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Changes of electric potential in pistils of *Petunia hybrida* Hort. and *Brassica napus* L. during pollination

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Abstract

The pattern of electric signals accompanying compatible and incompatible pollination were studied in pistils of petunia (*Petunia hybrida* L.) and rape (*Brassica napus* L). Electric potential was recorded for 4-7 hours with non-polarizable Ag/AgCl electrodes implanted into the ovary and beneath the sigma. At the end of measurements, pistils were fixed and the growth of pollen tubes was analyzed under a fluorescent microscope.

Action potentials appeared in both species. In rape the potential dropped by 10 mV for few minutes after pollination regardless of the compatibility of the cross. In this species, during compatible pollination action potentials with amplitudes of 15-20 mV were recorded up to one hour after pollination. They were followed by a long lasting decrease of the potential by 10 to 50 mV. Contrary, after the self-incompatible pollination, action potentials were rare and of lower amplitudes and the potential gradually raised in comparison to the initial level. During the first hour after the compatible pollination of *Petunia hybrida* series of action potentials with amplitudes reaching 10-20 mV were recorded. At the time corresponding to the pollen tubes entrance to the transmitting tissue of the style, action potentials reaching up to 40 mV were followed by a steady decrease of the potential. The electric signals traveled along the style with velocity of 25 mm/s. Incompatible pollination in petunia resulted only in minor oscillation and gradual increase of the potential up to 100 mV in comparison to the initial level.

The present investigation demonstrated that each phase of pollen-stigma recognition events, germination and growth of pollen tubes within the style have its characteristic pattern of electric changes which was species specific and depended on compatibility of the cross.

List of abbreviations: EP – electric potential; hAP – hours after pollination; AcP – action potential

Introduction

Various types of electric signals were described in plants (for review see: Pickard 1973, Davies *et al.* 1991, Roberts 1992). They are the manifestation of membrane depolarisation travelling with various speed along tissues to distant plant organs. Touching, wounding, chilling and treatment with chemicals induce specific waves or oscillation of the electric potential (EP). Changes of EP accompany pollination and germination of pollen on the stigma (Lysikov and Dukhovnyi 1966, Sinyukin and Britikov 1967, Linskens and Spanjers 1973, Spanjers 1981, Fromm *et al.* 1995, Wędzony and Filek 1996). Compatible and incompatible pollination, application to stigma of the alien or inactivated pollen and mechanical stimulation of the stigma resulted in different electric patterns (Lysikov and Dukhovnyi 1966, Linskens 1973, Spanjers 1981, Fromm 1995).

To our knowledge correlation between pollen tube growth and electric response of the pistil was studied only in wheat (Wędzony and Filek 1996). Ac-

tion potentials were not recorded during this study, only long lasting waves of depolarization occurred. However, morphology of a wheat pistil is not typical for most higher plants and there is no self-incompatibility system in this species. Therefore, it was decided to extend experience by recording EP in *Brassica napus* and *Petunia hybrida* styles following compatible and incompatible pollination and simultaneously analyse pollen tubes growth. *Brassica napus* L. bears the well known sporophytic system of self-incompatibility, while petunia represents the gametophytic system. Comparison of these two ways of the prevention of self-pollination can give additional information on the nature of signalling between the stigma and the ovary.

Material and methods

Self-incompatible population of petunia (*Petunia hybrida* L.) and winter rape (*Brassica napus* L. *ssp. oleifera cv.* 'Górczański') were grown in a climatic chamber under 16/8 day/night photoperiod at 21/17 °C respectively. Two days before anthesis flowers were emasculated and isolated. At the time of anthesis they were cut off and placed in Faraday's cage on plastic isolation beds with stocks deepen in APW (0.1 mmol·dm^{-3} KCl, 0.1 1 mmol·dm^{-3} NaCl, 0.2 mmol·dm^{-3} $CaCl_2$). Electric measurements were done with a pair of Ag/AgCl microelectrodes filled with 0,1 mol·dm^{-3} KCl in 0.6 % agar, and ended with glass capillaries with tip diameter under 1 μm, pulled with Sutter P-87. The reference electrode was implanted for approx. 1 mm into the ovary base while the measurement electrode was placed at the same depth immediately beneath the stigma. Implantation was performed with the use of a micromanipulator under the control of a stereomicroscope. Additionally, in ten petunia flowers the second measurement electrode was implanted into the style 15 mm beneath the first one. Electrodes were connected to a preamplifier with an input impedance over 10^{12} attached to a voltmeter (Axonprobe 1-A). EP values were continuously monitored on the voltmeter display and they were recorded in the computer every 30 seconds.

Compatible (18 in petunia, 10 in rape) and self-incompatible (11 in petunia, 20 in rape) pollination was done by shaking stamen heads over the stigmas without touching the latter. Electric records were taken for 4-7 hours. To control the electric stability of the system 10 unpollinated pistils of each, petunia and rape, were monitored for 7 and 5 h respectively.

At the end of the measurements, the pistils were fixed in ethanol : acetic acid mixture (3 : 1). Also pistils of both species pollinated in the climatic chamber and in Faraday's cage prepared in the same way as for measurements, were fixed at one hour intervals up to 7 hours after pollination (hAP). The latter were used to study the normal pattern of tubes growth and possible modifications of this process in experimental conditions. Squashed preparations of pistils were examined later in aniline blue according to Wędzony and Van Lammeren (1996).

Results

Pollen tubes growth

For both investigated species, the timing and the pattern of growth of pollen tubes were the same in the climatic chamber and in Faraday's cage. After compatible pollination of *Brassica napus* emerging pollen tubes were seen at 1 hAP, and they reached stigmatic tissue beneath papillae at 2 hAP (Fig. 1a). Five hAP the rape pollen tubes were half way from the stigma to the base of the style. After the self-pollination in *Brassica* the hydration of pollen was incomplete and it did not germinate. Stigmatic papillae which had directly contacted incompatible pollen grains deposited callose in their walls (Fig. 1b).

After compatible pollination of petunia flowers 90 % of pollen grains were already germinated at 1 hAP. Two hAP numerous tubes were seen in the stigmatic tissue where they showed a 'curly' pattern of growth (Fig. 2a). Three hAP first bunches of pollen tubes were seen in the transmitting tissue of the style and two hours later most germinated tubes were found there. The tubes in transmitting tissue had smooth surface and showed callose knobs at regular intervals (Fig. 2c). Growth rate of tubes in the transmitting tract was fast and at 7 hAP they were about twice as long as at 5 hAP. In self-

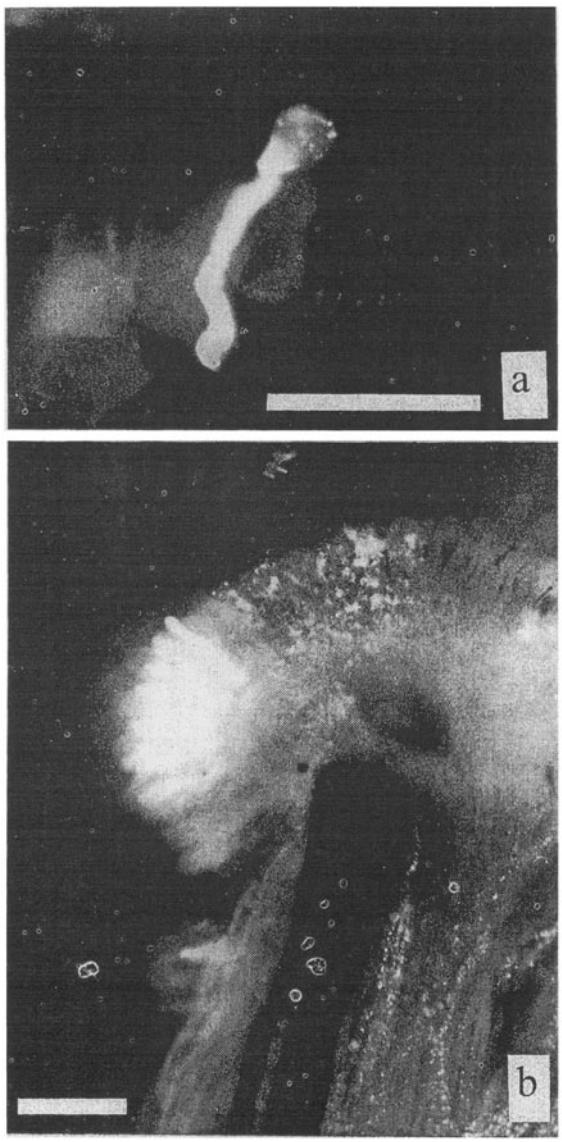

Fig. 1. *Brassica napus*. Squashed preparations of pistils stained with aniline blue and analyzed under fluorescent microscope with UV light. Bars = 50 μm. a). Compatible pollen grain on the stigma fixed 2 hours after pollination. Pollen tube had grown along pappilla and reached stigmatic tissue beneath. b). Style fixed five hours after incompatible pollination. Loosely attached pollen grains were washed away during fixation. Callose is visible in pappillae which had contacted pollen grains.

pollinated flowers of petunia about 50 % of pollen did not germinated within an hour. Later, the tubes growth was less regular and slower in the stigmatic tissue in comparison to compatible pollination, therefore only few incompatible pollen tubes reached the transmitting tract of the style up to 7 hAP (Fig. 2 b).

Measurements of the electric potential

The EP showed instability caused by the injury stress for 5 to 10 minutes after the microelectrodes implantation. Later, it was stable and showed only 3 mV slowly changing fluctuations in unpollinated pistils. The values of the membrane EP ranged from –90 to –100 mV in petunia and from –80 to –95 mV in rape. Pollination was always done after stabilization of EP *i. e.* about 25 minutes after the electrodes implantation. EP changes in form of action potentials (AcP) of relatively high amplitude, oscillation of lower amplitude and long lasting electric waves were recorded. The exact time of their appearance and the exact values of amplitudes varied from sample to sample, however, a general pattern characteristic for each species and type of pollination can be determined.

Brassica napus

A few minutes after pollination in *Brassica napus* EP dropped by 5 mV regardless of compatibility of the cross (Fig. 3a). That probably reflects pollen - stigma recognition events. During the first hour after compatible pollination AcPs reaching 15 - 20 mV and lasting a few minutes along with minor oscillations were recorded (Fig. 3b). The time of their appearance corresponds to hydration and germination of compatible pollen. Subsequently EP was decreased by 10 - 50 mV while pollen tubes grew along papillae of the stigma and entered the transmitting tract of the style.

After incompatible pollination long series of AcPs were not recorded, only single, minor AcPs sometimes occurred (Fig. 3b). About one hour after pollination the EP rose usually by 5-20 mV from the initial level and remained stable during further recordings.

Petunia hybrida

Electric potential recordings done on the pistils shown in Fig. 2 a are demonstrated in Fig. 4 a. Its pattern is typical for compatible pollination in petunia. Several major AcPs were usually noticed during the first 30 minutes (Fig. 4a). Their amplitudes reached 10-20 mV and impulses lasted for a few

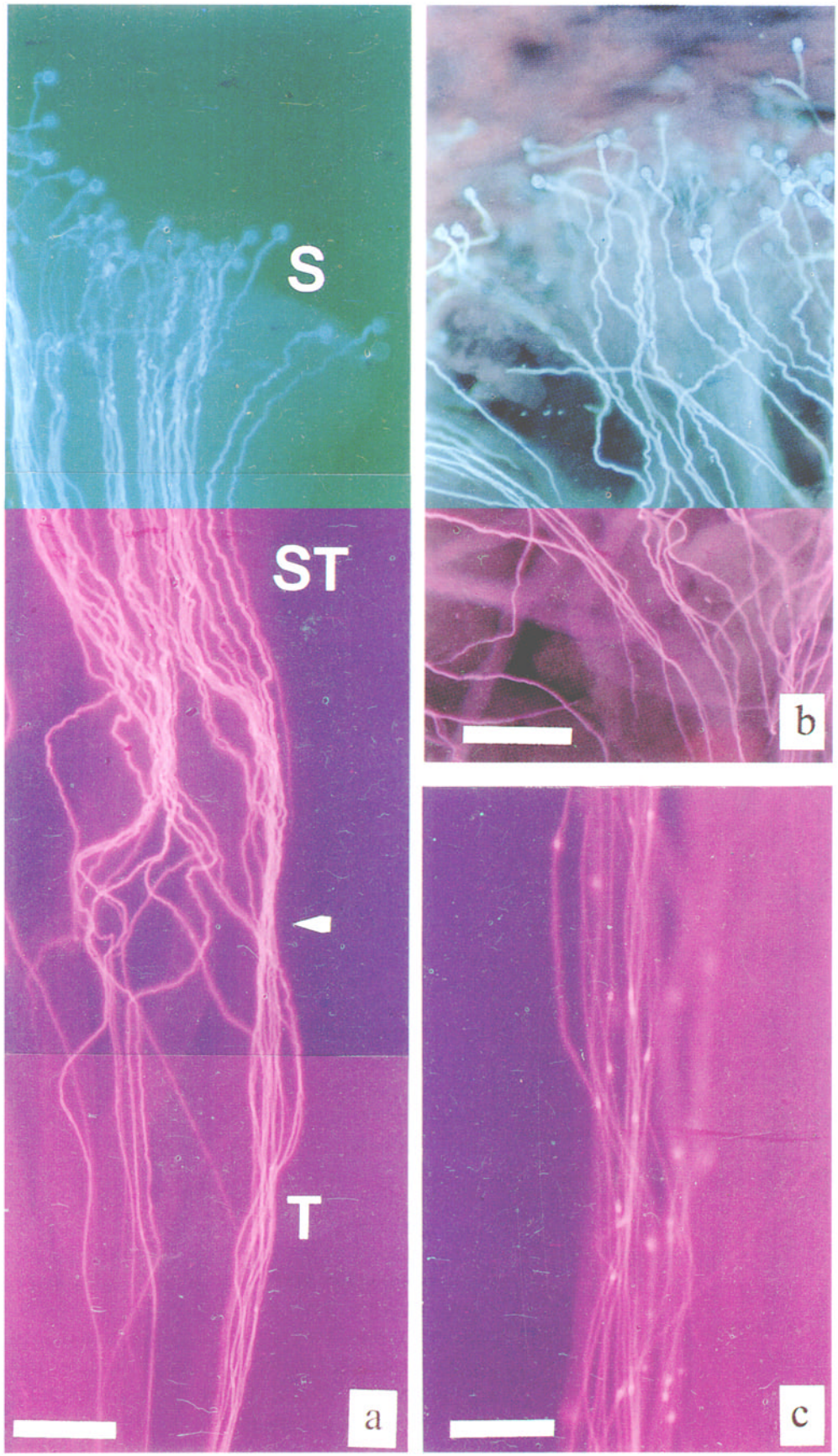

Fig. 2. *Petunia hybrida*. Squashed preparations of pistils after compatible (a, c) and incompatible (b) pollination stained with aniline blue and analyzed under fluorescent microscope with UV light. Bars = 50 μm. a). Pistil fixed after 5 hours electric recordings. Note changes in pollen tubes' morphology on their way from the stigma surface covered with exudate (S) through the region of the stigmatic tissue (ST) to the transmitting tissue (T). An arrow-head points to the area of transition from the stigmatic tissue to the transmitting tract. b). Pistil fixed after 7 hours of electric potential recordings during incompatible pollen tubes, growth. Picture shows region of stigma and stigmatic tissue. Tips of slowly growing pollen tubes visible at the bottom of the picture c). Fragment of transmitting tissue with passing pollen tubes fixed 7 hours after compatible pollination. Note smooth surface of pollen tubes and regular deposition of callose knobs.

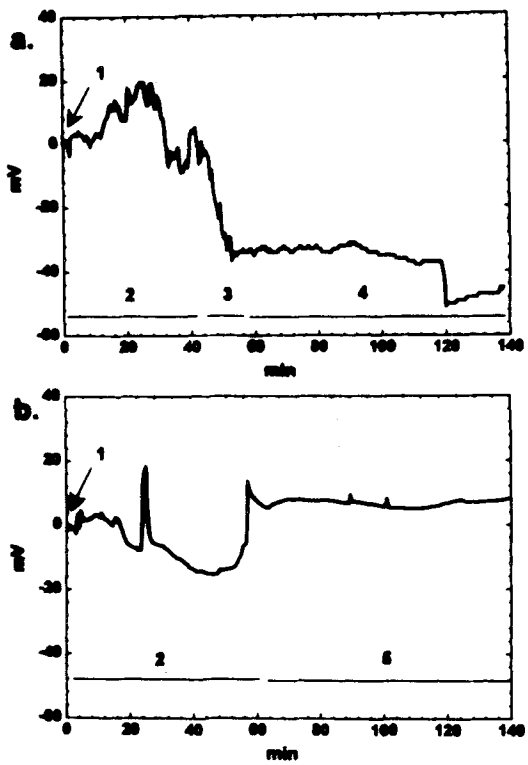

Fig. 3. *Brassica napus*. Examples of electric potential recordings after compatible (a.) and incompatible (b.) pollination. Numbers mark cytological events accompanying recordings. 1 – moment of pollination. Potential recorded at that time was regarded later as the 0 level. 2 – recognition and hydration of pollen grain; 3 – pollen tube emergence; 4 – pollen tube growth along pappilae and to the stylar tissue; 5 – rejection of pollen grains, callose reaction.

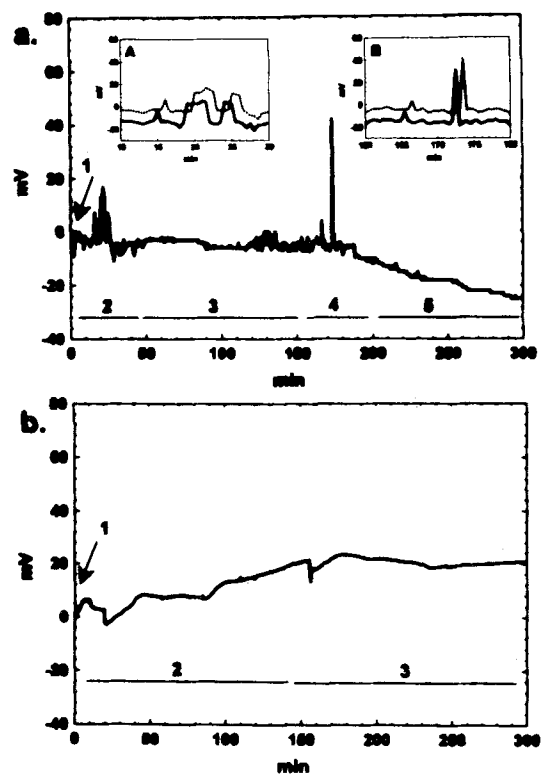

Fig. 4. *Petunia hybrida*. Examples of electric potential recordings after compatible (a.) and incompatible (b.) pollination. Numbers mark cytological events accompanying recordings. 1 – moment of pollination. Potential recorded at that time was regarded later as the 0 level. 2 – recognition, hydration and germination of pollen grains; 3 – pollen tubes' growth through stigmatic tissue; 4 – transition of tubes to the transmitting tissue; 5 – growth of pollen tubes along transmitting tissue.

minutes. The time of their appearance corresponds to pollen germination. The pollen tubes growth within stigmatic tissue was accompanied by numerous EP oscillations. The second series of AcP reaching the highest recorded amplitude, *i.e.* 40 mV, usually appeared 150-180 minutes after pollination (Fig. 4 a). The latter coincide with the pollen tubes penetration to the transmitting tissue of the style (Fig. 2 a). The AcPs in *Petunia* travelled along the style with the velocity of 25 mm/s as was registered with the two electrodes system (Fig. 4a A and B). The long lasting decrease of EP followed the second series of AcPs while the EP oscillation was considerably diminished at that period (Fig. 4a). The latter pattern corresponds to the phase of pollen tubes growth in the transmitting tract of the style (Fig. 2b).

During incompatible pollination in Petunia, the series of the AcPs and the major EP oscillation were absent (Fig. 4b). Minor single pikes were sometimes recorded at the time of pollen germination and during the incompatible pollen tubes growth in the stigmatic tissue. A few hours after pollination the EP usually increased to the level from 20 to 100 mV higher in comparison to the initial one (Fig 4b).

Discussion

The presented data show that AcPs of relatively high amplitudes appear in style a few minutes after compatible pollination in *Brassica napus* (Fig. 3a) and *Petunia hybrida* (Fig. 4a). Similar data were reported from *Zea mays* (Lysikov and Dukhovnyi 1966), *Incarvillea grandiflora, I. delvayi, Lilium martagon* (Sinyukhin and Britikov 1967) and *Hi-*

biscus rosachinensis (Fromm *et al.* 1995). Species investigated so far belong to various taxa and they have variable anatomy of styles. Therefore it can be suggested that the transduction of AcP is a universal way of fast spreading of information about the pollination event. In some studies, however, the early post-pollination AcPs were not reported (Linskens and Spanjers 1973, Spanjers 1981). That could be caused by imperfect methodology applied in the latter experiments. Especially the relatively large diameter of the capillary, *i.e.* 2-5 μm, and the high concentration of KCl (3 mole·dm^{-3}) is not in favour of precise recordings. The leakage of K^+ from the electrode tip to the surrounding tissue can cause local alterations of EP and it could influence the sensitivity of the system. To avoid those problems, lower concentration of the electrolyte additionally gellyfied with agar and low diameter of the capillary tip were applied in the current experiment.

During compatible pollination of *Petunia* in the present study the second wave of AcPs with high amplitudes was recorded 2-2,5 hAP (Fig. 4a), the time of pollen tubes penetration to the transmitting tissue of the style. Those impulses resemble AcPs found in *Lilium longiflorum* 220-400 min AP (Spanjers 1981). In both species representing the gametophytic type of incompatibility the final recognition of tubes probably takes place within the transmitting tract of the style which corresponds to the time of appearance of AcPs. Therefore, it can be supposed that the second wave of AcP expresses the acceptance of the pollen tubes in the pistil. As expected, such signals are absent in the case of incompatible pollination in petunia (Fig. 4b).

Compatible pollination in *Brassica* causes numerous oscillations and several AcP during the first hour after pollination and in that respect it is similar to Petunia (Fig. 3a). It agrees with an observation that in *Brassica* recognition and rejection or acceptance of the tubes is limited to the stigmatic area (Dickinson 1995). Probably therefore, the second wave of AcP is absent (Figs 3 a and b). In its place a long lasting decrease of potential accompanies pollen tubes growth in style of *Brassica* (Fig. 3 a) which resembles the EP decrease after the second wave of AcPs during the compatible pollination of Petunia flowers (Fig. 4 a). A similar long lasting polarisation of pistil was found previously in wheat (Wędzony and Filek 1996). This phenomenon expresses probably the prolonged changes in the pistils metabolism which were reported in petunia from biochemical and physiological studies (Roggen 1967, Linskens 1973, Donk, Van der 1974, Deurenberg 1976 a, b, Bednarska, Butowt 1995, Lenartowska *et al.* 1997). Among others, a reversed gradient of membrane bound calcium distribution was detected in petunia pistils after pollen tubes' passage (Bednarska 1995). Various physiological alterations following pollination but preceding fertilisation were also found in orchids (Arditti 1979) and in *Hibiscus* (Fromm *et al.* 1995).

During incompatible pollination in both studied species less oscillation of EP and opposite direction of the finale polarisation of pistils were recorded in comparison to the compatible pollination (Figs 3b, 4b). These remain in agreement with some findings of previous studies of incompatible pollination (Sinyukin and Britikov 1967, Linskens 1973, Linskens and Spanjers 1973, Spanjers 1981).

Analysis of pollen tubes' growth, along with electrical recordings, allows for the assumptions as to the area of generation of the signals. For both species it was the surface of the stigma during the first hour of recordings. That is in agreement with numerous data on pollen-stigma interaction which involves ions, peptides and water exchange (Gaude and Dumas 1987, Clarke *et al.* 1989, Bednarska 1992, Bednarska and Butowt 1995, Dickinson 1995). It was demonstrated here that the interaction between the male gametophyte and the stylar tissue is prolonged during further stages. It is represented by EP oscillations in both species and by the second wave of AcPs in Petunia. The occurrence of the latter suggests the presence of receptor area localised on the transition from stigmatic tissue to the transmitting tissue of the style. It agrees with the data of Bednarska (1995) and Lenartowska *et al.* (1997) who observed blockage of some pollen tubes in the upper one third part of the petunia pistil and massive Ca^{2+} displacements between cytoplasm, membranes and the apoplast of the transmitting tissue, which accompanied pollen tubes' growth in this area.

The present investigation demonstrated that each phase of pollen-stigma recognition events, germi-

nation and growth of pollen tubes within the style has its charcteristic pattern of electric changes. They are species specific, depend on compatibility of the cross and the type of incompatibility system. Those action potentials, oscillations and changes of polarization are the most probable form of early communication between style and ovary generating physiological changes in the latter necessary for complete fertilization.

References

Arditti J. 1979. Aspects of the physiology of orchids. Adv. Bot. Res. 7: 421-655.

Bednarska E. 1992. Calcium uptake from the stigma by germinating pollen in *Primula officinalis* L. and *Ruscus aculeatus* L. Sex. Plant Rep. 4: 36-38.

Bednarska E. 1995. Localization of membrane-associated calcium in unpollinated and pollinated pistil of *Petunia hybrida* Hort. Acta Soc. Bot. Poloniae 64(1): 19-24.

Bednarska E., Butowt R. 1995. Calcium in pollen – pistil interaction in *Petunia hybrida* Hort. III. Localization of Ca^{2+} ions and ATPase in pollinated pistil. Folia Hist. Cytob. 33(2): 125-132.

Clarke A. E., Anderson M. A., Atkinson A., Bacic A., Ebert P. R., Jahnen W., Lush W. M., Mau S.-L., Woodward J. R. 1989. Recent developments in the molecular genetics and biology of self-incompatibility. Plant Mol. Biol. 13: 261-271.

Davies E., Zawadzki T., Witters D. 1991. Electrical activity and signal transmission in plants: How do plants know? In: Plant Signaling, plasma membrane and change of state. ed. by Penel C., Greppen H. Universite de Geneve, Geneva pp.119-137.

Deurenberg J. J. M. 1976 a. *In vitro* protein synthesis with polysomes from unpollinated, cross- and selfpollinated Petunia ovaries. Planta 128(1): 29-33.

Deurenberg J. J. M. 1976 b. Activation of protein synthesis in ovaries from *Petunia hybrida* after compatible and incompatible pollination. Acta Bot. Neerl. 25(3): 221-226.

Dickinson H. 1995. Dry stigmas, water and self incompatibility in *Brassica*. Sex. Plant Reprod. 8: 1-10.

Donk, Van der, J. A. W. M. 1974. Synthesis of RNA and protein as a function of time and type of pollen tube-style interaction in *Petunia hybrida* L.. Molec. Genet. 134: 93-94.

Fromm J., Hajirezaei M., Wilke I. 1995. The biochemical response of electric signaling in the reproductive system of *Hibiscus* plants. Plant Physiol. 109: 375-384.

Gaude T., Dumas C. 1987. Molecular and cellular events of self-incompatibility. Int. Rev. Cytol. 107: 333-366.

Lenartowska M., Bednarska E., Butowt R. 1997. Ca^{2+} in the pistil of *Petunia hybrida* Hort. during growth of the pollen tube – cytochemical and radiographic studies. Acta Biol. Crac. ser. Bot. 39: 79-89.

Linskens H. F. 1973 . Activation of the ovary. Caryologia. Suppl. 25: 27-41.

Linskens H. F., Spanjers A. W. 1973. Changes of the electric potential in the transmitting tissue of *Petunia* styles after cross- and self-pollination. Incompatibility Newsletter. 3: 81-85.

Lysikov V., Dukhovnyi A. I. 1966. Izmenenie biopotentsialov v generativnykh organakh kukuruzy. Tr. Kishinievsk. Sel'skokhoz. Inst. 45: 72-75.

Pickard B. G. 1973. Action potentials in higher plants. Bot. Rev.39: 172-201.

Roberts K. 1992. Potential awareness of plants. Nature 360: 14-15.

Roggen H. P. J. R. 1967. Changes in enzyme activities during the progametic phase in *Petunia hybrida*. Acta Bot. Neerl. 16: 1-31.

Sinyukhin A. M., Britikov A. A. 1967. Action potentials in the reproductive systems of plants. Nature 215: 1287-1280.

Spanjers A. W. 1981. Bioelectric potential changes in the style of *Lilium longiflorum* Thumb. after self- and cross-pollination of the stigma. Planta. (153): 1-5.

Wędzony M., Filek M. 1996. Changes of electric potential in wheat pistils induced by pollination. Acta Soc. Bot. Poloniae 65(1-2): 97-100.

Wędzony M., Van Lammeren A. A. M. 1996. Pollen tube growth and early embryogenesis in wheat x maize crosses influenced by 2,4-D. Ann. Bot. 77: 639-649.

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