza progamiczna i zapłodnienie badano w warunkach naturalnych u 5 gatunków *Oenothera*: *hookeri* Torr. et Gray, *lamarckiana* de Vries, *brevistylis*, *biennis* L., *suaveolens* Desf. i *suaveolens sulfurea*. Zapylenie i wzrost łagiewki pyłkowej obserwowano przy użyciu mikroskopu fluoroscencyjnego w świetle UV 430 nm w macerowanych słupkach, barwionych błękitem anilinowym. Pyłek poszczególnych gatunków różnił się cechami morfologicznymi, np. pyłek *Oe. lamarckiana* bardzo często miał 4 porusy, a pyłek *Oe. suaveolens* wykazywał świecenie kalozy na wewnętrznej ścianie. Kielkowanie i rozwój łagiewki były podobne u wszystkich gatunków. W załączni łagiewki pyłkowe rozgałęziały się i zmieniały kierunek wzrostu. Taki typ wzrostu łagiewki pyłkowej uznano za charakterystyczny dla *Oenothera*.

SŁOWA KLUCZOWE: *Oenothera*, zapylenie, zapłodnienie, wzrost łagiewki pyłkowej.